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CERTIFICATE

This is to certify that the work presented in the thesis entitled **"Pre – Concentration and Quantitative Determination of Pharma Compounds Present in Water"**, submitted to the Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, for the award of **Ph.D.** degree by **Mr. Koppula Santhosh Kumar**, is the original research work carried out by him under my guidance and supervision.

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|| om shree ganeshaya namaha ||

|| anant koti brahmand nayak rajadhiraj yogiraj parbrahma shree

sachidanand sadguru shree sai nath maharaj ki jai ||

|| anant koti brahmand nayak rajadhiraj yogiraj parbrahma shree

sachidanand sadguru shree swami samarth maharaj ki jai ||

DEDICATED TO MY PARENTS

DECLARATION

I state that the work presented in this entitled , **"Pre – Concentration and Quantitative Determination of Pharma Compounds Present in Water"**, comprises of independent investigations carried out by me under the guidance of Dr. Prakash B. Samnani, Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, and are true to the best of my knowledge. Wherever references have been made to the work of others, it has been clearly indicated with the source of information under the reference section. The work presented in this thesis has not been submitted elsewhere for the award of any other degree.

Koppula Santhoshkumar

KOPPULA SANTHOSH KUMAR

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SUMMARY

There is a growing concern about the presence of pharmaceuticals, personal care products and illicit drugs in the aquatic environment (Kasprzyk – Hordern *et al.* 2009). Following use, pharmaceuticals / drugs are excreted as the parent compound, water soluble conjugate or as metabolites and thus enter sewage treatment works (STWs). Disposal of unused pharmaceuticals can also be a route to the aquatic environment either through dumping to sewer via the toilets or drain, or to land – fills in domestic refuse or as special water by licensed waste contractors (Kanda *et al.* 2003). Despite their likely continuous discharge, little is known about the ultimate fate and transport of many drug substances after their intended application. Fewer studies have documented the effect of drugs like salicylate, acetaminophen, and ibuprofen on fish. These are endocrine disruptors in fish and have the potential to impair the adaptive cortisol response to stressors (Gravel and Vijayan 2006). Such reports have led to pharmaceuticals attracting increasing attention as environmental pollutants due to their possible environmental effects (Jones et al. 2003). The type / group of pharmaceuticals detected in aquatic system are fairly broad e.g. contraceptive hormones, lipid regulators, pain – killers, antibiotics, anti – cancer drugs, anti – epileptic drugs and those regulating blood pressure. Where pharmaceuticals have been detected in sewage effluents or surface water, the levels are generally at the ng L^{-1} or at most, low mg L^{-1} level (Kanda *et al.* 2003).

Analyses of such trace level organic pollutants in environmental samples is usually carried out by Gas Chromatography – Mass Spectrometry (GC – MS) or Liquid chromatography – Mass Spectrometry (LC – MS) techniques (Jones *et al.* 2003; Batt and Aga 2005). However, these techniques are relatively expensive and not easily available at several places. Need of the hour; therefore, is to use simpler techniques which however, require higher concentration of target drug molecules. Therefore, prior to the instrumental analysis, attention has to be paid to the sample preparation and enrichment procedure.

Solid Phase Extraction (SPE) is the method of choice for sample enrichment / pre – concentration in environmental analytical chemistry. There are several solid phases on which the sample can be concentrated (Batt and Aga 2005; Psillakis *et al.* 2003).

Drugs have been shown to pass intact through conventional STPs, into water ways, lake and aquifers and discharged pharmaceuticals may end up at landfill sites posing a threat to underlying ground water (Jones *et al.* 2003). Presently, STPs are not designed to completely remove most pharmaceuticals and these compounds are consequently released into surface water, (Zuccato *et al.* 2008; Carballa *et al.* 2004; Stackelberg *et al.* 2007) making it important to develop new methods to treat water containing such pollutants. For removal of such organic compounds charcoal (activated charcoal) is often used as an adsorbent. This sorbent is highly inert, thermally stable, has porous structure and large internal surface area. Literature reports several studies on use of activated charcoal for removal of a variety of pollutants from water (Garcia – Araya *et al.* 2003; Safarik *et al.* 1997).

Aim of our work was to develop simple, accurate and cost effective techniques to determine such low concentration drugs present in aquatic environment. Such studies have not been done in India till now. For our pre – concentration studies we have selected five drugs of different categories: aspirin, paracetamol, esomeprazole magnesium, fenofibrate, and venlafaxine HCl.

Adsorption by activated charcoal is frequently the most efficient and most economical method for removing pollutants from water, particularly when these are present in low concentrations, whether it is a batch process or continuous flow treatment method. The removal efficiency of activated charcoal may be increased by presence of some metal complexes on the surface of activated charcoal. Concept was to use the activated charcoal which has previously been used for removal of metals in effluents. Since disposal of such carbon is a problem. So before disposing, whether we can use the metal loaded carbon once more for removing pharmaceuticals or organic compounds. Aspirin and paracetamol were considered as target drugs for the study of removal efficiency of commercially available activated charcoal (granular) and effect of metal complexes on it. Effect of oxygen on the efficiency of activated charcoal loaded with metal complex to remove these compounds was also studied. It was expected that in presence of metal complexes and oxygen the non – polar part of organic pollutants would be oxidized and become more polar, increasing its affinity for carbon. Hence its removal should be more complete from water. This is because

several transition metal ions and metal complexes are known to act as catalyst for oxidation of organic compounds in presence of oxygen (Jana *et al.* 2007; Silva *et al.* 2004; Silva *et al.* 2002).

The overall work is organized in five chapters. Chapter one describes the literature survey in the area. Chapter two contains pre – concentration method developed for aspirin and paracetamol. The drugs were analysed by UV – Visible spectrometer. Chapter three discusses pre – concentration method developed for esomeprazole magnesium, fenofibrate, and venlafaxine HCl. The drugs were analysed by HPLC. Chapter four describes validation of gradient HPLC method developed for simultaneous determination of esomeprazole magnesium, fenofibrate and venlafaxine HCl. Chapter five is concerned with treatment studies of drugs from water using activated charcoal loaded with metal and metal complex.

Aspirin and paracetamol are components of multidrug pharmaceuticals preparations for the therapy of pain and are widely used as analgesic and antipyretic drugs. There are various methods for the determination of aspirin and paracetamol in various pharmaceutical products, including derivative spectrophotometry (Nogowska *et al.* 1999). Presence of aspirin and paracetamol in water is also reported (Gravel and Vijayan 2006; Jones *et al.* 2003; Kasprzyk – Horden *et al.* 2008, 2009). For our work, Polystyrene divinylbenzene (PSDVB) beads were used as adsorbent for solid phase extraction. aspirin as target drug was pre – concentrated from aqueous solutions on PSDVB beads of gel type and macro – porous type with different cross – linkings and on anion – exchanger. Later it was recovered by different solvents and analysed by UV – Visible spectrometer. Pre – concentration using SPE was carried out using a glass column packed with adsorbent material. Flow rate was maintained using stop – cock attached with the column. Studies were conducted to work out the experimental conditions for the optimum adsorption and recovery.

A typical experiment was performed using synthetic sample of aspirin. A sample of 100mg L^{-1} aspirin aqueous solution of 100mL volume was prepared by diluting an appropriate aliquote of stock solution. The column packed with 1g of the adsorbent material (PSDVB polymer beads) was activated by passing 5mL acetonitrile through it followed by 5mL of acetonitrile: water (80:20) (v/v) and then

by 5mL of water. The aqueous drug sample was passed through the activated column at the rate of 0.66mL min^{-1}. The adsorbed drug was eluted with 10mL of methanol. Amounts of drug adsorbed and recovered in methanol were determined by recording absorbance at 225nm. Optimization was carried out by varying the following parameters one at a time while maintaining all others constant: initial volume of aqueous drug solution, amount of adsorbent, concentration of aqueous drug solution, volume of solvent for recovery and flow rate.

After optimizing the conditions the drug samples of aspirin and paracetamol individually; these conditions were used for analysis of mixture of aqueous solution of aspirin and paracetamol together. For recovery of adsorbed drugs different solvents were used. Later the method was applied for environmental samples collected from the outlet of secondary clarifier of the Sewage Treatment Plant operating with Up – Flow Anaerobic Sludge Blanket (UASB) principle.

Samples of aspirin and paracetamol were analysed by UV spectrometer and HPLC. For UV – Visible spectrometery, the two drugs were analysed together by using Beer – Lambert's law. A chromatographic method was also developed for the analysis of two drugs in mixture by HPLC using UV detector at 280nm and using a C – 18 column. Analytical performance characteristics studies were also performed for two methods.

It was concluded that method developed for pre – concentration of aqueous samples containing aspirin and paracetamol using UV – Visible spectrometer for quantification enabled the determination of the target pharmaceuticals in synthetic water samples at 0.025mg L^{-1}. By using commercially available macro – porous polymer of PSDVB with varying cross – linking and anion – exchanger (Amberlite IRA – 93), in simple laboratory conditions water samples containing aspirin and paracetamol can be pre – concentrated. Quantitative analysis of aspirin and paracetamol individually and in mixture can be done by UV – spectrometer even at low levels though in mixture, pre – concentration of paracetamol is not achieved. The treated water samples collected from STP (Vadodara – India) did not show presence of aspirin and paracetamol up to the detection level of 0.03mg L^{-1} using the developed method when the samples were analysed by HPLC method.

These optimized conditions were used as starting set of conditions for three other drugs: Esomeprazole magnesium, fenofibrate and venlafaxine HCl and further improved for each of the three drugs individually. Quantification of the pre – concentrated drugs from synthetic samples was carried out using HPLC method reported in literature and modified wherever necessary for out conditions. The methods were validated.

Esomeprazole magnesium is a proton pump inhibitor (PPI) developed as an optical isomer (*S* – Esomeprazole) for the treatment of acid – related diseases (Lind *et al.* 2000). Esomeprazole does not undergo chiral inversion in vivo (Andersson *et al.* 2001) and therefore can be determined using the same methodology as for its racemate, omeprazole. The literature survey reveals that omeprazole has been analysed in different products by various methods (Hernando *et al.* 2007). Fenofibrate is fibric acid derivative, used for regulating plasma lipids and treatment of hyperlipoproteinaemias (Sweetman 2002). Fenofibrate has been analysed in pharmaceutical products by different methods (Reddersen and Heberer 2003, Sacher *et al.* 2001; British Pharmacopeia 2007; Romanyshyn and Tiller 2001; Streel *et al.* 2000; Masnatta *et al.* 1996; Ramusino and Carozzi 1986; Rao and Nagaraju 2003; Lacroix *et al.* 1998). Vanlafaxine HCl is a non – tricyclic antidepressant. There are various methods reported in literature for determination of venlafaxine HCl for different purposes (Hicks *et al.* 1994; Vu *et al.* 1997; Schultz and Furlong 2008).

Omeprazole is excreted in an unaltered form in the low proportion and its presence in aquatic environment has been reported (Hernando *et al.* 2007). Presence of fenofibrate in aquatic environment has been reported recently (Redderson and Heberer 2003; Hernando *et al.* 2006). Venlafaxine HCl is soluble in water, which suggests that significant amount of active unused venlafaxine HCl may reach municipal sewage treatment plants through toilets and drains. Number of reports on the occurrence of a wide variety of antidepressants in the aquatic environment have been increasing steadily in recent times, (Weigel 2004) predominantly venlafaxine (Schultz and Furlong 2008).

Optimized conditions obtained in the pre – concentration study of aspirin and paracetamol viz. 1g adsorbent, 100mL aqueous solution of drug and 5mL solvent for

recovery were used as starting set of conditions for the drug esomeprazole magnesium. Aqueous solutions of the pure drug with known concentration were prepared and the pre – concentration conditions optimized by varying experimental parameters one – by – one. Synthetic aqueous samples for esomeprazole magnesium were analysed by HPLC using mobile phase which was prepared by mixing phosphate buffer and acetonitrile in a ratio 70: 30(v/v). Flow rate was 1.0mL min^{-1}. Detection was carried out at 302nm. Sample injection volume was 20μL. All determinations were performed at room temperature. Under these conditions the retention time of esomeprazole prepared in methanol and water was in the range of 15.0 to 15.3min.

Influence of changing the following parameters on pre – concentration of drug was studied in the sequence: i) flow rate of aqueous solution of drug, ii) volume of aqueous solution of drug, iii) amount of adsorbent, iv) different concentrations of aqueous drug solution, and v) different volumes of solvent for recovery. Initially effect of flow rate of aqueous esomeprazole solution on adsorption was studied. With increase in flow rate, adsorption of drug on adsorbent decreases. For subsequent experiments the 0.66mL min^{-1} flow rate was maintained, which resulted into maximum drug absorption of up to 70%. Effect of changing volume of aqueous solution containing the drug, effect of changing amount of adsorbent while keeping volume and concentration of drug solution constant have been studied and optimized in the present work for esomeprazole. Results show that when 100mL esomeprazole aqueous solution is passed through column containing 1g of polymer beads, 70% esomeprazole gets adsorbed on the polymer. While with 50mL of initial drug solution the percentage of drug adsorption increases to 87.95. With this percentage of adsorption, methanol was used for recovery. The percentage of recovery was studied with four different volumes of methanol (3, 5, 7 and 10mL). Maximum drug recovery of up to 102.91% was observed with 10mL of methanol resulting in pre – concentration factor of 6.05. With the decrease in volume of methanol for recovery the percentage of drug recovered decreases but the pre – concentration factor increases. Considering this trend, the condition for recovery of drug adsorbed on 1g of adsorbent was optimized to 5mL of methanol. With these optimized conditions for recovery, pre – concentration experiments were performed taking higher volumes of aqueous drug solutions keeping the amount of drug same. These experiments show

that with increase in volume of initial aqueous drug solution, the percentage of amount of drug adsorbed remains almost same but after its recovery with 5mL methanol, pre – concentration factor for respective experiments increases. However this trend changes with higher volume of initial drug solution. To study effect of initial volume different sets of experiments were carried out with 50, 100, 150, 250 and 500mL of initial aqueous drug solutions respectively. With 250mL initial drug solution, maximum of 96.51% drug was found to get adsorbed. Results also show that volume of 5mL methanol can recover 100% drug at lower amount up to 1.47mg.

The optimized conditions for maximum adsorption of drug and its recovery with better per – concentration factor for esomeprazole are: 250mL of initial aqueous drug solution passed through 1g PSDVB beads with flow rate 0.66mL min^{-1}, followed by 5mL methanol used for recovery of drug adsorbed.

For fenofibrate similar studies were carried out and conditions were optimized. Samples of fenofibrate were analysed by HPLC method using mobile phase which was prepared by mixing acetonitrile and milli – Q water in a ratio of 80:20(v/v) and adjusted to pH 4.0 using phosphoric acid. The flow rate was 1.5mL min^{-1}. Detection was carried out at 287nm. The injection volume was 20µL. All determinations were performed at room temperature. Under these conditions the retention time of fenofibrate prepared in methanol, water and acetonitrile was in the range of 6.0 to 6.3min.

Initially methanol was used for recovery of fenofibrate adsorbed on polymer beads. Due to poor recovery, methanol was replaced by acetonitrile. The optimized conditions for maximum adsorption of drug and its recovery with better pre – concentration factor for fenofibrate are: 50mL of initial aqueous drug solution passed through 1g PSDVB beads with flow rate 0.66mL min^{-1}, followed by 5mL acetonitrile used for recovery of drugs adsorbed.

Similar experiments were performed for venlafaxine HCl and the optimized conditions are: 50mL of initial aqueous drug solution passed through 1g PSDVB beads with flow rate 0.66mL min^{-1}, followed by 5mL methanol used for recovery of drugs adsorbed. Samples of venlafaxine HCl were analysed by HPLC using mobile

phase consisting of acetonitrile: phosphate buffer, 75:25(v/v) at a flow rate of 1.5mL min^{-1}. Detection was carried out at 224nm. All determinations were performed at room temperature. Under these conditions the retention time of venlafaxine HCl prepared in methanol and water was in the range of 2.7 to 2.9min.

The validity of these chromatographic procedures was established through studies of linearity, sensitivity, repeatability. Linearity was established with a series of working standard solutions prepared by diluting the stock solution with respective solvents individually to the final concentrations. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve. The calibration graphs involved at least five experiment points for each drug and they are described by the following equations: for esomeprazole in water: $y = 43243x + 3294.5$ ($r^2 = 1.0000$); for esomeprazole in methanol: $y = 43257 x + 2539.5$ ($r^2 = 1.0000$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area. LOD for esomeprazole in water and methanol was 0.09mg L^{-1} respectively and LOQ in water and methanol was 0.19mg L^{-1} respectively. Equations for standard curve: for fenofibrate in acetonitrile: $y = 39353x - 1379.7$ ($r^2 = 0.9997$); for fenofibrate in water: acetonitrile (60:40)(v/v): $y = 39385 x + 19766$ ($r^2 = 0.9991$). LOD for fenofibrate in water: acetonitrile (60:40)(v/v) and acetonitrile is 0.06mg L^{-1} respectively and LOQ in water: acetonitrile (60:40)(v/v) and acetonitrile is 0.48 mg L^{-1} respectively. Standard curve equations: for venlafaxine HCl in water: $y = 22423x + 157.57$ ($r^2 = 1.0000$); for venlafaxine HCl in methanol: $y = 23924 x + 167.52$ ($r^2 = 1.0000$). LOD for venlafaxine HCl in water and methanol is 0.03mg L^{-1} respectively and LOQ in water and methanol is 0.24mg L^{-1} respectively.

Developed optimized methods for pre – concentration of esomeprazole, fenofibrate and venlafaxine HCl were applied to environmental water sample which was also analysed by HPLC after pre – concentration. In this case, no peaks were observed in the chromatogram for all three drugs. The samples were spiked with a known amount of drugs (1mg L^{-1}) and analysed but the signal enhancement for 1mg L^{-1} added drug was not seen. Results indicate no presence of esomeprazole,

fenofibrate and venlafaxine HCl in the sample collected from the STP which was confirmed by a LC – MS method.

Accuracy of the pre – concentration methods for esomeprazole magnesium, fenofibrate and venlafaxine HCl was studied individually by fortifying the synthetic aqueous and live samples with a known amount of all three drugs respectively. The methods developed for pre – concentration of aqueous solutions containing esomeprazole, fenofibrate and venlafaxine HCl using HPLC method for quantification, are accurate, sensitive and reliable and enable the determination of the target drug in water sample at concentration levels of 0.006mg L^{-1} for esomeprazole, 0.046mg L^{-1} for fenofibrate and 0.024mg L^{-1} for venlafaxine HCl respectively. Thus, in simple laboratory conditions aqueous solution of esomeprazole, fenofibrate and venlafaxine HCl can be pre – concentrated by a factor of 30, 20 and 10 respectively by using, commercially available macro – porous polymer of PSDVB with 8% cross – linking.

The water sample collected from STP (Vadodara – India) after treatment does not show presence of esomeprazole, fenofibrate and venlafaxine HCl up to the detection level of 0.003mg L^{-1} considering the pre – concentration factor in optimized conditions. This means concentration of these drugs in the treated sewage water is below this level possibly due to dilution or STP is efficient in removing the drug effectively.

According to the information collected from literature there is no reported method for simultaneous determination of esomeprazole, fenofibrate and venlafaxine HCl using HPLC which can be applied for detection of these drugs present in water at low concentrations. In this chapter we report development and validation of a new HPLC method for simultaneous determination of esomeprazole, venlafaxine HCl and fenofibrate in a synthetic mixture. For recovery studies, treated sewage water collected from a STP, Vadodara, India was used.

The new HPLC method is simple and sensitive, with total run time less than twenty minutes for the simultaneous determination of esomeprazole, venlafaxine HCl and fenofibrate. Separation was carried out on a C18 column. Mobile phase A

contained a mixture of buffer and acetonitrile in the ratio 75:25(v/v). Mobile phase B consisted of buffer and acetonitrile in the ratio of 30:70(v/v). The buffer consists of 0.3% formic acid. The mobile phase was premixed, filtered through a 0.45µm nylon filter and degassed. The flow rate was kept at 1.1mL min^{-1} throughout. The LC gradient was time (min.) / mobile phase: 0.00 / A, 6.01 / B and 15.01 / A. The detection was monitored at 230nm. The injection volume was 10µL.

At the beginning of method development a chromatographic condition was set for the separation of esomeprazole, venlafaxine HCl and fenofibrate individually by BDS Hypersil C8 column (250 x 4.6mm, 5µ particle size) using a mixture of acetonitrile: buffer (0.13% formic acid, 15.50% 0.1mol L^{-1} ammonium acetate) in the ratio 25:75(v/v) (pH 3.8) as mobile phase A and acetonitrile as mobile phase B at a wavelength of 302nm with flow rate 1.0mL min^{-1} with run time 45 minutes. To reduce the run time chromatographic conditions were changed. This was achieved on a C18 (150cm x 4.6mm, 3.5µm particle size) column and mixture of acetonitrile: buffer (0.3% formic acid) in the ratio 25:75(v/v) as mobile phase A and in the ratio 30:70(v/v) as mobile phase B. At the wavelength of 230nm all the three drugs gave a good response. Under these conditions, sharp peaks that belong to esomeprazole, venlafaxine HCl and fenofibrate were obtained at retention time 3.25, 4.77 and 13.12 minutes respectively. The tailing factor for esomeprazole, venlafaxine HCl and fenofibrate was 1.288, 1.478 and 1.290 respectively.

The gradient RP – LC method developed for determination of esomeprazole, venlafaxine HCl and fenofibrate is precise, accurate and specific. The developed, validated method could separate esomeprazole, venlafaxine HCl and fenofibrate with good resolution.

The limit of detection of esomeprazole, venlafaxine HCl and fenofibrate was 1.02mg L^{-1}, 1.02mg L^{-1} and 1.05mg L^{-1} (for test concentration) respectively; the limit of quantification was 5.18mg L^{-1}, 5.09mg L^{-1} and 5.22mg L^{-1} (of test concentration) respectively. The method can be used for routine analysis.

For removal study from water, aspirin and paracetamol were considered as pollutant target drugs. The drug solutions containing a known concentration of aspirin and paracetamol were treated with activated charcoal loaded with metal complexes

(MACs) or metal ions (MCs). Effect of oxygen on removal efficiency of impregnated activated carbon was also studied. The amount of unadsorbed drug in filtrate was measured by UV – Visible spectrometer and the adsorbed percent was calculated. Metal complexes, copper bisacetylacetonate, manganese salen and copper salen were synthesized using reported methods, characterized by FTIR and CHN micro analysis and loaded on activated charcoal. The copper bisacetylacetonate complex was dissolved in chloroform (500mg L^{-1}). 5g of activated charcoal (AC) was placed in the copper bisacetylacetonate chloroform solution for 3 hours at room temperature with occasional swirling. The resulting material (MAC_1) was filtered off, washed, dried (100°C) and stored in bottle. Similarly manganese salen was loaded on activated charcoal by dissolving in acetonitrile to get manganese salen loaded activated charcoal (MAC_2) and copper salen by dissolving in chloroform to get copper salen loaded activated charcoal (MAC_3) respectively. The metal salts, copper sulphate, nickel sulphate, cobalt chloride and nickel chloride were loaded individually on activated charcoal to get MC_1, MC_2, MC_3 and MC_4 respectively. For determining percentage loading of metal ion or metal complex the amount of the metal complex or metal salt remaining in the filtrate was determined by recording absorbance of the solution at the λ_{max} of the respective metal complex and metal solution, and the concentration computed from corresponding calibration curves of respective metal complex or metal salt. The amount of metal complex or metal salt adsorbed on charcoal was computed using absorbance value for solution before passing it through charcoal. Analytical characteristics and linearity of calibration for each metal complex and metal solution were studied. These materials were used for removal of drugs from aqueous solution. The following two sets of experiments were applied to the three aqueous solutions of aspirin, paracetamol and mixture of aspirin and paracetamol.

Set 1: 10mL (50mg L^{-1}) aspirin solution was added into two different stoppered tubes containing AC and MAC respectively for 30 minutes with constant stirring. Resultant solution was filtered and absorbance was measured using UV – Visible Spectrometer.

Set 2: 10mL (50mg L^{-1})) aspirin solution was added into two different stoppered tubes containing AC and MAC respectively for 30 minutes with constant supply of oxygen.

Resultant solution was filtered and absorbance was measured using UV – Visible Spectrometer.

These set of conditions were applied individually to all activated charcoal samples loaded with three metal complexes and metal salts respectively. Results show that AC removes 14.29% aspirin and 47% paracetamol from water when the individual aqueous drug solutions where treated with it, but the percentage of removal increases for both drugs in presence of oxygen. The trend remains almost similar when the individual aqueous drug solutions were treated with metal complex loaded activated charcoal MAC_1, MAC_2 and MAC_3, except MAC_1 which separates 70% aspirin from water in presence of oxygen. When the mixture of aqueous drug solution was treated with AC the percentage removal of individual drug increases compared to the individual treatment with AC In case of paracetamol, however, adsorption from mixture decrease in absence of oxygen.

In case of individual drug solutions, MAC_1 separates maximum amount of aspirin from water when compared with other three adsorbents in absence of oxygen. In presence of oxygen the trend remains same but removal efficiency of MAC_1 for aspirin increases to 38% compared to that with absence of oxygen. Whereas MAC_3 separates maximum amount of paracetamol from water when compared with other three adsorbent in absence of oxygen. In presence of oxygen the removal efficiency of all four adsorbents increases.

In case of treatment of drugs in presence of each other, MAC_2 separates maximum amount of aspirin from water when compared with other three adsorbent in absence of oxygen, whereas in presence of oxygen MAC_1 separates maximum amount of paracetamol. When paracetamol is considered, MAC_3 adsorbs maximum amount of paracetamol in absence of oxygen and almost 100% in presence of oxygen.

The adsorption of drugs on impregnated AC increases in presence of oxygen compared to that in absence of oxygen. In case of MAC_1 a distinct difference on the adsorption behavior of aspirin and paracetamol is observed: aspirin and paracetamol in presence of each other get less adsorbed compared to individual treatment in absence of oxygen, but in presence of oxygen adsorption of aspirin increases and

paracetamol decreases in presence of each other. Similarly in case of MAC3 amount of aspirin adsorbs in less amount when treated with paracetamol in absence of oxygen compared to its individual treatment but in presence of oxygen adsorption efficiency of MAC_3 increases both for aspirin and paracetamol in presence of each other as compared to their individual treatment. At this juncture we are unable to provide suitable explanation for the observed trend.

All most in all cases of metal salts loaded activated charcoal, the adsorption of drugs increases in absence of oxygen with respect to activated charcoal which is not loaded with metal salts. The effect of oxygen was not observed on the absorption of adsorbents except AC. MC_2 removes maximum about of drugs in absence of oxygen and presence of oxygen increases removal efficiency of MC_2. In case of MC_1 and MC_3 a distinct difference on the adsorption behavior of aspirin and paracetamol is observed: When treated individual aspirin adsorbed more compared to paracetamol and where as paracetamol adsorbed more compared to aspirin when treated in presence of each other. In case of MC_4, the percentage removal of both aspirin and paracetamol in presence of each other decreases compared to its individual treatment.

From the removal study it can be concluded that metal complex and metal ion loaded activated charcoal can remove drugs present in water. Its removal efficiency increases in presence of oxygen. Metal loaded charcoal can be used for removal of drugs present in water as tertiary treatment in water treatment plants. However, no specific trend could be observed in terms of use of metal salt or metal complex.

CHAPTER 1

INTRODUCTION

WATER

Water is a ubiquitous chemical substance that is composed of hydrogen and oxygen and is essential for all known forms of life. Water on Earth moves continually through a cycle of evaporation or transpiration (evapotranspiration), precipitation, and runoff, usually reaching the sea. Over land, evaporation and transpiration contribute to the precipitation.

Clean, fresh drinking water is essential to human and other life forms. There is a clear correlation between access to safe water and GDP per capita. However, some observers have estimated that by 2025 more than half of the world population will be facing water – based vulnerability (Kulshreshtha 1998). Water plays an important role in the world economy, as it functions as a solvent for a wide variety of chemical substances and facilitates industrial cooling and transportation. Approximately 70% of freshwater is consumed by agriculture (Baroni *et al.* 2007).

WATER QUALITY

Water quality is one of the most relevant topics in efforts towards environmental and sustainable development. Still in many countries surface water is one of the most important sources of drinking water. Water intake is crucial to our survival. For example, drinking ample amounts of water has been tied to general good health. Also, water can be a specific antidote to some of the more troubling and inconvenient health problems, such as obesity and many types of cancer. Water has the potential to be one of the most useful and cost – effective medicinal substances available.

Unfortunately, dangerous chemicals, organic materials, and bacteria contaminate much of the water we drink. When combined with these elements, water, crucial to our survival as it is, can present a significant health risk. Despite several governmental efforts to clean, purify, and provide safer sources of water, dangerous contaminants continue to be present in our drinking water. These contaminants, many of which are undetectable by sight or taste, can lead to diseases ranging from asthma to the debilitating Parkinson's disease (www.historyofwaterfilters.com).

In order to understand drinking water contamination, it is necessary to first understand from where our drinking water comes. For most urban residents, relying upon municipal water systems, drinking water comes from two major sources: groundwater and surface water.

Each source of water has a unique set of contaminants; groundwater stores pesticide chemicals and nitrate while surface water contains most bacteria and other microorganisms. Because of the interconnectedness of groundwater and surface water, these contaminants may be shared between the two sources. Neither water source can ever be entirely free from water contaminants. Due to the cycle of water (hydrology), the two sources of drinking water feed each other, sharing contaminants.

Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and either does not support a human use, like serving as drinking water, and /or undergoes a marked shift in its ability to support its constituent biotic communities, such as fish. Natural phenomena such as volcanoes, algal blooms, storms, and earthquakes also cause major changes in water quality and the ecological status of water.

FACTORS INFLUENCING WATER QUALITY

Water pollution is the contamination of water bodies such as lakes, rivers, oceans, and groundwater. All water pollution affects organisms and plants that live in these water bodies and in almost all cases the effect is damaging not only to individual species and populations but also to the natural biological communities. It occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful constituents.

Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases, (Pink 2006; West 2006) and that it accounts for the deaths of more than 14,000 people daily (West 2006). Some 90% of China's cities suffer from some degree of water pollution, (Chinadaily.com.cn 2005) and nearly 500 million people lack access to safe drinking water (The New York Times 2007). In addition to the acute problems of water pollution in developing countries, industrialized countries continue to struggle with

pollution problems as well. In the most recent national report on water quality in the United States, 45% of assessed stream miles, 47% of assessed lake acres, and 32% of assessed bay and estuarine square miles were classified as polluted (EPA 2007).

Water pollution has many causes and characteristics. Point source pollution refers to contaminants that enter a waterway through a discrete conveyance, such as a pipe or ditch. Examples of sources in this category include discharges from a sewage treatment plant, a factory, a city storm drain or from a construction site. Non – point source (NPS) pollution refers to diffuse contamination that does not originate from a single discrete source. NPS pollution is often accumulative effect of small amounts of contaminants gathered from a large area. The leaching out of nitrogen compounds from agricultural land which has been fertilized is a typical example. Nutrient runoff in storm water from "sheet flow" over an agricultural field or a forest are also cited as examples of NPS pollution. Contaminated storm water washed off of parking lots, roads and highways, called urban runoff, is sometimes included under the category of NPS pollution. Often, this runoff is typically channeled into storm drain systems and discharged through pipes to local surface waters, and is a point source. However where such water is not channeled and drains directly to ground it is a non – point source.

The specific contaminants leading to pollution in water include a wide spectrum of chemicals, pathogens, and physical or sensory changes such as elevated temperature and discoloration.

Oxygen – depleting substances may be natural materials, such as plant matter (e.g. leaves and grass) as well as man – made chemicals. Other natural and anthropogenic substances may cause turbidity (cloudiness) which blocks light and disrupts plant growth, and clogs the gills of some fish species (EPA 2005).

Many of the chemical substances are toxic. Pathogens can produce waterborne diseases in either human or animal hosts. Alteration of water's physical chemistry includes acidity (change in pH), electrical conductivity, temperature, and eutrophication.

PHARMACEUTICALS IN WATER

The focus of environmental research has recently extended beyond classic environmental pollutants, such as PCBs, dioxins, and pesticides, to pharmaceuticals and personal care products (PPCPs), which enter the environment mainly via regular domestic use (Daughton and Ternes 1999) and other sources.

During the past three decades, research on the impact of chemical pollution has focused almost exclusively on the conventional 'priority' pollutants [i.e. persistent organic pollutants (POPs)] and this has been extensively reviewed recently (Birkett and Lester 2002). Today, these compounds are less relevant for many first world countries because emissions have been substantially reduced through the adoption of appropriate legal measures and the elimination of many of the dominant pollution sources. The focus has consequently shifted to compounds present in lower concentrations but which nevertheless might have the ability to cause harm (Larsen *et al.* 2004). One of the interesting characteristics of many of the chemicals that might cause this type of pollution is that they do not need to be persistent in the environment to cause negative effects (Ayscough *et al.* 2000). This is because their high transformation and removal rates can be offset by their continuous introduction into the environment, often through sewage treatment works (Suter and Giger 2001). This is one reason why there is an increasing consensus that this kind of contamination might require legislative action sooner rather than later (Petrovic *et. al.* 2003a; Hilton *et. al.* 2003).

The issue of pharmaceuticals (and their metabolites) in the environment, notably the aquatic compartment, has been a growth area in environmental chemistry for several years (Jones *et. al.* 2001). To date, most of the published literature has addressed the occurrence of drugs in sewage effluent and receiving waters. Although, the risks associated with exposure to drugs are probably most significant with regard to the natural environment; the public's concern is more focused on human exposure. This is especially important in areas that practice indirect water reuse, where sewage effluent is released to streams and rivers that are in turn used as a source of raw water for the production of potable supplies for communities living downstream (van Dijk – Looijaard and van Genderen 2000).

A few studies have documented the occurrence of such organic compounds in drinking – water supplies. Exceptions include documentation of low – level concentrations of such compounds in plant – scale studies of drinking water supplies (Loraine and Pettigrove 2006; Petrovic *et al.* 2003b; Adams *et al.* 2002; Ternes *et al.* 2002; Reddersen *et al.* 2002; Heberer and Stan 1997) and evaluation of their fate in laboratory – scale simulations of drinking – water – treatment (DWT) processes (Mompelat *et al.* 2009; Westerhoff *et al.* 2005; Huber *et al.* 2005; Pinkston and Sedlak 2004; Zwiener and Frimmel 2000).

It is often anticipated that pharmaceuticals are easily bio – degradeable in the environment, since they are transformed to some extent in humans. First findings of drugs in the aquatic environment were reported in the 1970s (Tabak and Brunch 1970; Garrison *et al.* 1976).

Some investigations showed the existence of drugs in public – owned treatment works effluents. They have been mainly carried out in the UK in the eighties (e.g. Aherne *et al.* 1990). The concentrations measured in surface waters and STP effluents were in the ng L^{-1} range. Similar substances were detected in effluents from sewage treatment plants as well as in the aquatic system, e.g. in small creeks and big river such as river Rhine, Elbe, Neckar, Danube, Po, and other (Ternes 1998; Zuccato *et al.* 2001) as well as lakes (e.g. Lake constane, swiss Lakes) (Poiger *et al.* 2001), in ground water (Herberer *et al.* 1995) as well as the North Sea and The Adriatic Sea (Buser and Muller 1998; Zuccato *et al.* 2001). Emissions from a landfill containing remainders from pharmaceuticals production were also reported (Holm *et al.* 1995).

The detected compounds include a wide variety such as hormones, lipid regulators, pain killers, antibiotics, anti – cancer drugs and other cytotoxic compounds, anti – epileptic as well as those regulating blood pressure (Ayscough *et al.* 2000). Tetracycline is one of the most important antibiotics used in agriculture. It was detected in topsoil (Hamscher *et al.* 2000) in high concentration (20mg kg^{-1} soil). This concentration is twice as high as the PEC (Predicted Environmental Concentration) set as a trigger value by the EU for the need of further investigations. Evidence of a wide variety of different active substances in the aquatic environment

as well as in liquid manure and in the soil also shows that the active substances are at least not completely eliminated in sewage treatment or in the environment (Kummerer 2001a). Drugs and disinfectants are applied, in contrast to many other chemical substances, because of their specific biological effect. Drugs used in veterinary medicine and husbandry for therapy as well as for prophylactic use and as growth promoters have been assessed (Montforts 2001). Up to now there is not sufficient data available on the occurrence, fate and effects of drugs in the environment and the risks for humans and the environment possibly connected with it (Halling – Sorensen *et al.* 1998; Stuer – Lauridsen *et al.* 2000; Kummerer 2001b).

According to present knowledge, for risk assessment most pharmaceuticals can be handled like pesticides. The mode of action should be taken into consideration when assessing effect of pharmaceuticals against organisms with standard tests. Some groups of compounds need special attention (Kummerer 2001b): Cytostatic agents and immunosuppressive drugs, because of their frequently evident carcinogenic, mutagenic or embryotoxic properties as well as other genotoxic compounds (e.g. some antibiotics); antibiotics and disinfectants, because of their pronounced bacterial toxicity and their potential of fostering resistance; Hormones, because of their high efficiency / low effect threshold; Chlorophenols, chlorine – releasing reagents such as sodium hypochlorite, dichloroisocyanuric acid and others used as disinfectants and as bleaching agents or diagnostics such as organic iodine – containing X – ray contrast media because they contribute to the absorbable organic halogen compounds (AOX); these are very often not biodegradable and spread widely in the aquatic environment and / or enter the food web; Heavy metals, e.g., as part of disinfectants and preservatives containing mercury, cytostatic agents containing platinum or MRI contrast media containing gadolinium, as they are not degradable and highly toxic in some oxidation states.

Other groups of drugs, for instance analgesics or sedatives, are also of interest. Barbiturates were reported to influence DDT – metabolism in fish. They also may modulate behavior and predator – prey relations by lowering swimming velocity and influencing reaction times (Kummerer 2001a).

TYPES OF DRUGS PRESENT IN WATER

During and after treatment, humans and animals excrete a combination of intact and metabolized pharmaceuticals. Consequently, many bioactive compounds enter wastewaters and the receiving water bodies without any test for specific environmental effects. In addition, the chemicals that compose personal care products also number in the thousands. The world's people consume enormous quantities of skin care products, dental care products, soaps, sunscreen agents, and hair styling products to name just a few. In the early 1990's annual production of these products exceeded 550, 000 metric tons for Germany alone (Daughton and Ternes 1999). Fragrances (e.g., nitro- and polycyclic – musks), UV blockers (e.g., methylbenzylidene camphor), and preservatives (e.g., parabens and isothiazolin derivatives) are included in personal care formulations, for chemical and biological stabilization (Ternes *et al.* 2004a). Unlike pharmaceuticals, personal care products do not have to pass through the human body. They enter the wastewater after their regular use during showering or bathing. The environmental fate of many cosmetic ingredients, such as preservatives and hair colorants, has not been studied, although considerable persistence and bioaccumulation potential have been reported (Geyer *et al.* 2000).

The occurrence of the Pharmaceutically active compounds (PhACs) in the aquatic environment has been investigated in several studies in Austira, Brazil, Canada, Croatia, England, Germany, Greece, Italy, Spain, Switzerland, The Netherlands and the U.S. More than 80 PhACs from various prescription classes have been detected up to the $\mu g\ L^{-1}$ level in sewage, surface and groundwater.

Analgesics and anti – inflammatory drugs

Most analgesics also have anti – inflammatory and antipyretic properties. Large amounts of pain killers are prescribed in human medical care but often they are sold at much higher quantities without prescription as so – called 'over – the – counter' (OTC) drugs.

Acetaminophen (paracetamol) and acetylsalicylic acid (aspirin) are the two most popular pain killers mainly sold as OTC drugs. As a pro – drug, aspirin is,

however, easily degraded by deacetylation into its more active from salicylic acid and into two other metabolites namely *ortho* – hydroxyhippuric acid and the hydroxylated metabolite gentisic acid. Ternes (1998), Ternes *et al.* (1998) detected salicylic acid, *ortho* – hydroxyhippuric acid and gentisic acid in sewage influent samples at concentrations up to 54, 6.8, and 4.6μg L^{-1} respectively. Ternes *et al.* (1998) observed that all three compounds were efficiently removed by the municipal STPs and only salicylic acid was detected at very low concentrations in sewage effluents and also in rivers. Heberer (2002a) also reported average concentrations of only 0.04μg L^{-1} for salicylic acid in sewage effluents. But in this study, the average influent concentrations of 0.34μg L^{-1} were relatively low, too. On the other hand, much higher concentrations of salicylic acid up to 13μg L^{-1} were detected in sewage effluents in Greece and Spain (Farre´ *et al.* 2001; Heberer *et al.* 2001a). Residues of salicylic acid do not necessarily have to derive from aspirin. Other sources such as the use of salicylic acid as keratolytic, dermatice, and preservative of food or its natural formation are even more likely to be responsible for the occurrence of this compound in the aquatic environment (Heberer 2002a).

Approximately, 75 tons of the prescription drug diclofenac were annually sold in Germany (Ternes 2001a). In long – term monitoring investigations of sewage and surface water samples from Berlin, Germany, Heberer *et al.* (2002b) identified diclofenac as one of the most important PhAC present in the water – cycle. Average concentrations of 3.02 and 2.51μg L^{-1} were detected in the influents and effluents of the municipal STPs, respectively. This low removal rate of only 17% demonstrates the persistence of diclofenac in the STPs and was also reported by Buser *et al.* (1998b), Stumpf *et al.* (1999), Zwiener *et al.* (2000), whereas Ternes (1998) reported a removal of 69% for diclofenac in the STPs. Diclofenac was also frequently detected at concentrations up to the μg L^{-1} level in investigations of sewage effluents and surface waters in Austria, Brazil, Germany, Greece, Spain, Switzerland, and the U.S. (Buser *et al.* 1998a; Heberer *et al.* 1997, 2001a; Ternes 1998; Stumpf *et al.* 1999; Ahrer *et al.* 2001; Farre *et al.* 2001; Ollers *et al.* 2001; Heberer 2002a). However, Buser *et al.* (1998b) also observed a significant elimination of diclofenac in the water of a natural lake in Switzerland presuming a possible photolytic degradation of the residues. In laboratory experiments with spiked lake water, Buser

et al. (1998b) confirmed a rapid and extensive photodegradation of diclofenac by sunlight. They also characterized several photoproducts but these could not be detected under natural conditions. Results from surface water monitoring in Berlin, Germany, also indicate a possible photodegradation of diclofenac. But seasonal differences of the concentrations of diclofenac are also due to more extensive application of such drugs during the winter period (Heberer *et al.* 2002a) because the cold and humid weather causes an increase of rheumatic diseases. In general, the reduction of diclofenac by natural photolytic degradation will also depend on some additional key parameters such as eutrophic conditions, degree of solid or particulate matter or the depth of the watercourse. Under recharge conditions, diclofenac has also been detected in ground water samples (Heberer *et al.* 1997; Sacher *et al.* 2001). First results from laboratory column experiments (Scheytt *et al.* 2001) and from observations (Brauch *et al.* 2000) and field experiments (Heberer *et al.* 2001b) on bank filtration indicate a significant sorption and an efficient attenuation of diclofenac residues in the subsoil (Verstraeten *et al.* 2002). To date, diclofenac was only sporadically found at trace – level concentrations in raw or treated drinking water (Brauch *et al.* 2000; Heberer *et al.* 2001a, b; Ternes 2001a). Zwiener and Frimmel (2000) have shown that diclofenac can be removed from drinking water by ozonation. Together with several other PhACs, diclofenac was also efficiently removed from surface or municipal sewage effluents using membrane filtration (Heberer *et al.* 2002b).

In Austria, Brazil, Germany, and Switzerland, ibuprofen is found in sewage effluents and rivers, usually at concentrations much lower than those observed for diclofenac (Heberer *et al.* 1997; Heberer *et al.* 2002b, Ternes 1998; Buser *et al.* 1999; Stumpf *et al.* 1999; Ollers *et al.* 2001). In Spain, Farre´ *et al.* (2001) detected 1.5, 0.87, and 85μg $L^{-1}$of ibuprofen in sewage effluent samples. In the same study, it was also found at relatively high concentrations of up to 2.7μg L^{-1} in Spanish surface waters. Ibuprofen is degraded in the human body to its principal metabolites hydroxyl – and carboxy – ibuprofen and to carboxy – hydratropic acid (Stumpf *et al.* 1998; Buser *et al.* 1999) which are found together with ibuprofen in raw sewage. Stumpf *et al.* (1998) observed a significant removal of ibuprofen and especially of carboxy – ibuprofen during sewage treatment, whereas the concentrations of

hydroxyl – ibuprofen in the sewage effluents (median: 0.92μg L^{-1}) were almost similar to those in the influents. Thus, hydroxyl – ibuprofen was found in 12 German surface waters at much higher concentrations (median: 0.34μg L^{-1}) than ibuprofen or carboxy – ibuprofen (median: 0.02μg L^{-1}, respectively) (Stumpf *et al.* 1998).

Several other analgesics namely 4 – aminoan – tiyrine, aminophenazone, codeine, fenoprofen, hydrocodone, indometacine, ketoprofen, mefenamic acid, naproxen, phenazone and propyphenazone have also been detected in sewage and surface water samples (Heberer *et al.* 1997, 2001a; Heberer *et al.* 2002b, Ternes, 1998; Stumpf *et al.* 1998; Stumpf *et al.* 1999; Ahrer *et al.* 2001; Farre *et al.* 2001; Ollers *et al.* 2001; Ternes *et al.* 2001; Heberer, 2002a, Kolpin *et al.* 2002).

Under recharge conditions or at land fill leachates several analgesics namely diclofenac, ibuprofen, ketoprofen, phenazone, propyphenazone, gentisic acid or N – methylphenacetin (both metabolites), have also been detected in groundwater samples in Croatia, Denmark and Germany (Holm *et al.* 1995; Heberer *et al.* 1997, 2001b; Ahel and Jelicic 2001; Sacher *et al.* 2001; Reddersen *et al.* 2002). In Germany, diclofenac, ibuprofen, and phenazone residues have been detected at trace – level concentrations in a few drinking water samples (Heberer *et al.* 2001a; Ternes 2001). In laboratory experiments, propyhenazone was adsorbed at sediments but there is also some evidence that it might be remobilized by particle transport (Scheytt *et al.* 2001). In the field experiments on bank filteration, propyhenazone was not totally removed. It was detected in the shallow wells and also reached the water supply wells (Heberer *et al.* 2001b).

In river Taff and River Ely, UK, paracetamol and tramadol have been quantified at concentrations exceeding single μg L^{-1}. Also codeine was found at relatively high concentrations reaching 0.5μg L^{-1}. The other anti – inflammatory / analgesics such as ibuprofen, diclofenac, ketoprofen, naproxen, mefenamic acid, aspirin and its metabolite salicylic acid were quantified at levels of less than 10ngL^{-1} to hundreds ng L^{-1} (Barbara *et. al* 2008).

Ketoprofen, naproxen, ibuprofen, diclofenac, mefenamic acid, paracetamol and aspirin were also analysed and determined at similar levels by several research

groups (Gros *et al.* 2006; Moldovan 2006; Vieno *et al.* 2005; Lindqvist *et al.* 2005; Bendz *et al.* 2005; Calamari *et al.* 2003; Kolpin *et al.* 2002, 2004; Glassmeyer *et al.* 2005). However, lower levels for naproxen, ibuprofen, diclofenac were obtained in surface water by Vanderford *et al.* (2003) and higher levels were obtained for ibuprofen by Roberts and Thomas (2006). Codeine (natural opiate) and tramadol (synthetic opioid) were not widely studied, although similar concentrations of codeine were observed by Glassmeyer *et al.* (2005) and Hummel *et al.* (2006).

Antibacterial drugs

Several studies have been carried out in Germany (Steger – Hartmann *et al.* 1997; Hirsch *et al.* 1999), Switzerland (Alder *et al.* 2001; Golet *et al.* 2001), and the U.S. (Lindsey *et al.* 2001; Kolpin *et al.* 2002) to investigate the occurrence and fate of antibacterial drugs in Sewage Treatment Plants (STPs) or surface waters. Macrolide antibiotics (clarithromycin, dehydro – erythromycin [metabolite of erythromycin], roxithromycin, lincomycin), sulfonamides (sulfamethoxazole, sulfadimethoxine, sulfamethazine, and sulfathiazole), chloroquinolones (cipro oxacin, nor oxacin, and enro oxacin), chloramphenicol, tylosin and trimethoprim have been found up to the low $\mu g\ L^{-1}$ level in sewage and surface water samples. In their monitoring investigations of various sewage, surface and groundwater samples in Germany, Hirsch *et al.* (1999) did not detect penicillins or tetracyclines. This result is no surprise as penicillins are easily hydrolyzed and tetracyclines readily precipitate with cations such as calcium and accumulate in sewage sludge's or sediments (Daughton and Ternes 1999; Stuer – Lauridsen *et al.* 2000). Nevertheless, Lindsey *et al.* (2001), Kolpin *et al.* (2002) also detected tetracycline drugs (chlortetracycline, oxytetracycline, and tetracycline) in U.S. surface water samples. Golet *et al.* (2001) analyzed fluoroquinolone antibiotics in primary and tertiary wastewater effluents in Switzerland. In these samples, ciprofloxacin and norfloxacin occurred at concentrations between 249 to 405ng L^{-1} and from 45 to 120ng L^{-1}, respectively. Antibiotics have also been identified at high concentrations in hospital effluents (Hartmann *et al.* 1998; Alder *et al.* 2001). Thus, Hartmann *et al.* (1998) detected between 3 and 87$\mu g\ L^{-1}$ of the fluoroquinolone antibiotic ciprofloxacin in hospital effluents. Sacher *et al.* (2001) reported the occurrence of sulfamethoxazole

(up to 410ngL^{-1}) and dehydroerythromycin (up to 49ngL^{-1}) in groundwater samples in Baden – Wurttemberg, Germany. Sulfamethoxazole and sulfamethazine have also been detected at low concentrations in a few groundwater samples in the U.S. and Germany (Hartig *et al.* 1999; Hirsch *et al.* 1999; Lindsey *et al.* 2001). Holm *et al.* (1995) found residues of different sulfonamides at high concentrations in groundwater samples collected down gradient of a land fill in Grinsted, Denmark.

Among antibacterial drugs studied, trimethoprim, erythromycin – H_2O and amoxicillin were found at high concentrations exceeding on an average 70ngL^{-1} and reaching 300ngL^{-1} during dry weather conditions in river Taff and River Ely, UK. All antibacterial drugs were found at similar levels in the two rivers studied. Their concentrations were found to be at the highest levels at sampling points just after a discharge of treated wastewater effluent into river water. (Barbara *et al.* 2008).

Similar levels of concentrations for erythromycin, trimethoprim and sulfamethoxazole were obtained by several research groups (Vanderford *et al.* 2003; Gros *et al.* 2006; Roberts and Thomas 2006; Bendz *et al.* 2005; Hirsch *et al.* 1999; Calamari *et al.* 2003; Kolpin *et al.* 2002, 2004; Glassmeyer *et al.* 2005). Trimethoprim, erythromycin – H_2O and amoxicillin were found in 100% of the samples at all sampling points located after WWTP discharge in the Taff River. Similar results were obtained for trimethoprim in the Ely River. A strong correlation was observed between the amount of pharmaceutical dispensed in Wales, its excretion as an unchanged drug and the concentration level in surface water. For example, amoxicillin as a pharmaceutical with the highest consumption (almost 9900kg per year) and high excretion as an unchanged drug (60–80%) was therefore expected to be present in surface water at concentrations higher than sulfamethoxazole (consumption: only 115kg per year; excretion as an unchanged drug: only 30%). Of the three antibiotics present in surface water at the highest concentrations (trimethoprim, erythromycin – H_2O and amoxicillin), only the level of amoxicillin was found to have decreased significantly downstream of treated wastewater discharge (100% decrease 25km downstream of sampling point). In the case of trimethoprim and erythromycin– H_2O, only a 56 and 12% reduction in their

respective concentration after 25km of river flow in the River Taff was observed (Barbara *et al.* 2008).

Antiepileptic drugs

The antiepileptic drug carbamazepine has frequently been detected in municipal sewage and surface water samples (Ternes 1998; Heberer *et al.* 2001a; Heberer *et al.* 2002b; Ahrer *et al.* 2001; Ollers *et al.* 2001; Heberer 2002a). Investigations of influent and effluent samples from different municipal STPs have shown that it is not significantly removed (less than 10%) during sewage treatment (Ternes 1998; Heberer 2002a). Thus, carbamazepine has been detected at concentrations up to $1075 ngL^{-1}$ in surface water samples in Berlin, Germany (Heberer *et al.* 2002b). Primidone, another antiepileptic drug, has also been detected in samples from municipal sewage influents and effluents and in surface waters (up to $635 ngL^{-1}$) in Germany (Heberer *et al.* 2001a; Heberer *et al.* 2002b, Heberer 2002a). Different field studies have shown that carbamazepine (Brauch *et al.* 2000; Heberer *et al.* 2001b) and primidone (Heberer *et al.* 2001b) are not attenuated during bank infiltration. Both compounds have been detected in the shallow wells and water supply wells of a transect build to study the behavior of PhACs during bank filtration (Heberer *et al.* 2001b). This also explains, why carbamazepine has been detected in a number of groundwater samples at a maximum concentration up to $1.1 \mu g\ L^{-1}$ (Seiler *et al.* 1999; Sacher *et al.* 2001; Ternes 2001a) and was also found with a concentration of $30 ng\ L^{-1}$ in drinking water (Ternes 2001 a). Carbamazepine and gabapentin were found to be ubiquitous (present in 100% of samples collected at sampling points downstream of WWTP discharge) and persistent in the river water.

Their removal in the River Taff accounted for approximately 50% of 27km downstream of the treated wastewater discharge. Despite similar quantities of both drugs dispensed in the Welsh community in 2006, gabapentin was found at much higher concentrations of up to $1 \mu g\ L^{-1}$ in both the River Taff and Ely than carbamazepine (maximum concentration $684 ngL^{-1}$ determined in the River Ely during dry weather conditions). This can be explained by the fact that gabapentin is excreted by the human body in unchanged form; while carbamazepine is excreted in approximately 3% as an unchanged compound and therefore its environmental

concentrations are much higher. Poor or no removal of both pharmaceuticals was observed after sewage treatment (Barbara *et al.* 2008). Similar observations in relation to carbamazepine were made by others (Vanderford *et al.* 2003; Vieno *et al.* 2006; Gros *et al.* 2006; Moldovan 2006; Bendz *et al.* 2005; Kolpin *et al.* 2002, 2004; Glassmeyer *et al.* 2005).

Beta – adrenoceptor blocking agents

Several beta-blockers (metoprolol, propanolol, betaxolol, bisoprolol, and nadolol) have been detected in municipal sewage effluents up to the μgL^{-1} level (Hirsch *et al.* 1998; Ternes 1998). Only metoprolol, propanolol, and bisoprolol have also been found at relatively low concentrations in surface water samples (Hirsch *et al.* 1998; Ternes 1998). As far as beta – blockers are concerned; Hirsch *et al.* (1998) did not recognize any relevance for groundwater recharge or drinking water supply. However, Sacher *et al.* (2001) also reported the detection of sotalol at maximum concentrations of 560ng L^{-1} in three groundwater samples from Baden – Wurttemberg, Germany.

In case of surface water in South Wales, UK, only atenolol was found to be present at levels exceeding $100ngL^{-1}$. This was expected due to its higher dispension (over 2300kg per year) and its high excretion rates as an unchanged drug (50%) when compared with the other beta – blockers studied (e.g., propranolol: only 463kg dispensed in Wales in 2006; excretion as an unchanged drug of <0.5%). Despite low concentrations, all β – blockers were found to be present in 100% of the analysed samples collected at sampling points located after treated wastewater discharge over the 10 month sampling regime, with limited decrease of concentration levels with the distance from wastewater discharge point (50%, 11% and 60% degradation of propranolol, metoprolol and atenolol, respectively, 25km downstream of sampling point). **The above outcome conforms that some β – blocker such as atenolol and propranolol also belong to the group of pharmaceuticals that are persistent and ubiquitous in the aqueous environment.** Comparable results were reported by the other researchers (Calamari *et al.* 2003; Gros *et al.* 2006; Bendz *et al.* 2005; Kolpin *et al.* 2002, 2004;

Glassmeyer *et al.* 2005). However, much higher or lower levels of β –blockers were observed by Vieno *et al.* (2006) and Roberts and Thomas (2006).

Lipid regulating agents

The first incidental findings of the drug metabolite clofibric acid (2 – (4) – chlorophenoxy – 2 – methyl propionic acid) in the aquatic environment in Germany and Switzerland (Heberer *et al.* 1997; Buser *et al.* 1998a) probably initiated most of the investigations on PhACs. However, the first detections of clofibric acid, the active metabolite of the blood lipid regulators clofibrate, etofyllin clofibrate, and etofibrate, in samples from STPs in the U.S. have already been reported in the 1970s (Garrison *et al.* 1976; Hignite and Azarnoff 1977). In Germany, it was found at concentrations up to $4\mu gL^{-1}$ in groundwater samples collected from former sewage irrigation fields near Berlin (Heberer *et al.* 1997). Underneath the sewage farm areas, it could even be found in samples from the fourth or fifth groundwater aquifer down to a depth of 125m. Up to $270ngL^{-1}$ of clofibric acid have been detected in Berlin drinking water samples (Heberer *et al.* 1997). Buser *et al.* (1998a) detected clofibric acid at the low ngL^{-1} range in Swiss lakes from populated areas and also in the North Sea. Clofibric acid was identified as refractory contaminant in several investigations of municipal sewage influents and effluents (Ternes 1998; Stumpf *et al.* 1999; Heberer *et al.* 2002b). Zwiener *et al.* (2000) carried out biodegradation studies using a pilot sewage plant and biofilm reactors operated under oxic or anoxic conditions. In spiking experiments with synthetic sewage water they confirmed the persistence of clofibric acid under anoxic and oxic conditions, as well. Meanwhile, a number of findings in sewage, surface, and groundwater have been reported for clofibric acid from Austria, Brazil and Germany (Heberer and Stan 1997; Heberer *et al.* 1997, 2001a; Ternes 1998, 2001; Stumpf *et al.* 1999; Ahrer *et al.* 2001; Ollers *et al.* 2001; Heberer 2002a) Bezafibrate, gemfibrozil, and fenofibric acid, the metabolite of fenofibrate, have also been detected up to the μgL^{-1} level in sewage effluents and surface water samples (Ternes 1998; Stumpf *et al.* 1999; Ahrer *et al.* 2001; Farre *et al.* 2001; Heberer *et al.* 2001b; Heberer *et al.* 2002b). Bezafibrate and gemfibrozil have also been found in ground water samples at maximum concentrations of 190 and 340ng L^{-1}, respectively (Ternes 2001; Heberer 2002a). In laboratory experiments

using soil columns (Scheytt *et al.* 2001), clofibric acid did not show any significant sorption. It leached almost tracer like through the soil columns without retardation. This observation was also confirmed in several studies on bank filtration where clofibric acid was reaching the water supply wells without being removed in the sub soil (Heberer *et al.* 2001b; Verstraeten *et al.* 2002). On the other hand, bezafibrate was found to be easily attenuated during bank filtration (Heberer *et al.* 2001b).

Lipid regulating agents were sporadically detected in River Taff at concentrations not exceeding 100ngL^{-1}. Concentration of clofibric acid (main metabolite of clofibrate) were found to increase in the aquatic environment in UK. (Kasprzyk – Horden 2008) Similarly to the other discussed groups of pharmaceuticals: bezafibrate, clofibric acid were found at comparable levels in surface water around the world (Gros *et al.* 2006; Vieno *et al.* 2005; Lindqvist *et al.* 2005; Calamari *et al.* 2003; Kolpin *et al.* 2002, 2004; Glassmeyer *et al.* 2005).

H2 – receptor antagonists

Ranitidine was found at lower concentrations in Italy, Spain and the USA (Gros *et al.* 2006, Calamari *et al.* 2003; Kolpin *et al.* 2002, 2004; Glassmeyer *et al.* 2005). However, the other H2 – receptor antagonists have rarely been reported.

Contrast Media

Iodinated X – ray contrast media, applied at high amounts mostly in hospitals but also in practical surgeries, have been identified by Gartiser *et al.* (1996) as the main contributors to the loads of total adsorbable organic halogens (AOX) in clinical wastewaters. Oleksy – Frenzel *et al.* (2000) used adsorbable organic iodine (AOI) detection and measured high concentrations up to 130μgIL^{-1} of organic iodine compounds in the influent and effluent of a municipal treatment plant in Berlin and up to 10mgI L^{-1} hospital wastewater observing no degradation or only minor attenuation during sewage purification. They assumed that AOI contamination of the aquatic environment was primarily due to the presence of iodinated X – ray contrast media. This has, however, not yet fully been confirmed by individual component identification. Putschew *et al.* (2001) identified 39% of the AOI detected in sewage effluents as contrast agents.

In Berlin, Germany, high AOI values of more than 10μgI L^{-1} have not only been measured in sewage and surface waters but also in bank filtrate and raw drinking water samples (Putschew *et al.* 2000). In surface waters between 18 and 33% of the AOI could be identified as being iodinated contrast media, whereas in bank filtrate and raw drinking water samples only between 3.4 and 25% of the AOI were identified (Putschew *et al.* 2000; Putschew and Jekel 2001). Although many of the postulated metabolites have also been analyzed, only one of these compounds was also identified in the samples (Putschew *et al.* 2001). Thus, it was assumed that the majority of the AOI consists of several other, still unknown, metabolites of iodinated contrast media (Putschew *et al.* 2000). The five X – ray contrast agents diatrizoate, iohexol, iopamidol, iopromide, and iomeprol were found at or up to μgL^{-1} concentrations in municipal sewage effluents and in surface water samples (Ternes and Hirsch 2000; Putschew *et al.* 2001). Iothalamic and ioxithalamic acid have sometimes also been detected at ngL^{-1} concentrations in influents and effluents of STPs and in surface waters (Ternes and Hirsch 2000). Generally, the loads of the X – ray contrast media are significantly increased on weekdays, because X – ray examinations are performed in hospitals and radiological practices predominately from Monday to Friday (Ternes and Hirsch 2000). Ternes and Hirsch (2000) stated that compared to the other drug residues, the iodinated X – ray contrast media exhibited generally higher maximum levels in STP effluents. Nevertheless, considering their high maximum levels, the average contamination was not as high as it was expected. Thus, the median concentrations of the X – ray contrast media of 50.75μg L^{-1} measured by Ternes and Hirsch (2000) are at least more than one order of magnitude lower than the corresponding maximum concentration levels. In all countries with a developed medical care system, it can be expected that X – ray contrast media are present at appreciable quantities in the sewage effluents and hence lead to a contamination of receiving waters. Iodinated contrast agents are very persistent in the aquatic environment and also easily leach into the groundwater aquifers. Thus, diatrizoate, iopromide, iopamidol, and amidotrizoic acid were detected up to μg L^{-1} level in groundwater and bank filtrate samples (Ternes and Hirsch 2000; Putschew *et al.* 2000; Sacher *et al.* 2001). Iothalamic acid and

ioxithalamic acid have also been detected in a few samples at low ngL^{-1} concentrations by Ternes and Hirsch (2000).

Ternes (2001), Putschew *et al.* (2000) also reported positive findings of diatrizoate, iopromide, and iopamidol in drinking water or raw water used for drinking water production. The rare earth element Gadolinium (Gd), used in the form of organic complexes in magnetic resonance imaging (MRI), is also consecutively discharged via hospital effluents and public sewage systems into the receiving surface waters (Kummerer 2001). It was detected in hospital effluents at concentrations between a few and up to $100\mu gL^{-1}$ (Kummerer and Helmers 2000). In rivers influenced by STP discharges, Gd has been found at concentrations of about $0.2\mu gL^{-1}$, significantly higher than the natural background value of approximately $0.001\mu g\ L^{-1}$ (Bau and Dulsk 1996; Kummerer and Helmers 2000).

Cytostatic drugs

The word cytostatic describes the way some anti – cancer drugs work. Most drugs that are used to treat cancer kill the cancer cells. The word 'cytotoxic' means toxic to cells, or cell – killing. Cytostatics are frequently used in chemotherapy. Thus, residues of cytostatic drugs almost exclusively originate from hospital applications and may occur in hospital sewage at concentrations up to the low μgL^{-1} level (Steger – Hartmann *et al.* 1997). In effluents from those municipal STP's receiving and purifying hospital effluents, cytostatic drugs have been found at trace concentrations mostly at the low ngL^{-1} level (Steger – Hartmann *et al.* 1996; Kummerer *et al.* 1997; Ternes 1998). Steger – Hartmann *et al.* (1996) detected ifosfamide and cyclophosphamide in sewage samples from a university hospital at concentrations of 24 and $146ngL^{-1}$, respectively. Kummerer *et al.* (1997) found ifosfamide at mean concentrations of $109ngL^{-1}$ in effluents from an oncologic hospital. In the influents and effluents of the receiving municipal STP, it was measured at mean concentrations between 6.2 and 9.3ng L^{-1} without observing any significant reduction during sewage treatment. In four out of 16 effluent samples from German STPs, Ternes (1998) detected cyclophosphamide at maximum concentrations of 20ng L^{-1}. Ifosfamide was only detected in two samples but in one of these samples with a concentration of $2.9\mu gL^{-1}$. Until now, cytostatics have not been detected in surface

waters but Kummerer *et al.* (1997) calculated a predicted environmental concentration (PEC) of 0.8ng L^{-1} for ifosfamide in German surface waters. Due to their high pharmacological potency, such compounds often exhibit carcinogenic, mutagenic or 'embryotoxic properties. Thus, further investigations on their occurrence and fate may be interesting regarding their risk potential for humans and the environment (Kummerer 2001).

Oral contraceptives (17α – ethinylestradiol and mestranol)

Synthetic steroids are frequently prescribed as oral contraceptives but because of their high pharmacological potency the total amounts annually sold are relatively low. Ternes *et al.* (1999b) estimated the annual prescriptions of 17α – ethinylestradiol in Germany at only 50kg per year. Thus, synthetic steroid hormones such as the estrogens 17α – ethinylestradiol (EE2) and mestranol can only appear at trace level concentrations at the low ngL^{-1} range in the sewage effluents. This presumption was confirmed by results from several investigations of STPs in Brazil, Canada, Germany, England, Italy, The Netherlands and the U.S. (Desbrow *et al.* 1998; Belfroid *et al.* 1999; Ternes *et al.* 1999a; Baronti *et al.* 2000; Kuch and Ballschmiter 2000; Johnson *et al.* 2000; Adler *et al.* 2001; Huang and Sedlak 2001; Xiao *et al.* 2001; Heberer 2002a). Mestranol has only sporadically been detected in sewage effluents at concentrations up to $4ngL^{-1}$ (Ternes *et al.* 1999a). The median concentration of EE2 in sewage effluents in Germany, England, The Netherlands and the U.S. reported by these authors is approximately between 1 and $3ngL^{-1}$ or even lower (below the analytical detection limit). Canadian sewage effluent samples contained EE2 at a median concentration of $9ngL^{-1}$ (Ternes *et al.* 1999a). In recent investigations of six activated sludge STPs in Rome area, Italy, Baronti *et al.* (2000) determined average concentrations of 3.0ng L^{-1} for EE2 in sewage influent samples. The median sewage effluent concentration of EE2 was $0.45\mu gL^{-1}$ Baronti *et al.* (2000) calculated a removal rate of 85% for EE2 and concluded that activated sludge treatment efficiently removed EE2. However, Ternes *et al.* (1999b) did not observe a significant reduction of EE2 concentrations in aerobic batch experiments containing diluted slurry of activated sludge from a STP near Frankfurt (Germany). In the same experiment, mestranol was rapidly eliminated. On the basis of the daily human

excretion of conjugated estrogens, Baronti *et al.* (2000) presumed that deconjugation of estrogens preferentially occurs in sewers. However, in investigations of influents and effluents from German STP's, Adler *et al.* (2001) observed that conjugated steroids contributed up to 50% of the total steroid concentration. Analysis of a water sample from the Tiber river (Italy) downstream of small towns whose sewages are treated by percolating filter STPs or directly discharged into the river revealed the presence of EE2 at 0.04ng L^{-1} (Baronti *et al.* 2000). In general, EE2 was only detected in a few surface water samples at maximum concentrations up to 4.3ngL^{-1} (Belfroid *et al.* 1999; Adler *et al.* 2001) but most of the samples were below the limits of detection (Belfroid *et al.* 1999; Huang and Sedlak 2001; Adler *et al.* 2001; Xiao *et al.* 2001). Although the detected concentrations are very low, they may still be important for the aquatic environment because 'in vitro' studies have shown that exposure of fishes to only 0.1ng L^{-1} of EE2 (Purdom *et al.* 1994) may provoke feminization in some species of male wild fishes. Due to their physico – chemical properties, estrogenic steroids should be adsorbed in aquatic sediments. Thus, it seems unlikely that they will leach through the subsoil and should therefore; also not appear in groundwater aquifers. Nevertheless, Adler *et al.* (2001) reported several positive detections of EE2 in ground and drinking water in Germany. They determined EE2 in groundwater and in raw and purified drinking water at maximum upto concentrations of 2.4ngL^{-1}.

Illicit drugs

Two illicit drugs amphetamine, cocaine and its main metabolite benzoylecgonine were found in the River Taff, UK at low levels of single ngL^{-1} (Kasprzyk – Hordern 2008). Similar levels of the studied illicit drugs were found in Italy (Zuccato *et al.* 2008). Their presence in the River Taff is again strongly associated with the discharge of treated wastewater effluent. Benzoylecgonine, on the other hand, was often found in the River Taff at levels 10 times higher than the parent compound. Both amphetamine and benzoylecgonine were found with 100% frequency in river water downstream of WWTP, which indicates both high, steady consumption of illicit drugs in the region and a new environmental problem as already indicated by Zuccato *et al.* (2008). Amphetamine was present at very low

concentrations and its presence varied at different sampling points (from 13% to 83% frequency). Benzoylecgonine was again quantified at the highest concentrations reaching 50ngL^{-1} (Kasprzyk – Hordern 2008)

Personal care products and endocrine disruptors

Benzophenone – 4 (sunscreen agent), methylparaben (preservative), 4 – chloroxylenol (disinfectant/ antiseptic) and 4 – tert – octylphenol were found at concentrations exceeding 100ngL^{-1} in rivers of UK. Concentrations of 4 – tert – octylphenol were found to be much higher in the River Ely than the River Taff because Coslech WWTP discharging treated wastewater effluent into the River Ely treats (as opposed to Cilfynydd WWTP) both communal and industrial wastewater. Triclosan was found at concentrations not exceeding 50ngL^{-1} (Kasprzyk – Hordern, 2008). Similar results were obtained by other researchers (Vanderford *et al.* 2003; Moldovan 2006; Bendz *et al.* 2005; Kolpin *et al.* 2002, 2004; Glassmeyer *et al.* 2005).

Other pharmaceuticals

The bronchodilator drugs (β_2 – sympathomimetics) salbutamol (albuterol in the U.S.) and terbutaline, and in a few cases clenbuterol and fenoterol were reported by Ternes (1998) to occur at concentrations < 20ngL^{-1} in municipal sewage effluents. For all four compounds, the median sewage effluent concentrations were below the detection limits. In surface waters, only sporadic detections have been reported (Hirsch *et al.* 1998; Ternes 1998). In investigations of STP effluents and surface waters, Ternes *et al.* (2001) detected the tranquilizer diazepam, the antidiabetic drug glibenclamide, and the calcium influx inhibitor nifedipine. All three compounds were only found in a few samples at maximum concentrations clearly below 100ngL^{-1}. In terms of surface water investigations in the U.S., commissioned by the U.S. Geological Survey, Kolpin *et al.* 2002 detected low ngL^{-1} concentrations of several other drugs such as the histamine H_2 – receptor antagonists cimetidine and ranitidine, the calcium ion influx inhibitor diltiazem, the angiotensin converting enzyme inhibitor enalaprilat, the nifedipine metabolite dehydronifedipine, the antidiabetic drug metformin, and the antidepressant fluoxetine. Eckel *et al.* (1993) detected

pentobarbital at a concentration of $1\mu gL^{-1}$ in groundwater from a land fill in Florida, USA. In groundwater samples near Reno (Nevada, USA), Seiler *et al.* (1999) identified residues of the antidiabetic drug chlorpropamide and the anticonvulsant phensuximide. 5, 5 – Diallylbarbituric acid was found together with several other pharmaceuticals and drug intermediates in groundwater from a land fill in Grinsted, Denmark (Holm *et al.* 1995).

Low concentrations or levels below the detectable limit of bendrofluumethiazide, digoxin, its metabolite digoxigenin and salbutamol were found in surface water of UK. Furosemide as well as valsartan and diltiazem were commonly found in both River Taff and River Ely, UK at levels usually not exceeding $100ngL^{-1}$ (Kasprzyk – Hordern 2008).

SOURCES OF DRUGS IN WATER

The most significant entry route for drugs into aquatic environments is their release from wastewater treatment plants. Hence different human derived residues and drugs have been reported in different matrices like sewage, ground water, surface water and marine water. Naturally occurring hormones like β – estradiol and synthetic steroids like ethynylestradiol are regularly excreted in urine, making their way into natural environments via wastewater treatment plant effluents. Similarly, substantial amounts of medication are also excreted unmodified which travel via urine and feces into wastewater. As a result, these compounds are commonly detected at elevated level in wastewater influents (Singh *et al.* 2010).

There is no data available about the total worldwide use of pharmaceuticals. The consumption and application of pharmaceuticals may vary considerably from country to country (Verbrugh and de Neeling 2003; Goossens *et al.* 2005, 2007; Schuster *et al.* 2008). If there are legislative changes imposed on the health system it may happen that some compounds are not used any more or others gain more importance, e.g., for economical reasons. According to United Nations' figures, 2.3% of Japanese women of reproductive age take a contraceptive pill containing ethinylestradiol as the main active compound, campared to 16% in North America and up to 59% in Europe (United Nations 2004). Some Pharmaceuticals are sold over

the counter without prescription in some countries, while in other they are only available by prescription. Some antibiotics such as streptomycins are used in the growing of fruits (pomology) while others are used in bee – keeping. Again, the situation may vary from country to country. In Germany, the use of these antibiotics for this purpose has been banned. Antimicrobials are among the most widely used pharmaceutical compounds in animals (Boxall *et al.* 2003, 2004; Sarmah *et al.* 2006). These drugs are used in animals husbandry for veterinary purposes, or as growth promoters (particularly in large – scale animals farming and intensive livestock treatment).

Manufacture

Because of good manufacturing practice (GMP) regulations (required for the manufacturing of pharmaceuticals) and the frequently high economic value of the active substances, the amount of emissions occurring during manufacturing has been thought to be negligible. Indeed, such emissions are assumed to be low in Europe and North America. However, manufacturers have not yet published data with regards to this. It has only recently been found that in Asian countries concentrations for single compounds up to several mg L^{-1} can be found in effluents (Larsson *et al.* 2007; Li *et al.* 2008a, b). A study was done in southern part of India, taking samples from a common effluent treatment plant near Patancheru near Hyderabad (Fick *et al.* 2009). About 21 pharma compounds were detected. However, even in Norway the input from a local manufacturer was high (Thomas 2008).

Hospitals

As to be expected, pharmaceuticals are present in hospital wastewater (Brown *et al.* 2006; Steger – Hartmann *et al.* 1996; Kummerer and Helmers 1997; Hartmann *et al.* 1999; Kummerer 2001a, b; Gomez *et al.* 2006; Seifrtova *et al.* 2008; Schuster *et al.* 2008). The concentrations of pharmaceuticals in hospital wastewater are higher than in municipal sewage. However, the total substance flow is much lower because of the much lower share of effluent from hospitals in municipal effluent in developed countries. The dilution of hospitals wastewater by municipal wastewater is by much more than a factor of 100 (Kummerer and Helmers 1997, 2000).

Private Households

Outdated medicines or their remainders are sometimes disposed off down household drains. In accordance with EU legislation, the discarding of unused drugs via household waste has been permitted since 1994. A recently conducted poll has found that 17.7% of those surveyed get rid of excess and outdated pill by pouring them into the toilet, and about 20% do the same with liquid pharmaceuticals (Gotz and Keil 2007). A survey carried out in the UK investigating the household disposal of unused and expired pharmaceuticals interviewed members of 400 households, predominantly from south – eastern England, and was the basis for a conceptual model to assess the pathways of human pharmaceuticals in to the environment. The model demonstrated that the disposal of unused pharmaceuticals, either by household waste or via the sink or toilet, may be a prominent route that requires greater attention (Bound and Voulvoulis 2005). More than half of the patients surveyed in a study conducted in the US reported storing unused and expired medications in their homes, and more than half had pushed them down a toilet. Only 22.9% reported returning medication to a pharmacy for disposal. Less than 20% had ever been given advice about medication disposal by a health care provider (Seehusen and Edwards 2006).

In a study performed in Kuwait (Abahussain *et al.* 2006) almost half of the respondents (45.4%) obtained medicines by prescription more than three times a year and almost all had unwanted medicines in their homes. The reasons for possessing unused medication were mostly due to a change of medication by the doctor (48.9%), or self-discontinuation (25.8%). Their most common method of disposal was to throw unwanted medicines in the trash (76.5%) or flush them down the drain (11.2%). The results of this study suggests that there is a role for patient education on the proper disposal of unused and expired medications in all countries. In some countries take – back systems are already in place (Niquille and Bugnon 2008). In the EU and the US (http://www.whitehousedrugpolicy.gov/drugfact/factsht/proper_disposal.html) it is legal to throw unused, unneeded or expired drugs in the trash. If the trash is incinerated this is probably the most effective and environmentally sound way to

handle the problem. If the waste is land filled it is a bad solution which only postpones the problem. The APIs will probably show up after some years in the effluent of the land fill. The US FDA advises without an additional explanation that some drugs be pushed down the toilet instead of throwing the trash http://www.whitehousedrugpolicy.gov/drugfact/actsht/proper_disposal.html), which is surprising as the APIs will directly end up in STPs. We could not trace any policy for unused drugs in India.

Landfills

If disposed off along with household waste, compounds end up on landfill sites where they can enter the landfill effluent (Eckel *et al.* 1993; Holm *et al.* 1995; Ahel and Jelicic 2001; Metzger 2004). If there is no collection of the effluent, this may be a source for contamination of surface water or ground water. The contribution to this from disposed, unused drugs is not known in many countries, just as the amount released during manufacturing remains unknown.

POLLUTION DUE TO PHARMACUETICALS IN WATER

Ground Water Pollution

A few references may be found in the literature concerning findings of metabolites originating from medical substances in ground water. A landfill in Florida which received wastes from the Jackson Naval Air Station in 1968 and 1969 including wastes from the naval base hospital, has contaminated a nearby shallow ground water (Eckel *et al.* 1993). The authors reported the presence and persistence of pentobarbital, meprobanate and phensuximide in the 21 years old anaerobic aground water plume. Holm *et al.* (1995) describes finding and distributions of organic compounds originating from waste from the pharmaceutical industry in the down gradient of a landfill. The authors reported findings of e.g., different sulfonamides (concentrations up to 5mg L^{-1}), propylphenazone (1, 2 – dihydro – 1, 5 dimethyl – 4 – (1 – methylethyl) – 2 – phenyl – 3 H – pyrazol – 3 – one) (concentrations upto 4mg L^{-1}), 5, 5 – Diallylbarbituric acid (concentrations up to 0.2mg L^{-1}). All these medical substances which have been used for treatment of humans in the period

between 1940's and 1970's. As a common practice in that period, waste from pharmaceutical industries was disposed off at landfills with no leachate collection systems. The chemicals may have entered the surrounding aquifers as part of the leachates (Holm *et al.* 1995). Contamination of tap water by clofibric acid was observed in the samples taken from different districts of Berlin, all containing clofibric acid in concentration between 10 and 165ngL^{-1}. Clofibric acid was additionally detected in all surface water samples taken from the Berlin area. Clofibric acid was also found in samples of surface waters taken from several rivers in other areas of Germany. These findings support the hypothesis that clofibric acid is a substance of considerable persistence in the environment and that regular therapeutic use is the source of clofibric acid which is carried by sewage effluents into the aquatic system. (Halling – Sorensen *et al.* 1998)

River Water Pollution

Watts *et al.* (1983) reported the presence of several antibiotics (erythromycine, sulphamethoxazole, tetracyclines) and the rophylline, in river water samples. They used field desorption mass spectrometry and high performance liquid chromatography. Aherne *et al.* (1985) has used immunoassay techniques for the detection of methotrexate, progesterone, noretheisterone and ethinyloestradiol in various river and potable water samples. Detection limit of between 5 and 10ng L^{-1} were achieved. Several findings of antineoplastic agents (chemotherapeutics) are found in treated hospital waste water effluents (Steger – Hartmann *et al.* 1996; Aherne *et al.* 1990). This indicates that genotoxic agents might find way to the receiving waters.

Sediment Pollution

Several investigations describe finding of antibiotics in sediment cores from medication in fish farms (Bjorklund *et al.* 1990; Coyne *et al.* 1994; Weston *et al.* 1994; Samuelsen *et al.* 1992). Oxytretracycline, an antibiotic agent, was found in concentrations varying between 0.1 and 4.9mg Kg^{-1} dry matter (Jacobsen and Berglind 1988).

Soil Pollution

Chlortetracyclines were found in soil amended with poultry manure (Warman and Thomas 1981). It was demonstrated that drug metabolites excreted by medicated livestock (e.g., as glucuronides) are decomposed by bacterial action in the liquid manure and reconverted into the active drugs. Due to the application of manure to agricultural soils, multiple drug resistance developed in livestock micro flora, even in the intestinal flora of untreated pigs. Thus, multiple – resistant strains found their way into the food chain. Shore *et al.* (1988) presented findings of testosterone and estrogen, used as growth promoters, in chicken manure.

TOXIC EFFECTS

Pharmaceuticals have adverse effects on living organisms. These chemicals in the environment may have endocrine – disrupting effects in living organisms, including humans. The incidence of endocrine – related diseases and adverse physiological effect in wildlife is increasing, and there are indications that changes in the reproductive health of humans, including declining male fertility, birth defects and breast and testicular cancer, could be linked to exposure to endocrine disrupting chemicals (Nikolaou *et al.* 2007). When assessing environmental and health risks from exposure to chemical pollutants, it is important to clearly distinguish between humans and ecosystems in terms of both exposure and effects. The effects of mg L^{-1} or lower concentrations on ecosystem can range from changes in gene expression to changes in population structure, although little evidence exists for such adverse effects from most pharmaceuticals. However, two extraordinary examples do exist. The best known was the dramatic decrease in vulture populations in India and Pakistan (95% in 3 years), where vultures that fed on carcasses of cattle treated with diclofenac died from rental failure because they were unable to excrete the drug. The other case involved the deliberate dosing of an entire experimental lake with low levels of the active ingredient in birth control pills, ethinyl estradiol. Within the first year, fathead minnows showed evidence of responses at the cellular and tissue levels and declines in the population; by the second year, the fist population had collapsed completely (Rodriguez – Mozaz and Weinberg 2010).

Micro – organisms

Ibuprofen, 2 -(4 – isobutylphenyl) propionic acid, has analgesic, anti – inflammatory and antipyretic properties (Reynolds 1989), and is taken orally to treat mild to moderate pain of rheumatism and other musculo – skeletal disorder. Sanyal *et al.* (1993), drew to attention the potential antimicrobial activity of Ibuprofen in terms of Minimum Inhibitory Concentration (MIC values), against certain dermatophyte fungi. Anti – fungal activity of ibuprofen was enhanced by lowered pH. These authors also noted that *Staphylococcus aureus* was suscetpiblie to ibuprofen. Elvers and Wright (1995) showed that Ibuprofen inhibited growth of the Gram – positive bacteria, but that two Gram – negative species were unaffected. Growth of *Staphylococcus aureus* was suppressed by ibuprofen concentrations greater than 150μg mL^{-1} at initial pH 7. At pH 6, such concentration prevented growth. The antibacterial activity of ibuprofen was affected by pH, being more effective at values below pH 7.

Phytoplankton

Streptomycin prevented growth of six blue – green algae species in an investigation performed by Harrass *et al.* (1985), at concentrations (0.09 to 0.86mg L^{-1}) substantially lower than needed to prevent growth of 7 of 8 green algae tested. *Chlorella vulgaris*, *Scenedesmus obliquus* and *Ulothrix sp.* grew in active streptomycin concentrations less than 21mg L^{-1}, while *Chlamydomonas reinhardtii* growth was prevented at concentration of 0.66mg L^{-1}. Algal growth in sublethal concentration of streptomycin was slowed or delayed, and the maximum density attained by several species was decreased. Result published by Lanzky and Halling – Sorensen (1997) showed that *Chlorella sp.* are sensitive (EC_{10} = 2.03mg L^{-1} and EC_{50} = 12.5mg L^{-1}) to metronidazole.

Plants

Antibiotics chlortetracycline and oxytetracycline effects on plants vary from species to species (Batchelder 1981; 1982). The most sensitive plant species in the Batchelder study was pinto beans when they were grown on sandy loam soil.

Crustaceans/copepods

The acute toxicity of furazolidone, 3 – [(5 – Nitrofurfurylidene) amino] – 2 – oxazolidinone, which are largely used in medicated fish feed, have been investigated by Macri *et al.* (1988). The authors found a significant toxicity of the compound on *Daphnia Magna*, while *Artemia salina* proved to be the less sensitive. Migliore *et al.* (1997) showed the toxicity of several agricultural antibiotics to *Artemia*. Acute toxicity studies of four antibiotics; aninosidine, bacitracin, erythromycin and lincomycin, all used as food additive or mass therapy in intensive farming, on *Daphnia magna* Straus have been performed by Dojmi di Delupis *et al.* (1992). EC_{50} values after 48 hours were found in the range of 30mg L^{-1} to 500mg L^{-1} with Bacitracin as the most potent.

It was fond that the calanoid copepods *Temora turbinate*, if raised in pharmaceutical waste concentrations above 1mg L^{-1}, resulted in smaller adult size, reduced egg production rate and an abnormal growth pattern (Lee and Arnold 1983).

Crustaceans/amphipod

Lee and Arnold (1983) studied the toxic effects of ocean – dumped pharmaceutical wastes on the marine amphipod *Amphitoe valida*. The toxic effects increased with increasing duration of exposure to waste concentration. Amphipods chronically exposed to waste concentration above 1% had lower survival rates and reduced fecundity when compared to control groups. The parent amphipods exposed to 3% waste had 100% mortality after three weeks, while those exposed to less than 2% waste were able to survive over 2 months. Larvae of the amphipod survived shorter periods than the parents. No offspring were recorded for amphipods exposed to 3% waste (Lee and Arnold 1983).

Fish

Relatively less details are outlined in the literature concerning the effects of medical substances on fish species. Fewer studies have documented the effect of drugs like salicylate, acetaminophen, and ibuprofen on fish. These are endocrine

disruptors in fish and have the potential to impair the adaptive cortisol response to stressors. (Gravel and Vijayan 2006). Researchers have uncovered new environmental effects, such as feminization or masculinization by hormones or structurally related compounds (xenoesterogens) that exhibited effects on fish down to 1ng L^{-1}.(Lange *et al.* 2001, Routledge *et al.* 1998)

Mosquito Larvea

Macri *et al.* (1988) showed that furazolidone had a significant toxic effect on the mosquito larvae *Culex pipiens* Larvac.

Insects

The potential of animal excreted residues of anthelmitics to adversely affect the development and survival of non – target organisims, important in the process of dung degradation and nutrient cycling, was first recognized in the 1970's. Whereas drugs such as piperazine, thiabendazole and levamisole had little or no effect on dung beetle breeding, formulations of coumaphos, dichlorvos and phenothiazine adversely affected their survival and reproduction for at least 4 to 5 days after treatment (Blume *et al.* 1976). Phenothiazine was also implicated in deleterious changes in the botanical compositions of pastures (Southcott 1980).

The 1980's saw the introduction of a new class of compounds known as macrocyclic lactones. Comprising of the avermectin (doramectin, abamectin and avermectin) and the milbemycins (moxidectin), these drugs are excreted in faeces of treated livestock, partly as unaltered drug. No data are available on the insecticidal properties of doramectin residues in dung, but effects of faecal residues of abamectin and avermectin are reported on a wide range of arthropods. In the late 80's Wall and Strong (1987) discovered that avermectin, an antiparasitic drug for cattle treatment, had an effect on dung degrading insects and delay in degradation of pats from cattle treatment was observed. The environmental aspects and effects of avermectin have also been investigated by e.g. Sommer *et al.* (1992); Sommer and Overgaard Nielsen (1992) and Holter (1993). Results show that the duration of effects after treatment of

avermectin on dung degrading organisms is depended on factors like species, temperature, soil composition and type of livestock.

Resistance development

Antibacterial resistance is a threat to the efficacy of antibacterial substances. Since it is generally agreed that the extent of usage of antibacterial substances is closely related to the development of antibacterial resistance, it is important to investigate this feature. The development of resistance to antimicrobial agents by many bacterial pathogens has compromised traditional therapeutic regimens, making treatment of infections more difficult. Three factors have contributed to the development and spread of resistance: mutation in common genes that extend their spectrum of resistance, transfer of resistance genes among diverse micro – organisms, and increases in selective pressures that enchance the development of resistant organisms. Resistance to kanamycin and neomycin in the bacterial assemblage of a coastal plain stream of South Carolina, US, was detected by growth of colonies on media containing antibiotics (Leff *et al.* 1993). Attrassi *et al.* (1993) reported resistance of bacterial flora to some antibiotics from water, mussels and sediments sampled at three marine sites, lacalised in Marocco. Resistance to penicillin and ampicillin common to all sites polluted or not, was frequent. However bacterial resistance to erythromycin, tobramycin, chloramphenicol and tetracycline was limited to polluted sites by slaugher house effluents or sewage. Multi – resistance was frequent: more than 55% of strains resisted at least one antibiotic and more than 20% carried plasmids. Antibiotic resistance in sediment bacteria was often found in locations with fish farms. Several papers e.g. Samuelsen *et al.* (1992); Husevag *et al.* (1991); Sandaa *et al.* (1992); Nygaard *et al.* (1992), have all reported findings of sediment bacteria resistant to various antibiotics used as feed additives in fish farms.

Genotoxicity

In recent years there has been an increasing interest in the genotoxicological effects connected to the spreading of genotoxins in the environment. The attention has been focused on the aquatic environment especially testing of surface water samples used as drinking water, waste water and sludge samples. Several researchers also

report on bacterial mutagens in the urine of patients in therapeutic treatment with medical substances e.g. tinidazole, prescribed against protozoal infections, (Espinosa – Aquirre *et al.* 1996), metronidazole (Connor *et al.* 1977) and general studies (Monteith *et al.* 1987).

Giuliana *et al.* (1996) analysed the genotoxic potential of 800 native (unconcentrated) waste water samples from a hospital with the umuc test. Genotoxic activity was found in 13% of the samples. The highest genotoxic acitivity occurred in the morning hours, but genotoxic potential without growth inhibition of the bacteria monitored as OD600, in the same way as antineoplastic drugs like mitomycin C or cisplatin. 4% of the genotoxic waste water samples showed combined cytotoxic and genotoxic activities as seen in control experiments using glutaraldehyd containing disinfectants and certain antibiotics. Due to the fact that the samples were unconcentrated a considerable amount of genotoxic substances is released to the environment especially during morning hours.

FATE AND EFFECT OF DRUGS IN WATER

In the beginning research was focused on the analysis of these micro-pollutants. However, it is important to stress that in the few examples in which we really know that drugs have effects on the environment (estrogens and their effects on fish and the effects of diclofenac on vultures), biological effects studies preceded chemical analyses. This is important to stress in order to demonstrate that chemical analyses following up biological effect data may also be an efficient way to find the most problematic chemicals. Later, research into their fate and (eco-) toxic effects came into the foreground. Currently, risk assessment and risk management issues are gaining momentum. However, in the context of India, even chemical analysis data is not available.

Small molecules and biopharmaceuticals

Pharmaceutically active compounds (sometimes called active pharmaceutical ingredients or APIs) are complex molecules with different functionalities and physico – chemical and biological properties. They are

developed and used because of their more or less specific biological activity. Most of them are polar compounds. The molecular weights of the chemical molecules range typically from 200 to 500/1000 Da. Such APIs are called ''small molecules''. These are the ones which are currently being researched and detected in the environment. They are part of the compounds called ''micro-pollutants'' because they are often found in the μgL^{-1} or ngL^{-1} range in the aquatic environment. Some medicines contain molecules based on proteins (''biopharmaceuticals'').

Biopharmaceuticals may be defined as medical drugs produced using biotechnology by means other than direct extraction from a native (i.e. non – engineered) biological source. Examples are proteins (including antibodies) and nucleic acids. The first and best – known example was recombinant human insulin. Biopharmaceuticals are not typically regarded as biopharmaceuticals by the industry. Not all of the naturally occurring compounds which are used as drugs are biopharmaceuticals. For example estrogen is not regarded as a biopharmaceutical.

The environmental relevance of biopharmaceuticals is not yet clear and they are not the focus of environmental research and risk management. One view is that they are not relevant because they are closely related to natural products and are therefore expected to be quickly biodegraded or are denatured, i.e. inactivated in the environment. The other view is that naturally occurring compounds are not in every case easily biodegraded and modified natural compounds even less so. Structurally related compounds such as plasmids have been found in the environment (Schluter *et al.* 2007; Kummerer 2009a).

Furthermore, it is known that the protein structures known as prions are very stable. Besides the active substances, formulations may also incorporate adjuvants and in some instances pigments and dyes. They are often of minor importance for the environment. Some medicines contain endocrine disrupting chemicals as adjuvants, e.g. Di – n – butylphthalat (DBP) (Koch *et al.* 2005).

Structure Matters

Pharmaceuticals and disinfectants can be classified according to their purpose and biological activity (e.g. antibiotics, analgesics, anti – neoplastics, anti

– inflammatory substances, antibiotics, anti – histamines, X-ray contrast media, surface disinfectants, etc.). The classification of small molecule APIs by their chemical structure is used mainly for the active substances within subgroups of medicines, e.g. within the group of antibiotics or subgroups within the antibiotics such as β – lactams, cephalosporins, penicillins or quinolones. In this case one may expect that the compounds can be treated as groups with respect to chemical behavior. However, even smaller changes in the chemical structure may have a significant impact on solubility and polarity as well as other properties that govern their environmental fate to some extent. Other classifications refer to the mode of action (MOA), e.g. anti-metabolites or alkylating agents within the group of cytotoxics / anti – neoplastics. In the case of classification according to MOA, chemical structures of molecules within the same group can be very different and therefore their environmental fate can differ too. In this case, compounds cannot be handled as a group with respect to environmental issues.

A closely related chemical structure may be accompanied by an identical or at least a similar mode of action (e.g. β – lactam antibiotics). However, as the example of anti – neoplastics shows, it might also be very different: alkylating, anti-metabolic, mitosis-inhibiting or intercalating substances can, but need not necessarily, belongs to different chemical classes. Compared to most bulk chemicals, pharmaceutically active compounds are often complex molecules with special properties, e.g. dependence of the octanol–water partition coefficient (K_{ow}) on pH (Cunningham 2008). APIs often have basic or acidic functionalities, sometimes even within the same molecule.

Under environmental conditions molecules can be neutral, cationic, anionic, or zwitterionic. The p*K*a values ¼ log 10 K_a (where K_a is the acid dissociation constant) of ciprofloxacin are 6.16 and 8.63. At a pH of 7.04, the iso – electric point of ciprofloxacin, the molecule carries both a negative and a positive charge, i.e. it is neutral as an entity despite the charges within the molecule. The log K_{ow} of ciprofloxacin at pH 7.04 is calculated to be about 1.74 and was experimentally determined to be 0.28 (Meylan and Howard 1995). Other compounds such as ceftazidime are inner salts, i.e. they are already zwitter ions and can additionally

from other zwitterions. This makes their environmental behavior even more complex. Not only are different pharmaceuticals of special interest with respect to the compounds themselves, but also because of the differences in their occurrence, their fate and their effects on humans or on other target organisms such as bacteria or parasites, and on non- target organisms in the environment.

Parent compounds, metabolites, transformation products and their elimination

In recent years it has been learnt that not only are the APIs themselves important, but also the molecules resulting from these parent compounds due to structural changes taking place in the environment. A chemical can undergo different structural changes by a variety of biotic and non-biotic processes after its introduction into the environment. Structural transformations may also be a result of effluent treatment (Ravina *et al.* 2002; Schroder 2002; Zuhlke *et al.* 2004; Lee *et al.* 2007; Trautwein *et al.* 2008; Mendez – Arriaga *et al.* 2008).

Many pharmaceuticals are bio – transformed by organisms such as bacteria and fungi in the environment (Haiß and Kummerer 2006; Groning *et al.* 2007). Nomenclature used by different authors is often somewhat confusing (Langin *et al.* 2008). For example, the term biodegradation is very often used. However, primary degradation, partial degradation and full mineralization are only rarely differentiated. With the advent of pharmaceuticals as environmental contaminants the situation got even more complicated. Many pharmaceuticals undergo a structural change in the body of humans and animals, respectively. This could be due to micro – organisms in the gut or by human enzymes such as cytochromes. Metabolites are the result of such a process. However, the naming and meaning of ''metabolite'' in publications is somewhat confusing. The term metabolite is used for compounds resulting from the structural change of pharmaceuticals within the human body, not differentiating biochemical processes performed by human enzymes from the ones due to bacterial activity in the alimentary system and the ones present on skin or non – biotic processes such as hydrolysis in the stomach. The term is also used for molecules resulting from structural change by fungi and bacteria in the environment and sometimes even for structural changes that are the

result of non – biotic processes such as oxidation, hydrolysis and photolysis (e.g. Mendez – Arriaga *et al.* 2008) in different environmental compartments such as surface water, soil or sewage treatment. As with metabolism, the chemical structure of the active molecules can be changed by biotransformation, biodegradation, and non-biotic transformation such as photo transformation and hydrolysis. Such a structural change results in a change in their physico – chemical and pharmaceutical properties. It is normally assumed that metabolism and other transformation processes of APIs leads to decreased toxicity. In some cases however, metabolism leads to more active compounds (e.g. in the case of pro-drugs). The same has been found for photo transformation and other oxidizing processes (Burhenne *et al.* 1997).

The proper and adequate use of the terms related to the fate of organic chemicals in the environment is advised in order to prevent confusion in the assessment of the fate and risks connected to the presence of these molecules in the environment (Kummerer 2009a).

METHODS OF ANALYSIS OF DRUGS PRESENT IN WATER

Quantitative evaluation of the fate and behaviour of pharmaceutical compounds in the aquatic environment requires sensitive and reliable analytical methods with detection limits in the lower ngL^{-1} range (Gomes *et al.* 2004). In the past, the analytical determination of pharmaceuticals has been mainly limited to biological samples such as blood, tissue and urine (Neill *et al.* 1991). A simple adaptation of these methods to environmental studies is not generally appropriate because the therapeutic dose of pharmaceuticals is usually much higher than the levels found in the environment and elevated levels of potentially interfering compounds such as humic substance often have to be separated out. This also means that until relatively recently, few analytical methods to detect these compounds in environmental samples at relevant concentrations had been developed (Lai *et al.* 2002).

Analytical procedures for the determination of pharmaceuticals in aqueous samples utilize both gas and liquid chromatography after extraction and clean up

procedures (Sacher *et al.* 2001; Ollers *et al.* 2001; Lindsey *et al.* 2001). A detailed review of analytical methods has recently been undertaken. (Ternes 2001b). However environmental studies frequently require data on the distribution of contaminants within several phases.

The application of advanced measurement technologies (e.g., gas chromatography with mass spectrometry (GC – MS) and GC with tandem MS (GC – MS^2) or liquid chromatography with MS (LC – MS) and LC with tandem MS (LC – MS^2)) to environmental analysis has allowed the determination of a broader range of compounds, including pharmaceuticals, and has therefore permitted more comprehensive assessment of environmental contaminants. LC – MS^2 is becoming more commonly used in pharmaceuticals analysis because of its high sensitivity and its ability to confirm compounds (as compared with conventional LC with ultraviolet (UV) or fluorimetric detection). LC – MS^2 allows separation and detection of compounds having the same molecular mass but different product ions, even if they co – elute. MS^2 detection is therefore preferred for increased analytical sensitivity and selectivity in complex matrices, such as wastewaters (Diaz – Cruz and Barcelo 2005). From the various studies reviewed by Fatta *et al.* 2007, it is concluded that GC – MS was used in 17 studies, LC – MS^2 in 12, high – performance LC with diode-array detection (HPLC – DAD) in two, HPLC – fluorescence in two, and GC – MS^2 and LC – MS in one each.

As mentioned by Petrovic *et al.* (2002), both GC – MS and LC – MS methods have some drawbacks. Prior to GC – MS analysis, derivatization of polar pharmaceuticals is necessary, performed using highly toxic and carcinogenic diazomethane or, less frequently, acid anhydrides, benzyl halides and alkylchloroformates. This step can also affect the accuracy of the method. Ternes (2001b) directly compared GC – MS and LC – electrospray ionization (ESI) – MS^2, and showed that only LC – (ESI) – MS^2 allows the analysis of extreme polar compounds (e.g., β – blockers atenolol and sotalol) due to an incomplete derivatization of the functional groups.

Farre *et al.* (2007) compared LC – (ESI) – MS and GC – MS (after derivatization with BF_3 – MeOH) for monitoring some acidic and very polar analgesics (salicylic acid, ketoprofen, naproxen, diclofenac, ibuprofen and gem brozil) in surface water and wastewater. The results showed a good correlation between methods, except for gem brozil, for which derivatization was not completely achieved in some samples. In general, the limits of detection (LODs) achieved so far with LC – MS^2 methods are slightly higher than those obtained with GC – MS methods (Diaz – Cruz and Barcelo 2005); however, LC – MS methodology showed advantages in terms of versatility and sample preparation being less complicated (i.e. derivatization is not needed).

SAMPLE PREPARATION

The sample preparation procedure is an important step in such analyses due to lower concentration of target compound and complex nature of matrix. In the case of pharmaceuticals containing acidic groups in their structure and existing largely in their ionized form at neutral pH, acidification of water samples is necessary (Renew 2004). The presence of natural organic matter in the samples may reduce the extraction efficiencies. In general, the water samples are filtered through 0.45μm or 0.2μm glass – fiber filters. Several techniques have been developed and optimized, with SPE being the most frequently used. Also solid – phase microextraction (SPME), liquid – phase microextraction (LPME) and lyophilization have been applied (Hirsch *et al.* 1998; Ternes 2001b; Kolpin *et al.* 2002).

Of the 32 studies reviewed by Fatta *et al.* (2007), sample extraction of water and wastewater was achieved using SPE in 28, SPME in two (in one study both SPE and SPME were applied), LPME in one and lyophilization in two. SPE sorbents in the form of commercially available cartridges (e.g., ENV+, Oasis HLB, Strata – X, Lichrolut C18, and Lichrolut EN) have been assessed for pre – concentration as well as for clean – up of pharmaceuticals in water samples. These were employed most because they give better recovery of both polar and non – polar compounds and have greater capacity than alkyl bonded silicas. However, these are very costly.

SPE is typically performed manually, but there are some significant disadvantages with this approach:

- manual (off – line) SPE is time – consuming as well as labor intensive and costly, which compromises productivity;
- exposure to hazardous or infectious matrices (such as sewage) involves safety issues; and,
- the recovery of the analyte can vary from batch to batch, causing reproducibility problems.
- by automating the process, these problems can be eliminated, with the following benefits:

    - direct injection of untreated samples;
    - automatic sample clean-up and/or analyte enrichment;
    - elimination of conventional manual sample pretreatment steps;
    - faster procedures;
    - methods are less prone to errors, resulting in better reproducibility;
    - reduction of health risks; and,
    - samples can be run unattended (e.g., overnight or over the weekend).

A review on the current aspects and future prospects for automating SPE was published by Rossi and Zhang (2000). However, only a few studies have so far used automated procedures for extraction (e.g., accelerated solvent extraction (ASE) (Richter *et al.* 1996), on-line coupled continuous flow liquid membrane extraction (CFLME) with a C18 pre – column system (Liu *et al.* 2003), or sequential injection analysis (SIA) with a lab – at – valve (LAV) approach for on line liquid–liquid micro-extraction (Burakham *et al.* 2005). These studies focused on the determination of organic pollutants (e.g., polyaromatic hydrocarbons (PAHs) and bisphenol A).

PRE – CONCENTRATION TECHNIQUE

Despite the current availability of advanced detection instrumentation, the rapid, accurate and perhaps simultaneous determination of a large number of pharmaceuticals in complex environmental matrices continues to constitute a major

and fascinating challenge for researchers not only because of the diversity of chemical properties of the pharmaceutical compounds, but also because of the generally low concentrations (usually $\mu g\ L^{-1}$ or $ng\ L^{-1}$ levels) and the complexity of matrices. Further improvements are needed in order to lower LODs. Moreover, increasing and intensive scientific research is needed to assess the impact of pharmaceuticals, in order to establish limits for their presence in waste- water discharges or drinking water. Current knowledge concerning their impacts on the environment refers to information on individual compounds, and this needs to be extended to more complex environmental mixtures with the help of improved analytical methods. For this reason, probably most of the analytical procedure reported in literature make use of SPE followed by GC – MS or LC – MS.

However, these techniques are relatively expensive and not easily available at several places. Need of the hour; therefore, is to use simpler techniques which however, require higher concentration of target drug molecules. Therefore, prior to the instrumental analysis, attention has to be paid to the sample preparation and enrichment procedure.

Solid Phase Extraction (SPE) is the method of choice for sample enrichment / pre – concentration in environmental analytical chemistry. There are several solid phases on which the sample can be concentrated. (Batt and Aga 2005; Psillakis *et al.* 2003).

INSTRUMENTAL TECHNIQUES USED IN THE PRESENT WORK

Spectroscopy

Spectroscopy was originally the study of the interaction between radiation and matter as a function of wavelength (λ). In fact, historically, spectroscopy referred to the use of visible light dispersed according to its wavelength, e.g. by a prism. Later the concept was expanded greatly to comprise any measurement of a quantity as a function of either wavelength or frequency. Thus it also can refer to a response to an alternating field or varying frequency (ν). A further extension of the scope of the definition added energy (E) as a variable, once the very close relationship $E = h\nu$ for photons was realized (h is the Planck constant). A plot of the response as a function of

wavelength or more commonly frequency is referred to as a spectrum; and also spectral linewidth.

Spectrometry

Spectrometry is the spectroscopic technique used to assess the concentration or amount of a given species. In this case, the instrument that performs such measurements is a spectrometer or spectrograph. Spectroscopy/spectrometry is often used in physical and analytical chemistry for the identification of substances through the light emitted from or absorbed by them.

The type of spectroscopy depends on the physical quantity measured. Normally, the quantity that is measured is an intensity, either of energy absorbed or produced. Electromagnetic spectroscopy involves interactions of matter with electromagnetic radiation, such as light.

Most spectroscopic methods are differentiated as either atomic or molecular based on whether or not they apply to atoms or molecules. Along with that distinction, they can be classified on the nature of their interaction. Absorption spectroscopy uses the range of the electromagnetic spectra in which a substance absorbs. This includes atomic absorption spectroscopy and various molecular techniques, such as infrared, ultraviolet-visible and microwave spectroscopy.

Most organic compounds and many inorganic ions and complexes absorb radiation in the UV – visible region (180 – 780nm). A plot of this absorption of a compound against wavelength is called an absorption spectrum. The shape of the absorption spectrum is a characteristic of a particular compound or class of compounds. Absorption spectrometry is a non – destructive technique and is extremely sensitive and is therefore, ideal for the characterisation of small amounts of precious compounds. The most important application of the technique is as a means of measuring concentration.

Trace compounds can be measured in presence of other components if there is sufficient difference in their absorption spectra. The technique is best suited for dilute

solutions. Solubility in a suitable solvent is a prerequisite for the accurate measurement of a particular sample.

A very popular application is the monitoring of the effluent from HPLC columns. The concentration of a particular component can be followed by measuring the absorbance at a suitable wavelength, or compounds eluted from the column can be identified by making rapid spectral scans. Absorbance measurements are also the most popular means of following the kinetics of reaction systems since they do not interfere with the progress of the reaction in any way. Another important application of rapid absorption measurement is in the clinical field, where colorimetric assays have been worked out for many biologically important compounds.

Absorption of radiation

When UV – visible radiation encounters a molecule or a molecular ion, an interaction between the radiation and the latter may take place. This absorption is very specific and results in an attenuation of the radiation and an increase in the energy of electrons of the molecule. This may be regarded as the promotion of one the outer electrons from a 'ground state' energy level into one of higher energy level. These levels are separated by discrete energy increment, E, which is determined by the nature of the molecule and only parcels of radiation of energy E can be absorbed.This parcel of radiation is termed as quantum and its energy is related to the frequency and wavelength of radiation by

$$E = h\nu = hc / \lambda$$

Where, h is the plank's constant (6.63×10^{-13} J s), c is the velocity of light (2.998×10^{8} m s^{-1}) and λ is wavelength.

According to molecular orbital theory, the absorbed wavelength is used for the transfer of an electron from a bonding or non – bonding molecular orbital to anti – bonding molecular orbital. The various electronic transitions involved in the UV – visible region for organic compounds are $\sigma - \sigma^*$, $n - \sigma^*$, $\pi - \pi^*$, $n - \pi^*$ in decreasing order of energy requirement. The energy required for $\sigma - \sigma^*$ and $n - \sigma^*$ transitions are relatively high with absorption in the vacuum ultraviolet region. Conjugation causes

delocalization of π electrons, thus making them more mobile and easy to excite. Greater the conjugation with the chromophores, the easier it is to excite the electrons and higher is the wavelength i.e. in the visible region and the compound appears coloured.

Radiation of a particular wavelength, which is characteristic of the molecule, is absorbed by it. The amount of radiation absorbed will be proportional to the amount (concentration) of the absorbing species, when the pathlength is constant.

According to Beer – Lambert law,

$$A = \varepsilon b c$$

Where,

A = absorbance

ε = Molar extinction coefficient (L mol^{-1} cm^{-1})

b = Pathlength of the cell (cm)

c = Molar concentration of solution (mol. L^{-1})

ε is a characteristic constant for a given absorbing species and the path length b is a constant for a given set of experimental conditions.

Therefore, absorbance is directly proportional to its concentration. This is the basis of quantitative measurement in spectrophotometry. A calibration curve of concentration of analyte versus absorbance is constructed by using standard solutions for the concentration range obeying Beer's – Lambert's law and from the slope of the curve the concentration of the unknown is calculated.

The amount of the individual compounds present in mixture can be derived by solving the equation (1) and (2), which are obtained adding Beer – Lambert's law. (A = ε b c), for two component mixture of X and Y, the mixtures absorbance, Am is

$$(Am)_{\lambda 1} = (\varepsilon_x)_{\lambda 1} bC_x + (\varepsilon_y)_{\lambda 1} bC_y \qquad \text{-----(1)}$$

Where λ_1 is the wavelength at which the absorbance of component X is measured.

$$(Am)_{\lambda 2} = (\varepsilon_x)_{\lambda 2} bC_x + (\varepsilon_y)_{\lambda 2} bC_y \qquad \text{-----(2)}$$

Where λ_1 is the wavelength at which the absorbance of component Y is measured.

ε is absorptivity (L g^{-1} cm^{-1}); its value is determined for each compound at both wavelengths. b is path length (cm) and C the concentration (M).

Chromatography

Chromatography is one of the most powerful and versatile analytical techniques available to the modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in a mixture in one single analytical run. Its versatility comes from its capacity to handle a wide range in complexity: from a single substance to a multi component mixture containing widely different chemical species. Another aspect of versatility is that the analysis can be carried out on a very costly complex instrument and on the other hand on a simple inexpensive thin layer plate.

The word chromatography is derived from greek letters *Chromos* meaning colour and *graph* meaning writing. Although colour has little to do with modern chromatography, the name has persisted, and despite its irrelevance, is still used to describe all separation techniques that employ a mobile phase consisting of different solvents and stationary phase involving suitable adsorbents. In a classical manner it can be defined as "A separation process that is achieved by distribution of substances between two phases i.e. a stationary phase and a mobile phase". The resolved compound can be further determined by suitable detector system.

There are ways of classifying different chromatographic methods based on physical state of phases used or mechanism controlling separation or kind of techniques used and or sample development. One such classification is presented in Table 1.1. This classification is based on nature of mobile phase which can be gas or liquid yielding gas chromatography or liquid chromatography, with solid or liquid as stationary phase. We have gas – liquid, liquid – liquid or liquid – solid chromatography. The later addition is supercritical fluid (SFC) chromatography. In gas chromatography, stationary phase is nonvolatile and is coated or bonded to porous support, while inert gas is used as mobile phase. The separation occurs due to difference in vapour pressure of components. In liquid – liquid partition

chromatography stationary phase is liquid coated on porous support (Table 1.1). While mobile phase is second liquid which is immiscible with the first phase. In Gas Solid chromatography, the separation occurs due to difference or equilibrium distribution of difference in adsorption on the stationary phase. GSC i.e. gas –solid chromatography uses solid stationary phase (e.g. granulated activated charcoal GAC) and gas mobile phase. The use of supercritical fluid as mobile phase leads to newer methods. This new technique has gained importance very recently.

Table 1.1. Classification of chromatographic methods

S.No	Mobile Phase	Stationary Phase	Mechanism	Technique
1.	Gas	Liquid (GLC)	Sorption	Column
2.	Gas	Solid (GSC)	Adsorption	Open bed
3.	Liquid	Liquid (LLC)	Partition	Column
4.	Liquid	Solid (LSC)	Adsorption	Batch
5.	Supercritical fluid	Liquid (SFLC)	Gas – Liquid	Column
6.	Supercritical fluid	Solid (SFSC)	Gas - Solid	Column

The classification based on a technique is one which involves use of either a column (Table 1.3) or open bed. The column may be packed column or capillary column also called as open tubular columns. The paper and thin layer techniques are examples of plane bed chromatography. Flow of mobile phase is due to capillary wetting. Open bed chromatography is restricted to use of liquid as mobile phase.

Table 1.2. Alternative way of classification on basis of mechanism

S.No	Kind	Adsorption	Partition	Exclusion	Ion Exchange
1.	Support	Solution – Solid	Solution – Liquid	Solution – Solid	Resin material
2.	Phases	Gas – Solid	Gas – Liquid	Gas – Solid	Inorganic substances
3.	Technique	Adsorption Chromatography	Gas Chromatography	Exclusion Chromatography	Ion Exchange Chromatography
4.	Mode	Gas – Solid Chromatography	Liquid – Liquid partition	-	Inorganic Exchangers

Table 1.3. Classification based on separation mechanism

S.No	Kind	Adsorption	Partition
1.	Adsorption Chromatography	Columnar method	LC
		Gas – Solid Chromatography	GSC
2.	Partition Chromatography	Liquid – Liquid partition	LLPC
		Paper	PC
		Thin layer	TLC
		Gas Liquid Chromatography	GLC
		Reversed phase partition	RPPC
3.	Ion Exchange	Cation exchange	CEC
		Anion exchange	AEC
		Ion chromatography	IC
4.	Exclusion Chromatography	Gel – permeation	GPC
		Ion exclusion	IEx
		Molecular sieve / Gel Filtration	MSC
5.	Electrochromatography	Zone electrophoresis	ZE
		Boundary layer method	BLE
		Curtain chromatography	CC
		Capillary electrophoresis	CZE

The planar chromatographic technique is simple, flexible and best for characterization. Column chromatography has better capacity, ease of operation and excellent efficiency. It is used in preparative and quantitative work. However, the most logical way of classification is based upon the mechanism of retention of solute or analyte in the stationary phase. It forms the backbone of chromatographic separations (Table 1.3.). The processes involved are either, the sorption by adsorption, partition and exclusion e.g. GLC, LLPC involve partition, while adsorption deals with sample fixation on stationary support involving some forces like London forces, dipole induced dipole interaction or the molecular interaction. The distribution ratio is most important factor in the consideration of partition chromatography. While exclusion mechanism is based upon segregation of species by taking the advantage of the difference in size and geometry of the molecules or species as seen in Gel permeation chromatography. The stationary phase is porous medium. Small species permeate faster in comparison to large size particles e.g. protein or polymers. More than one mechanism of retention is usually considered best. It is briefly summarized in Table 1.3.

It is interesting to note that HPLC i.e. high performance liquid chromatography or IC – ion chromatography are both highly instrumental techniques, do not figure in the above classification. The reason is simple; they are techniques and not a class of chromatography. For instance, we can use HPLC for any four kinds of chromatography described in Table 1.3(1 – 4).

Thus, chromatography methods have a few things in common. There is a stationary support in the form of a column packed with inert material, then a phase moving down the column, called the mobile phase and the phase which usually adheres to the stationary support called the stationary phase. Separation is feasible on account of a differential migration front; developed by exploring differences in adsorbility, partition coefficient, exchange potential, molecular size or ionic mobility. Further, most terms used while describing chromatographic methods are common to all the method.

Specifically, if the stationary phase is a liquid, depending on the polarity of the mobile phase in comparison with the stationary phase it may be either a normal phase HPLC or a reverse phase HPLC. The stationary phase is packed in a column and the mobile phase is pumped at a constant flow rate or at a constant pressure by means of a pump. The solute is introduced at the head of the column. The mobile phase carries the solute on to the column, where separation actually occurs. The separated components are carried further to the detector, which in turn is connected to an integrator or a data station. The components are detected by the detector and recorded in the form of a chromatogram (Khopkar 2008).

Mass spectrometry

The mass spectrometer is an instrument that sorts out charged gas molecules or ions according to their weight or mass. This technique has nothing to do with spectroscopy; however, the name spectrometer was chosen because of the similarity between photographic records and optical lines in spectra. As mass spectrum is obtained by converting the compound of a sample into rapidly moving ions (+ ve in nature) and resolving them on the basis of their mass to charge (*m/e*) ratio. The ionisation process produces positives particles, the mass distribution of which is

characteristic of the parent compound. In elucidation of molecular structure, mass spectras are useful for determining molecular weight. Since the ion current at various mass settings is proportional to concentration, quantitative analysis can also be easily carried out. The sample is bombarded with a beam of electrons which in turn produces ion molecules or ionic fragments of the original species; such charged fragments can be separated according to their mass.

Qualitative analysis with mass spectrometry

The identification of an unknown compound is possible by mass spectrometry. This is possible by calibration with a known compound like mercury varpour (m /e = 198 – 204) or perfluorokerosine (PFK) with peaks CF3 (69), C3F3 (93), CC4F3 (131). The mass spectrometer is thus useful for determination of molecular weight or molecular formula, or identification of a compound from the fragmentation patterns.

In determination of molecular weight, a volatile compound is essential as the molecular ion peak has to be identified as (M + 1) peak with chemical ionisation with methane but peaks due to impurities should be ignored. The molecular weight determined by the mass spectrometer may not be the same as that calculated form atomic weights if the parent compound contains certain elements with high isotopic abundances.

With the use of very efficient microprocessor the huge data obtained can be quickly processed. Further, for GC – MS, libraries of mass spectra of compounds using electron ionization are available which are used to identify the compounds.

STATISTICAL METHODS IN ANALYTICAL CHEMISTRY

Analytical science accumulates enormous amounts of data out of different measurements. This data has no value unless one examines how much of it is reliable and how much is reproducible. Statistics, a branch of mathematics, deals with the presentation and analysis of numerical data. The application of many statistical methods does not require the services of a 'statistician' or a 'mathematician' to convert chemical data into useful information.

Exploratory data analysis

Exploratory data analysis is a term used to describe a group of techniques (largely graphical in nature) that sheds light on the structure of the data. Without this knowledge a researcher, or anyone else, cannot be sure that he is using the correct form of statistical evaluation.

Summary statistics

Summary statistics are used to make sense for large amount of data. Typically, the mean, sample standard deviation, range, confidence intervals, quantities and measures for skewness and spread of the distribution are reported.

The mean

The mean, $\bar{x}$ can be shown to be best estimate of the true value; it is calculated as the arithmetic mean of n observations

$$\bar{x} = \sum (x_i) / n$$

The median

This is the value or observation, which subdivides the numerical, ordered data into two halves. If the number of observation (n) is odd, (n - 1) / 2 observations are smaller than the median and the next higher value is reported as the median. If n is even, then average of the middle two observation is reported.

The most useful characeteristics of the median is the small influence exerted on it by extreme values, that is, its robust nature.

Standard deviation

The standard deviation is a measure of the spread of data (dispersion) about the mean. It is the positive square root of the variance.

$$SD = \sqrt{\frac{(x_i - \bar{x})^2}{(n-1)}}$$

Standard error

The standard error is the standard deviation of an estimate. Thus, the standard error of the mean (SEM) is the standard deviation of the mean. If s is the standard deviation of a sample then the SEM is given by the formula.

$$SEM = \frac{SD}{\sqrt{n}}$$

Coefficient of variation

The ratio of the standard deviation to the mean is the coefficient of variation (CV). It is expressed as a percentage.

$$CV = 100 \left(\frac{SD}{\bar{x}}\right)$$

Regression and calibration

Calibration is fundamental to achieving consistency of measurement. Often calibration involves establishing the relationship between an instrument response and one or more reference values. Once the relationship between input value and the response value is established, the calibration model is used in reverse that is to predict a value from an instrumental response.

In statistics, the term regression is used to describe a group of methods that summaries the degree of association between one variable and another variable. The most common statistical method used to do this is least – square regression, which works by finding the "best curve" through the data that minimises the sums of square of the residuals. There are a number of least – square regression models, for example, linear, logarithmic, exponential and power.

Statistics of the straight line

The general equation of straight line can be expressed as

$$y = bx + a$$

where,

y is the dependent variable

a is the y intercept

b is the slope of the line

x is the independent variable

$$b = \frac{\sum x_i y_i - \sum x_i \sum y_i / n}{\sum (x_i - x)^2}$$

Using the method of calculus, the slope and intercept of the line are determined.

Objectives in a simple linear regression analysis

Various inferences and estimates may be made from regression line:

- Estimation of variability associated with the slope (b) and intercept (a)
- Test whether there is any association between the two variables x and y.
- Estimate the variability associated with a given y value.
- Make prediction about y value for a given x value within the range studied.

Error analysis for linear regression

The various numeric tools to determine how well a regression equation fits the data are as follows

$$a = \bar{y} - b\bar{x}$$

- The standard error of Y estimates (S_{yx})

It is measure of the certainty with which the independent values in the sample can be used to predict the dependent values. That is, S_{yx} is an estimate of the error in a single value for y calculated using the regression equation. S_{yx} is the standard deviation of the difference observed and calculated values and is calculated with the equation:

$$S_{yx} = \sqrt{\frac{\sum (y_i - y_i(x_i))^2}{(N-2)}}$$

Where $y_i (x_i)$ is the value predicted from the curve, y_i is the observed value and (N – 2) is the degree of freedom.

- Coefficient of determination (r^2)

It is a measure of the Goodness – of – Fit for a straight line regression. The value of r square can range from 0 to 1. If the relationship between two variables is strong, the r square value will be close to 1. The square root of r square is called correlation coefficient.

The following equation can be used for manually determining the sample correlation coefficient (r)

$$r = \frac{\sum x_i y_i - \sum x \sum y_i / n}{\sqrt{\sum (x_i - x)^2 \sum (y_i - y)^2}}$$

- The standard error of coefficient

It is an estimate of the standard deviation of the coefficient of an independent variable in an equation. Generally, the larger the standard error of coefficient in relation to the corresponding X coefficient, the less certain that one can be about prediction. The equation used:

$$S_b = \sqrt{\frac{S^2}{\sum (x_i - \bar{x})^2}}$$

Weighted linear regression

In analytical science it is often found that the precision changes with concentration. In particular the standard deviation of the data is proportional to the magnitude of the value being measured. When this relation is observed weighted linear regression is used for obtaining the regression line. The potential benefit of weighed regression analysis is improved quantification at low concentration.

Polynomial regression

When error statistics or a scatter diagram show that a linear characterization of the association between observed value of two variables appears ineffectual, an equation of higher order may be investigated to see if it improves the fit. This equation will be of the general form.

$$Y(x) = a_0 + a_1 X + a_2 X^2 + \ldots\ldots + a_n X^n$$

Polynomial curve fitting is an extension of the fitting techniques described for linear regression analysis.

Calculations used for the present study

Determination of concentration of drug by UV- Visible spectrometery

The concentration is determined by the respective equation of standard curve, by graphical extrapolation method.

Determination of concentration of drug by HPLC

The concentration is determined by the respective equation of standard curve.

$$\text{Concentration of drug} = \frac{(\text{Peak area} - \text{y intercept})}{\text{Slope}}$$

Determination of amount of drug present in the solution

$$\text{Amount of drug in milligrams} = \frac{\text{Concentration of drug solution in ppm}}{\text{Volume of drug solution}} \times 1000$$

Determination of Amount of drug adsorbed by adsorbent

Amount of drug adsorbed = (Amount of drug present in initial drug solution) – (Amount of drug present in the solution which is collected after passing through the adsorbent)

Determination of percentage of drug adsorbed

$$\text{Percentage of drug adsorbed} = \frac{\text{Amount of drug adsorbed}}{\text{Amount of drug present in initial drug solution}} \times 100$$

Determination of amount of drug recovered

Amount of drug recovered = (Amount of drug adsorbed) – (Amount of drug present in the recovery solvent)

Determination of percentage of drug recovered

$$\text{Percentage of drug recovered} = \frac{\text{Amount of drug adsorbeAmount of drug recovered}}{\text{Amount of drug present in initial drug solution}} \times 100$$

Determination of pre – concentration factor (PF)

$$\text{Pre} - \text{concentration factor (PF)} = \frac{\text{Concentration of drug in recovered solvent in ppm}}{\text{Concentration of initial drug solution in ppm}}$$

AIM OF THE WORK

It is, thus clear from the foregoing discussion that determination of pharma compounds in water system has not been undertaken as an study in INDIA. Further, most researchers have employed expensive GC – MS and LC – MS^2 methods for the purpose which are rather expensive and not easily available in all laboratories. Therefore, the present study was undertaken with the following objectives.

- To develop simple, accurate and cost effective techniques to determine such low concentration drugs present in aquatic environment. For our pre – concentration studies we have selected five drugs of different categories: Aspirin, Paracetamol, Esomeprazole Magnesium, Fenofibrate, and Venlafaxin HCl.

- To develop and validate a HPLC method for simultaneous determination of Esomerazole Magnesium, Fenofibrate and Venlafaxine HCl.

- To develop a LC – MS method for the simultaneous determination of Esomerazole Magnesium, Fenofibrate and Venlafaxine HCl in environmental water samples.

- To study the effect of metal ions and metal complex loaded activated charcoal in presence of oxygen to remove drugs from waste water.

CHAPTER 2

PRE – CONCENTRATION AND QUANTITATIVE DETERMINATION OF ASPIRIN AND PARACETAMOL

Aspirin, also known as acetylsalicylic acid, is a salicylate drug, used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti – inflammatory medication.

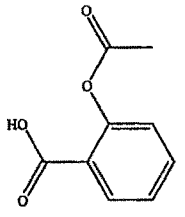

Figure 2.1. Chemical structure of aspirin

Aspirin also yields an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damage of the walls within blood vessels. Because the platelet patch can become too large and also block blood flow, locally and downstream, aspirin is also used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at high risk for developing blood clots (Lewis *et al.* 1983). It has also been established that low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue (Julian *et al.* 1996; Krumholz *et al.* 1995).

The main undesirable side effects of aspirin are gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in higher doses. In children and adolescents, aspirin is no longer used to control flu – like symptoms or the symptoms of chickenpox or other viral illnesses, because of the risk of Reye's syndrome (Macdonald 2002). Aspirin was the first discovered member of the class of drugs known as non – steroidal anti-inflammatory drugs (NSAIDs), not all of which are salicylates, although they all have similar effects and most have inhibition of the enzyme cyclooxygenase as their mechanism of action.

Paracetamol or acetaminophen is a widely used over – the – counter analgesic (pain reliever) and antipyretic (fever reducer).

It is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In

combination with non – steroidal anti – inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of more severe pain such as postoperative pain (Sign Guidelines).

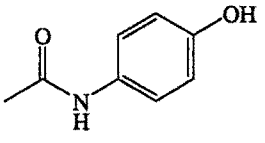

Figure 2.2. Chemical structure of paracetamol

While generally safe for human use at recommended doses (1000mg per single dose and up to 4000mg per day for adults, up to 2000mg per day if drinking alcohol) (http://www.drugs.com), acute overdoses of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. Paracetamol toxicity is the foremost cause of acute liver failure in most part of the world, and accounts for most drug overdoses (Daly *et al.* 2008; Khashab *et al.* 2007; Hawkins and Edwards 2007; Larson *et al.* 2005).

Paracetamol is derived from coal tar, and is part of the class of drugs known as "aniline analgesics"; it is the only such drug still in use today (Bertolini *et al.* 2006). It is the active metabolite of phenacetin, once popular as an analgesic and antipyretic in its own right, but unlike phenacetin and its combinations, paracetamol is not considered to be carcinogenic at therapeutic doses (Bergan *et al.* 1996).

High concentrations of aspirin and paracetamol have been detected with 100% frequency in raw sewage and aquatic system. Aspirin and its metabolites salicylic acid directly enter in to aquatic system through inefficient STPs whereas, paracetamol is excreted mainly as conjugate which can undergo hydrolysis during wastewater treatment resulting in the release of the parent compound (Kasprzyk – Horden *et al.* 2008, 2009).

There are various methods reported for the determination of aspirin and paracetamol in mixture, including derivative spectrophotometry (Nogowska *et al.* 1999), flow injection partial – squares UV spectrophotometry (Ruiz – Medina *et al.* 1999; Bouhsain *et al.* 1997), stopped – flow Fourier – transform infra – red

spectrometry (Bouhsain 1996), planar chromatography (Franeta *et al.* 2001; Simon *et al.* 2001), solid phase spectroscopy (Ruiz – Medina *et al.* 2000), and micellar electrokinetic chromatography (Boonkerd *et al.* 1995).

This chapter describes pre – concentration of aspirin and paracetamol using Polystyrene Divinyl Benzene or anion – exchanger as solid phase from synthetic aqueous solution. The adsorbed drugs were recovered by different solvents and later analyzed by UV – Spectrometer.

After optimizing the conditions the drug samples of aspirin and paracetamol individually; the optimized conditions were used for synthetic mixture aqueous solution of aspirin and paracetamol together. Later the optimized method was applied for environmental sample. To verify the results the samples were also analysed by HPLC.

EXPERIMENTS

Chemicals and Reagents

Aspirin and Paracetamol were obtained from a local drug industry, India; macro – porous and gel polystyrene divinyl benzene beads (8% and 12%cross – linking) were a kind gift from Doshi Ion – Exchange, Ahmedabad, India. Strong anion – exchanger (Amberlite IRA – 93), all other reagents and solvents were purchased from Qualigens and were of analytical or HPLC grade. These were used as obtained.

Instrumentation

For Pre – concentration

Pre – concentration by SPE was carried out using a glass column packed with adsorbent material. Flow rate was maintained using stop – cock attached with the column. The arrangement of the pre – concentrations experiments is given in Figure 2.3.

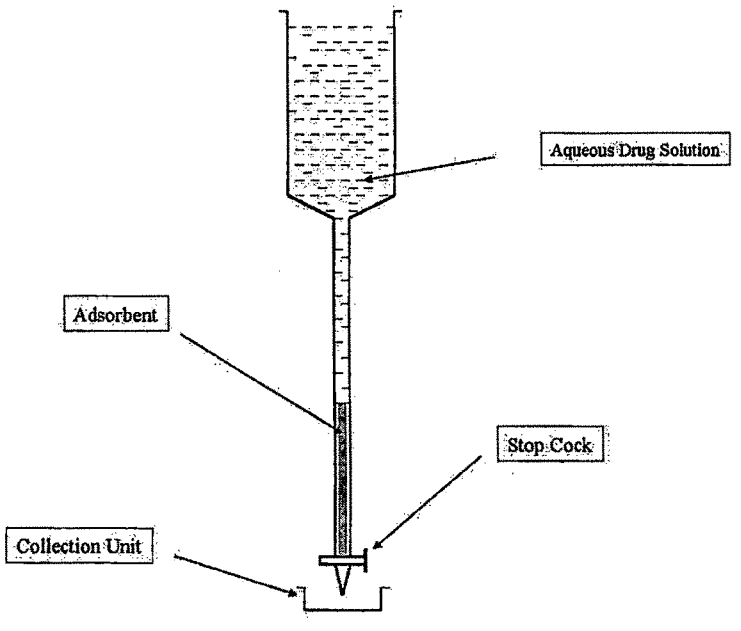

Figure 2.3. Pre – concentration method

For UV – Visible spectrophotometer

A UV – Vis spectrometer (Perkin Elmer Lambert 35) equipped with 1cm quartz cells (4mL each) was used for all absorbance measurements.

For Chromatographic method

Chromatographic determination was carried out with HPLC (Waters – Model number 2965 Seperation Module with empower pro software) using UV (2487 Channel 1) with measurements at 280nm and using a chromatographic column [Hyperssil BDS C18 (250 x 4.6) mm 5μ].

Stock Solutions

Stock solutions of aspirin and paracetamol (1000mg L^{-1}) were prepared by separately dissolving 100mg of each compound in 100mL DDW (Double Distilled Water). Working standard solutions were obtained by diluting stock solution with DDW to obtain the concentration of 100mg L^{-1}. The standard mixture solutions were prepared by mixing the working standard solutions to obtain 100mL, 25mg L^{-1} of aspirin and 25mg L^{-1} paracetamol mixture respectively. To obtain standard curve, solutions of different concentrations were prepared from stock standard solutions.

Treatment to PSDVB beads

10g of PSDVB beads were washed in a soxhlet with 350mL methanol for 3 h followed by 350mL of water (10h) and again with 350mL of methanol (3h). Then the beads were dried in vacuum oven at 50° C for 3 hours. These treated beads were used for pre – concentration experiments.

Pre – concentration Studies

Pre – concentration using PSDVB beads

Preliminary studies were conducted to work out the experimental conditions for the optimum adsorption and recovery. A typical experiment was performed using synthetic sample of aspirin. A sample of 100mg L^{-1} aspirin aqueous solution of 100mL volume was prepared by diluting an appropriate aliquot of stock solution. The column packed with 1.0g of the adsorbent material (PSDVB polymer beads) was activated by passing 5mL acetonitrile through it followed by 5mL of acetonitrile: water (80:20) (v/v) and then by 5mL of water. The aqueous drug sample was passed through the activated column at the rate of 0.66mL min^{-1}. The adsorbed drug was eluted with 10mL of methanol. Amounts of drug adsorbed and recovered in methanol were determined by recording absorbance at 225nm and using calibration graph developed for the purpose. To optimize the experimental conditions for pre – concentration of aspirin different experimental parameters where changed one – by – one, keeping other factors constant. Following experimental parameters were considered for optimization: initial volume of aqueous drug solution, amount of adsorbent, concentration of aqueous solution, volume of solvent for recovery and flow rate.

The developed optimized conditions for pre – concentration of aspirin were applied for pre – concentration of paracetamol. Amount of paracetamol adsorbed and recovered was determined by recording absorbance at 244 nm and using its calibration graph.

Aqueous solution containing both aspirin and paracetamol was pre – concentrated using the optimized conditions. Amount of aspirin and paracetamol adsorbed and recovered was determined by recording absorbance at 225nm and 244nm for aspirin and paracetamol respectively for simultaneous determination by

solving the equations obtained adding Beer – Lambert's law as mentioned in chapter 1 (*Page No. 56*).

Pre – concentration using anion – exchanger

1g of the anion – exchanger was soaked overnight in 20mL DDW in beaker. After decantation, the slurry was packed in glass column which was washed with 20mL DDW and activated by passing 50mL 2M HCl solution. Then the column (containing anion – exchanger) was washed with 50mL DDW. Through this washed activated column, 100mg L^{-1}, 100mL aspirin aqueous solution was allowed to pass at flow rate of 1mL min^{-1}. The adsorbed drug was recovered with 10mL 1%HCl. Amounts of drug adsorbed and recovered in 1%HCl were determined by recording absorbance at 225nm.

The same conditions were used to pre – concentrate paracetamol using anion – exchanger. Amount of paracetamol adsorbed and recovered was determined by recording absorbance at 244nm.

Aqueous solution containing both aspirin and paracetamol was pre – concentrated using the anion – exchanger. Amount of aspirin and paracetamol adsorbed and recovered was determined by recording absorbance at 225nm and 244nm for aspirin and paracetamol respectively for simultaneous determination.

Environmental Sample

Treated waste water sample was collected from STP operating with Up – Flow Anaerobic Sludge Blanket (UASB) principle. The plant has working capacity of 43MLD and is located in Vadodara, Gujarat, India. 2.5L (volume) sample was collected from the outlet of secondary clarifier of the treatment plant in a glass container. For sample preparation, the collected water sample was filtered through Whatman filter paper (No. 41) and into the filtrate for extraction, 75µL of 40% H_2SO_4 and a scoop of disodium ethylene diamine tetra acetate (Na_2EDTA) were added (Lindsey *et al.* 2001). An aliquot of the sample was then subjected to analysis as such whereas other was subjected to optimized pre – concentration step, and was analysed for the aspirin and paracetamol by UV – Visible spectrophotometer and HPLC.

Chromatographic Procedure

Synthetic samples of known concentration of aspirin and paracetamol were analysed by HPLC using Buffer 80%(v/v) (1mL H_3PO_4 / Liter H_2O) plus Acetonitrile 20%(v/v) as mobile phase, volume of sample injected was 20µL. The mobile phase flow rate was kept at 1.5mL min^{-1}. Under these conditions the retention times were 2.4 min for paracetamol and 7.4 min for aspirin. Chromatogram showing peaks of aspirin and paracetamol for 100mg L^{-1} solution is given in Figure 2.3.

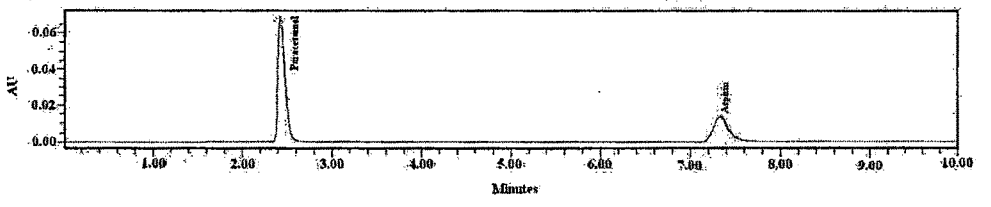

Figure 2.3. Chromatogram showing peaks of aspirin and paracetamol for 100mg L^{-1}

Under these chromatographic conditions Limit of Quantification (LOQ) and Limit of Detection (LOD) for aspirin and paracetamol were determined as 0.3mg L^{-1} and 0.015mg L^{-1} respectively. Peak of aspirin and paracetamol for 0.3mg L^{-1} (LOQ) and 0.06mg L^{-1} (LOD) is given in Figure 2.4. and Figure 2.5.

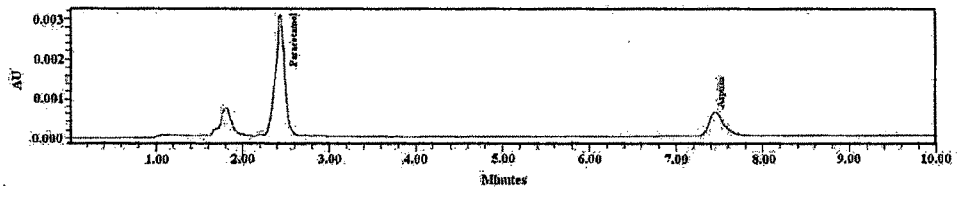

Figure 2.3. Chromatogram showing peak of aspirin and paracetamol at LOQ level

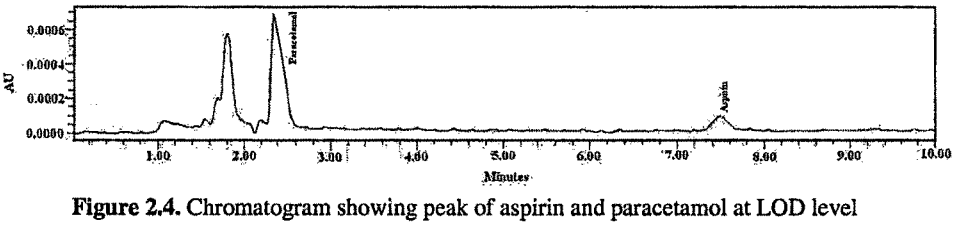

Figure 2.4. Chromatogram showing peak of aspirin and paracetamol at LOD level

Synthetic aqueous samples of mixture aspirin and paracetamol and environmental samples of, before and after optimized pre – concentration method using anion – exchanger were also analysed under these HPLC conditions.

RESULTS AND DISCUSSION

The preliminary UV – Visible experimental data indicate that the adsorption capacity of macro – porous polymer of PSDVB is better compared to gel type polymer. Table 2.1 shows that macro – porous PSDVB beads adsorb 5.5% of aspirin whereas gel type PSDBV adsorbs 0.4% of drug. This is expected since macro – porous polymer has higher surface area compared to gel type polymer.

Table 2.1. Pre – concentration studies of aspirin: Comparison between gel type and 12% cross – linking macro – porous type PSDVB beads.

Sr. No.	Adsorbent		Flow rate	Before		After	Percentage of Drug adsorbed
	Type	Weight		Volume	Amount of drug present in solution	Amount of drug present in solution	
		g	mL min^{-1}	mL	mg	mg	%
1.	Macro	1.00	1.00	100	10.00	9.45	5.5
2.	Gel	1.00	1.00	100	10.00	9.96	0.4

To get more adsorption macro – porous polymers of two different cross – linking: 12% and 8% of PSDVB were used to for pre – concentration. Study shows that macro – porous polymer with 8% cross – linking is better adsorbent. Table 2.2. shows that 12% PSDVB adsorbs 5.5% and 8% PSDVB adsorbs 12.0% drug. In general, increase in cross – linking in polymer results into decrease in surface area and porosity.

Table 2.2. Pre – concentration studies of aspirin: Comparison between different 8% and 12%cross – linking PSDVB beads.

Sr. No.	Adsorbent		Flow rate	Before		After	Percentage of Drug adsorbed
	Macro – porous	Weight		Volume	Amount of drug present in solution	Amount of drug present in solution	
		g	mL min^{-1}	mL	mg	mg	%
1.	12%	1.00	1.00	100	10.00	9.45	5.5
2.	8%	1.00	1.00	100	10.00	8.8	12.0

Type of solvent for recovered – H_2O, Volume of solvent for recovery – 10mL.

For further experiments macro – porous polymer having 8% cross – linking was used. The adsorption of aspirin from aqueous solution increases when the amount of adsorbent increases. However, the amount of drug recovered remains almost same for 10mL recovery solvent as shown in Table 2.3.

Table 2.3. Pre – concentration studies of aspirin: Effect of amount of macro – porous polymer

Sr. No.	Amount	Flow rate	Before	After	Percentage of Drug adsorbed	Recovered	
	Weight		Amount of drug present in solution	Amount of drug present in solution		Weight	Percentage
	g	mL min^{-1}	mg	mg	%	mg	%
1.	0.5	1.0	10.00	9.6	4.0	0.066	16.5
2.	1.0	1.0	10.00	8.8	12.0	0.083	6.92
3.	1.5	1.0	10.00	8.6	14.0	0.082	5.86
4.	1.0	0.1	10.00	7.9	21.0	0.083	3.95

Initial volume of drug solution – 100mL, Type of solvent for recovered – H_2O, Volume of solvent for recovery – 10mL.

Data of Table 2.3., (Sr. No. 4) show that by decreasing the flow rate the adsorption is better but the weight of recovered drug after adsorption is same as that in case of 1mL min^{-1} flow. Keeping the flow 1mL min^{-1}, the nature of recovering solvent was changed by taking 5% and 20% methanol / water respectively.

Table 2.4. Pre – concentration studies of aspirin: Effect of nature of solvent for recovery

Sr. No.	Amount	Before	After	Percentage of Drug adsorbed	Recovered		
	Weight	Amount of drug present in solution	Amount of drug present in solution		Type of Solvent	Weight	Percentage
	g	mg	mg	%		mg	%
1.	1.0	10.00	8.8	12.0	H_2O	0.083	6.92
2.	1.0	10.00	8.8	12.0	5% CH_3OH	0.083	6.92
3.	1.0	10.00	8.8	12.0	20% CH_3OH	0.084	6.92

Initial volume of drug solution – 100mL. Volume of solvent for recovery – 10mL.

From Table 2.4. it is seen that the recovery remains same even if solvent polarity is varied. So considering all these observations conditions for 100mL aqueous solution of aspirin for pre – concentration were optimized to 1g of adsorbent and 10mL volume of water (solvent for recovery) with flow of 1mL min^{-1}. Same optimized conditions were used for pre – concentration of paracetamol from synthetic aqueous solution Table 2.5.

Table 2.5. Pre – concentration studies of paracetamol: Pre – concentration of paracetamol using optimized conditions of aspirin

Sr. No.	Amount	Before	After	Percentage of Drug adsorbed	Recovered		
	Weight	Amount of drug present in solution	Amount of drug present in solution		Type of Solvent	Weight	Percentage
	g	mg	mg	%		mg	%
1.	1.0	10.00	9.7	3.0	H_2O	0.016	5.33

Initial volume of drug solution – 100mL, Flow rate – 1mL min^{-1}, Solvent for recovery – H_2O.

Data shows that, 8% macro – porous PSDVB adsorbs 3% Paracetamol from it aqueous solution and by 10.0mL water, 5.33% paracetamol is recovered.

To get better adsorption anion – exchanger was used as adsorbent keeping all above optimized experimental conditions same. Adsorption of the drug was excellent on anion – exchanger as shown in Table 2.6. However, recovery using water or methanol was negligible. For better recovery of drug adsorbed on anion – exchanger, 1% HCl aqueous solution was used. Pre – concentration factor is calculated by the formula:

$$\text{Pre - concentration factor (PF)} = \frac{\text{Concentration of solvent after recovery}}{\text{Concentration of initial drug solution}}$$

Table 2.6. Pre – concentration studies of aspirin and paracetamol: Individual

Sr. No.	Before			Percentage of Drug adsorbed	Recovered				PF
	Drug	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
		mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	ASP	10.00	100.00	99.00	10	0.59	59.00	5.96	0.59
2.	PAR	10.00	100.00	14.00	10	0.33	33.00	23.57	0.33

ASP – Aspirin, PAR – Paracetamol, Initial volume of drug solution – 100mL, Solvent for recovery – 1% HCl, Flow rate – 0.66mL min^{-1},.

Same optimized conditions were used to pre – concentrate aspirin and paracetamol individually at lower concentration. From results show in Table 2.7, it is observed that the percentage of adsorption of paracetamol increases at lower concentration.

Table 2.7. Pre – concentration studies of aspirin and paracetamol: At lower concentrations

Sr. No.	Before			Percentage of Drug adsorbed	Recovered				PF
	Drug	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
		mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	ASP	2.5	25.00	100.00	10	0.42	42.00	16.8	1.68
2.	PAR	3.4	34.00	29.41	10	0.28	28.00	28.0	0.82

ASP – Aspirin, PAR – Paracetamol, , Initial volume of drug solution – 100mL, Solvent for recovery – 1% HCl, Flow rate – 0.66mL min^{-1}

Same optimized conditions were used to pre – concentrate aspirin and paracetamol in presence of each other. From results shown in Table 2.8., it is also

observed that the percentage of adsorption of aspirin decreases in presence of paracetamol in water.

Table 2.8. Pre – concentration studies of aspirin and paracetamol: In presence of each other

.Sr. No.	Before			Percentage of Drug adsorbed	Recovered				PF
	Drug	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
		mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	ASP	3.79	37.9	71.63	10	0.80	80.0	29.54	2.11
2.	PAR	2.18	21.8	25.90	10	0.18	18.0	31.26	0.83

ASP – Aspirin, PAR – Paracetamol, , Initial volume of drug solution – 100mL, Solvent for recovery – 1% HCl, Flow rate – 0.66mL min^{-1}

In subsequent experiments the volume of solvent for recovery was doubled to increase the drug recovery keeping all other factor same. Table 2.9. shows the recovery of drug increases; at the same time pre – concentration factor decreases.

Table 2.9. Pre – concentration studies of aspirin and paracetamol: In presence of each other, with (10 + 10)mL 1% HCl for recovery

.Sr. No.	Before			Percentage of Drug adsorbed	Recovered				PF
	Drug	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
		mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	ASP	3.85	38.5	76.83	10 + 10	1.00	50.0	33.76	1.30
2.	PAR	2.18	21.8	29.90	10 + 10	0.32	16.0	56.15	0.73

ASP – Aspirin, PAR – Paracetamol, Initial volume of drug solution – 100mL, Solvent for recovery – 1% HCl, Flow rate – 0.66mL min^{-1}.

Effect of solvent for recovery was further studied taking 2% HCl. Table 2.10. shows 2% HCl recovers less amount of drug compared to 1% HCl.

Table 2.10. Pre – concentration studies of aspirin and paracetamol: In presence of each other, with 10mL 2% HCl for recovery

.Sr. No.	Before			Percentage of Drug adsorbed	Recovered				PF
	Drug	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
		mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	ASP	3.77	37.7	73.85	10	0.28	28.0	10.16	0.74
2.	PAR	2.14	21.4	18.57	10	0.15	15.0	38.22	0.70

ASP – Aspirin, PAR – Paracetamol, Initial volume of drug solution – 100mL, Solvent for recovery – 2% HCl. Flow rate – 0.66mL min^{-1}

Volume of 2% HCl was increased to 20mL for better drug recovery. Table 2.11. show that 20mL 2% HCl recovers more drug as compared to 10mL 1% HCl.

Table 2.11. Pre – concentration studies of aspirin and paracetamol: In presence of each other, with (10 + 10)mL 2% HCl volume of solvent for recovery

.Sr. No.	Before			Percentage of Drug adsorbed	Recovered				PF
	Drug	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
		mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	ASP	3.76	37.6	75.59	10 + 10	0.98	49.00	34.41	1.29
2.	PAR	2.14	21.4	21.41	10 + 10	0.33	16.5	71.46	0.77

ASP – Aspirin, PAR – Paracetamol, Initial volume of drug solution – 100mL, Solvent for recovery – 2% HCl, Flow rate – 0.66mL min^{-1}.

The conditions in Table 2.8., were selected as optimized conditions for maximum adsorption and recovery with better pre – concentration factor for aspirin and paracetamol in presence of each other from aqueous solution. The optimized conditions for maximum adsorption of aspirin and paracetamol in presence of each other and their recovery with better pre – concentration factor are: 100mL of initial aqueous drug solution pass through 1g anion – exchanger with flow rate 0.66mL min^{-1}, followed by 10mL 1% HCl used for recovery of drug adsorbed.

Developed optimized method for pre – concentration of aspirin and paracetamol was applied to environmental water sample collected from STP. Later the samples were also analyzed by the HPLC method. The result shows that aspirin and paracetamol in the environmental water samples were very low beyond the detection limit of the HPLC method i.e. 0.039mg L^{-1}.

Analytical Performance Characteristics

For UV – Visible Spectrometer

The validity of procedure was established through a study of linearity, sensitivity, repeatability. Linearity was established with a series of working standard solutions prepared by diluting the stock solution with respective solvents individually to the final concentrations. Calibration curves were obtained by measuring the UV absorbance of the standard solutions of aspirin and paracetamol in a concentration range of 20 – 100mg L^{-1} at wavelengths 225 nm for aspirin and 244nm for paracetamol. Precision of the method was determined by replicate measurements (n =6) of the absorbance of the pure aspirin and paracetamol solutions (six each of same

concentration). Validity of the methods for the analysis of aspirin and paracetamol was examined using the proposed procedures.

Aspirin in water

Linearity experiment in the range of 20 – 100mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 2.12

Table 2.12. Linearity experiment for aspirin in water: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	20	0.7046
2.	40	1.4800
3.	60	2.1679
4.	80	2.9517
5.	100	3.6327

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.13. Plot of absorbance Vs concentration for aspirin in water is shown in Figure 2.4.

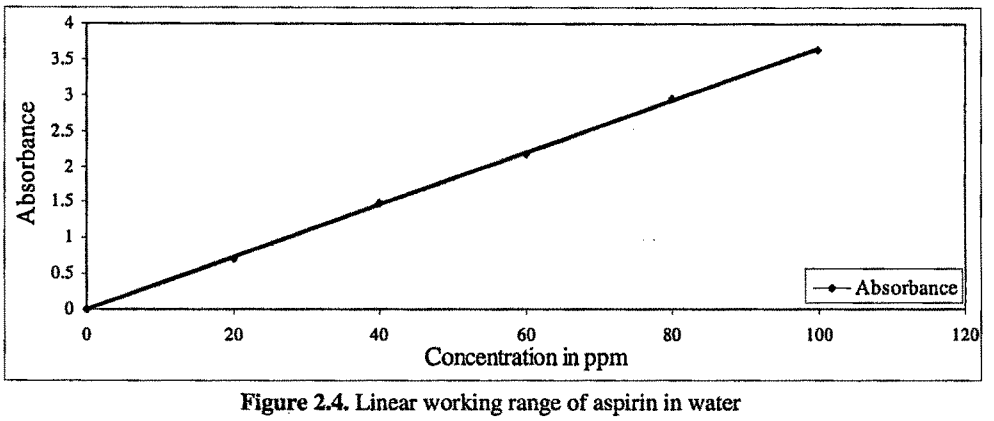

Figure 2.4. Linear working range of aspirin in water

Table 2.13. Results of regression analysis: Aspirin in water

Parameters	Aspirin in water
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9997
Slope, a	0.0366
Intercept	– 0.0052
No. of observations	5

The calibration graphs involved at least five experiment points for compound and they are described by the following equations: for aspirin in water: $y = 0.0366x - 0.0052$ ($r^2 = 0.9997$).

Aspirin in 1% HCl

Linearity experiment in the range of 10 – 50mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 2.14.

Table 2.14. Linearity experiment for aspirin in 1% HCl: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	10	0.4336
2.	20	0.8512
3.	30	1.2557
4.	40	1.4800
5.	50	2.0992

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.15. Plot of absorbance Vs concentration for aspirin in 1% HCl is shown in Figure 2.5.

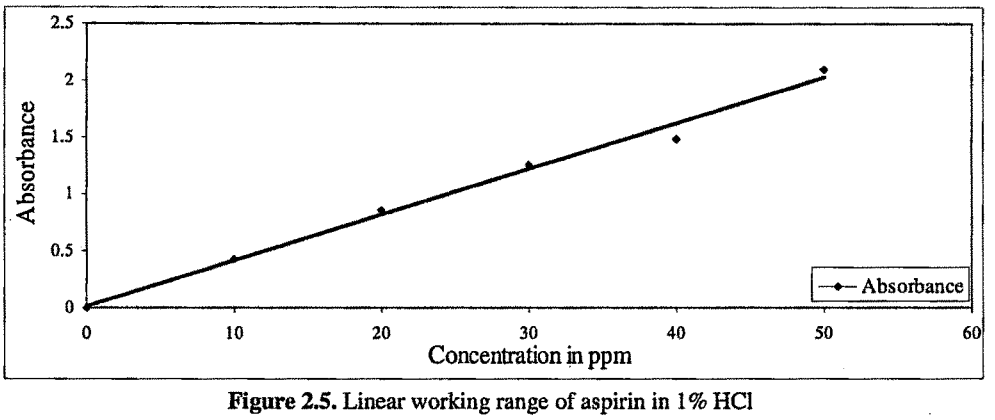

Figure 2.5. Linear working range of aspirin in 1% HCl

Table 2.15. Results of regression analysis: Aspirin in 1% HCl

Parameters	Aspirin in 1% HCl
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9899
Slope, a	0.0401
Intercept	0.0171
No. of observations	5

The calibration graphs involved at least five experiment points for compound and they are described by the following equations: for aspirin in 1% HCl: $y = 0.0401\ x + 0.0171$ ($r^2 = 0.9899$).

Aspirin in 2% HCl

Linearity experiment in the range of 10 – 50mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 2.16.

Table 2.16. Linearity experiment for aspirin in 2% HCl: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	10	0.4059
2.	20	0.7936
3.	30	1.0936
4.	40	1.4724
5.	50	1.8659

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.17. Plot of absorbance Vs concentration for aspirin in 2% HCl is shown in Figure 2.6.

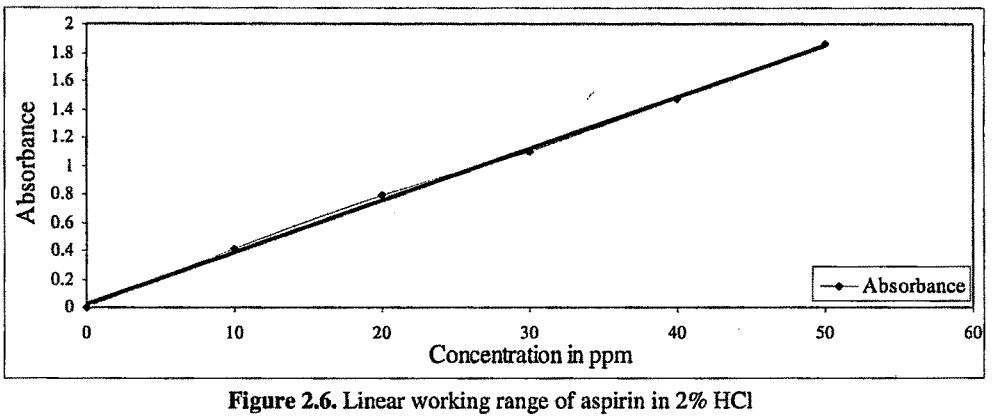

Figure 2.6. Linear working range of aspirin in 2% HCl

Table 2.17. Results of regression analysis: Aspirin in 2% HCl

Parameters	Aspirin in 2% HCl
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9985
Slope, a	0.0367
Intercept	0.0222
No. of observations	5

The calibration graphs involved at least five experiment points for compound and they are described by the following equations: for aspirin in 2% HCl: $y = 0.0367 x + 0.0222$ ($r^2 = 0.9985$).

Aspirin in 5% methanol

Linearity experiment in the range of 10 – 50mg L^{-1} was conducted. The absorbance values with respective concentrations are tabulated in Table 2.18.

Table 2.18. Linearity experiment for aspirin in 5% methanol: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	10	0.3832
2.	20	0.7506
3.	30	1.1309
4.	40	1.5000
5.	50	1.8505

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.19. Plot of absorbance Vs concentration for aspirin in 5% methanol is shown in Figure 2.7.

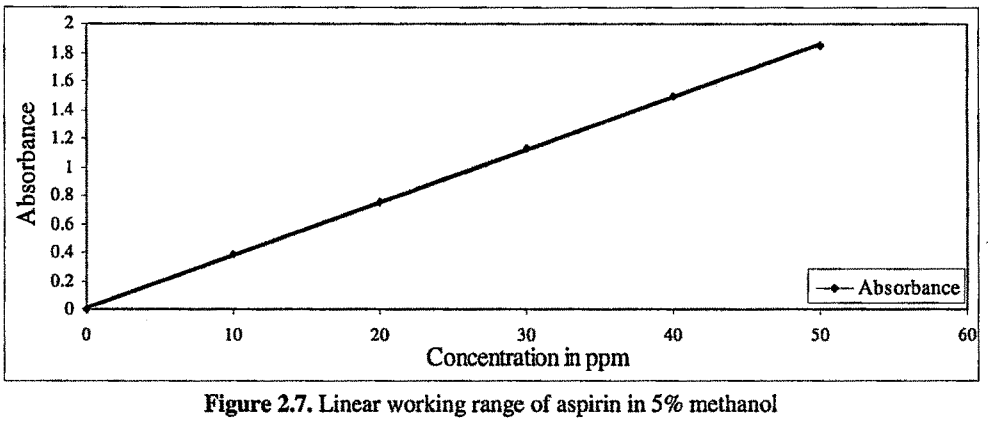

Figure 2.7. Linear working range of aspirin in 5% methanol

Table 2.19. Results of regression analysis: Aspirin in 5% methanol

Parameters	Aspirin in 5% methanol
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9998
Slope, a	0.0371
Intercept	0.0085
No. of observations	5

The calibration graph is described by the following equation: for aspirin in 5% methanol: $y = 0.0371x + 0.0085$ ($r^2 = 0.9998$).

Aspirin in 20% methanol

Linearity in the range of 10 – 50mg L^{-1} was determined. The absorbance values with respective concentrations are tabulated in Table 2.20.

Table 2.20. Linearity experiment for aspirin in 20% methanol: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	10	0.4671
2.	20	0.9251
3.	30	1.3559
4.	40	1.8389
5.	50	2.2447

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.21. Plot of absorbance Vs concentration for aspirin in 20% methanol is shown in Figure 2.8.

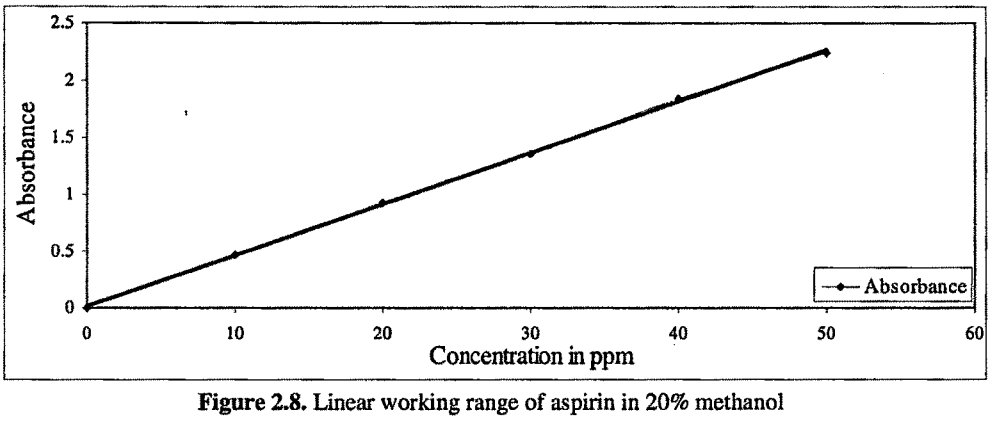

Figure 2.8. Linear working range of aspirin in 20% methanol

Table 2.21. Results of regression analysis: Aspirin in 5% methanol

Parameters	Aspirin in 20% methanol
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9996
Slope, a	0.0451
Intercept	0.0122
No. of observations	5

The calibration graph is described by the following equation: $y = 0.0451x + 0.0122$ ($r^2 = 0.9996$).

Acceptability of linearity data is judged by examining the coefficient of determination and the y – intercept as follows.

(a) The plot of concentration Vs absorbance (mean of three observations) for the linear working range is depicted in Table 2.12 for aspirin in water, in Table 2.14 for aspirin in 1% HCl, in Table 2.16 for aspirin in 2% HCl, in Table 2.18 for aspirin for 5% methanol and in Table 2.20 for aspirin for 20% methanol. The plot shows that a linear relationship exists between concentration and absorbance in the range of concentration 20 – 100mg L^{-1} for aspirin in water and 10 – 50mg L^{-1} for aspirin in 1% HCl, 2% HCl, 5% methanol and 20% methanol obeying Beer's – Lambert's law for its determination in the respective solution.

(b) The coefficient of determination i.e. 0.9997 for aspirin in water, 0.9899 for aspirin in 1% HCl, 0.9985 for aspirin in 2% HCl, 0.9998 for aspirin in 5% methanol and 0.9996 for aspirin in 20% methanol, means that almost 99.9% of variation in y i.e. the change in the response of the analyte can be explained by the change in x i.e. concentration of the analyte in the respective solutions. The correlation coefficient is a measure of goodness of the fit of the calculated line to the sample data.

(c) The slope of the regression line is 0.0366 for aspirin in water, 0.0401 in 1% HCl, 0.0367 for aspirin in 2% HCl, 0.0371 for aspirin in 5% methanol and 0.0451 for 20% methanol, this indicates that one unit increase in the concentration of aspirin in solution will result in an increase in the absorbance value by 0.0366, 0.0401, 0.0367, 0.0371 and 0.0451 units respectively.

Paracetamol in water

Linearity in the range of 20 – 100mg L^{-1} was determined. The absorbance values with respective concentrations are tabulated in Table 2.22.

Table 2.22. Linearity experiment for paracetamol in water: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	20	1.3586
2.	40	2.7087
3.	60	4.0588
4.	80	5.4087
5.	100	6.7586

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.23. Plot of absorbance Vs concentration for paracetamol in water is shown in Figure 2.9.

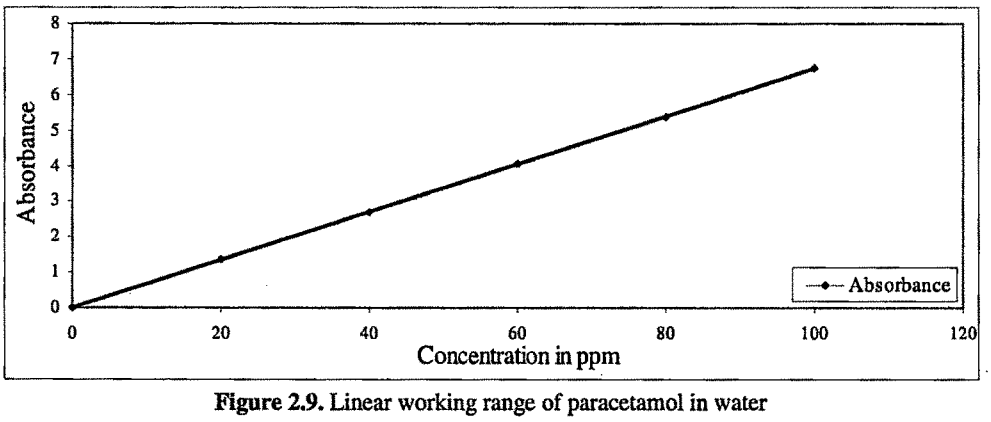

Figure 2.9. Linear working range of paracetamol in water

Table 2.23. Results of regression analysis: Paracetamol in water

Parameters	Paracetamol in water
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9989
Slope, a	0.0675
Intercept	0.0087
No. of observations	5

The calibration graph is described by the following equation: $y = 0.0675x + 0.0087$ ($r^2 = 0.9989$).

Paracetamol in 1% HCl

Linearity experiment in the range of 10 – 50mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 2.24.

Table 2.24. Linearity experiment for paracetamol in 1% HCl: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	10	0.4627
2.	20	0.9072
3.	30	1.3722
4.	40	1.8165
5.	50	2.2825

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.25. Plot of absorbance Vs concentration for paracetamol in 1% HCl is shown in Figure 2.10.

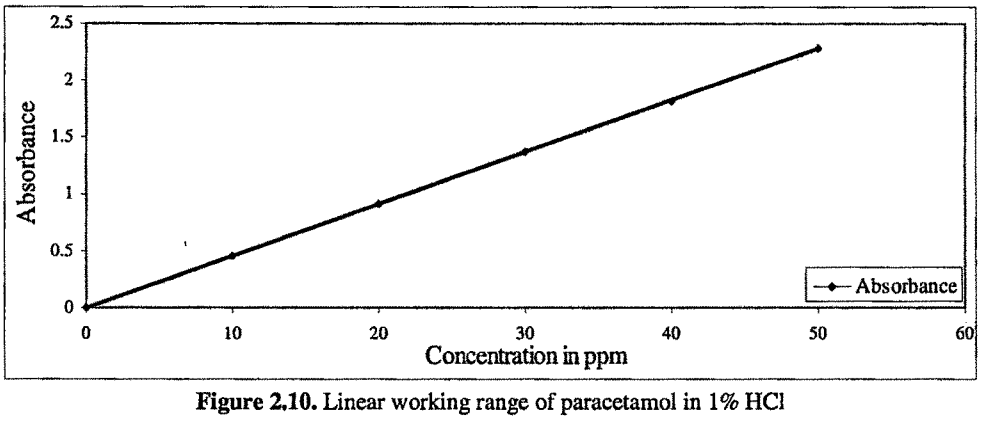

Figure 2.10. Linear working range of paracetamol in 1% HCl

Table 2.25. Results of regression analysis: Paracetamol in 1% HCl

Parameters	Paracetamol in 1% HCl
Regression Equation (y)	
Correlation Coefficient (r^2)	1.0000
Slope, a	0.0455
Intercept	0.0017
No. of observations	5

The calibration graph is described by the following equation: $y = 0.0455x + 0.0017$ ($r^2 = 1.0000$).

Paracetamol in 2% HCl

Linearity experiment in the range of 10 – 50mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 2.26.

Table 2.26. Linearity experiment for paracetamol in 2% HCl: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	10	0.7133
2.	20	1.3683
3.	30	2.107
4.	40	2.7029
5.	50	3.3453

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.27. Plot of absorbance Vs concentration for paracetamol in 2% HCl is shown in Figure 2.11.

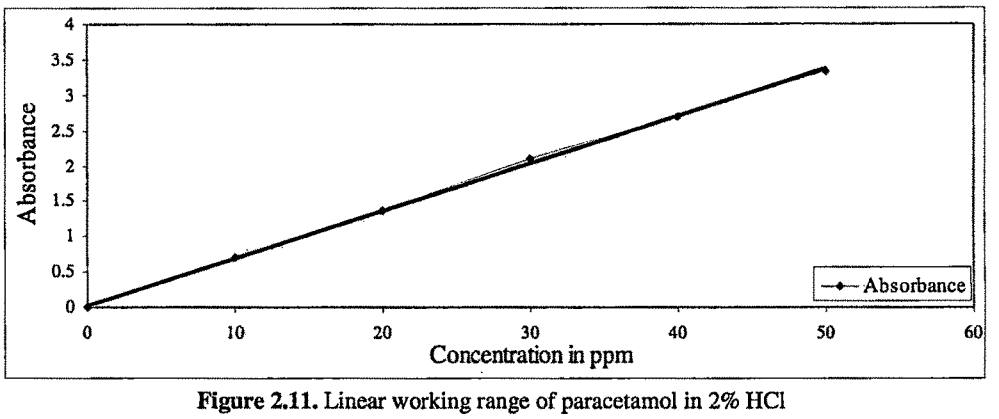

Figure 2.11. Linear working range of paracetamol in 2% HCl

Table 2.27. Results of regression analysis: Paracetamol in 2% HCl

Parameters	Paracetamol in 2% HCl
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9991
Slope, a	0.0670
Intercept	0.0323
No. of observations	5

The calibration graph is by the following equation: $y = 0.067x + 0.0323$ ($r^2 = 0.9991$).

Paracetamol in 5% methanol

Linearity in the range of 10 – 50mg L^{-1} was determined. The absorbance values with respective concentrations are tabulated in Table 2.28.

Table 2.28. Linearity experiment for paracetamol in 5% methanol: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	10	0.8498
2.	20	1.5154
3.	30	2.3616
4.	40	2.9612
5.	50	3.5850

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.29. Plot of absorbance Vs concentration for paracetamol in 5% methanol is shown in Figure 2.12.

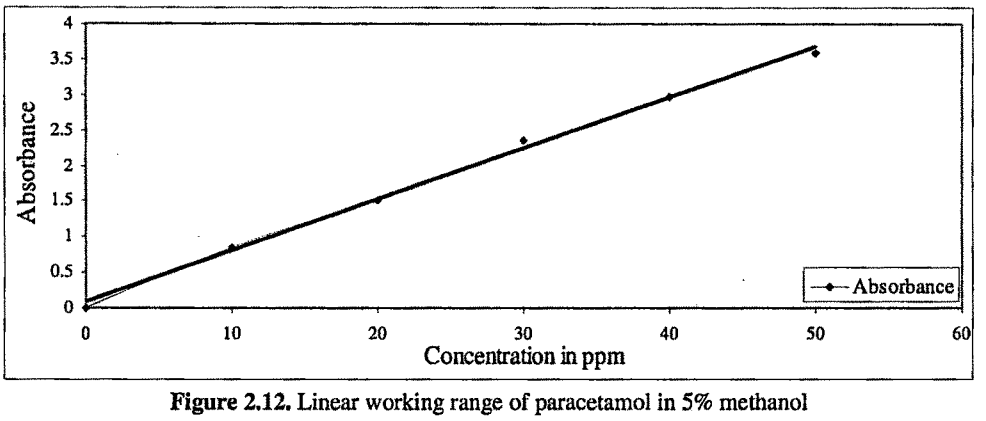

Figure 2.12. Linear working range of paracetamol in 5% methanol

Table 2.27. Results of regression analysis: Paracetamol in 5% methanol

Parameters	Paracetamol in 5% methanol
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9964
Slope, a	0.0717
Intercept	0.0865
No. of observations	5

The calibration graph is described by the following equation: $y = 0.0717\ x + 0.0865$ ($r^2 = 0.9964$)

Paracetamol in 20% methanol

Linearity experiment in the range of 10 – 50mg L^{-1} was conducted. The absorbance values with respective concentrations are tabulated in Table 2.30.

Table 2.30. Linearity experiment for paracetamol in 20% methanol: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	10	0.869
2.	20	1.5883
3.	30	2.4396
4.	40	3.1266
5.	50	3.6899

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.31. Plot of absorbance Vs concentration for paracetamol in 20% methanol is shown in Figure 2.13.

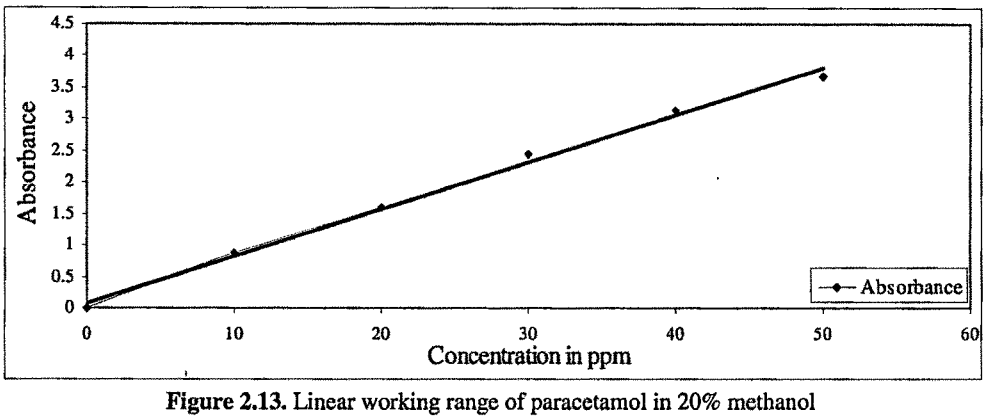

Figure 2.13. Linear working range of paracetamol in 20% methanol

Table 2.31. Results of regression analysis: Paracetamol in 20% methanol

Parameters	Paracetamol in 20% methanol
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9958
Slope, a	0.0745
Intercept	0.0898
No. of observations	5

The calibration graph is described by the following equation: $y = 0.0745x + 0.0898$ ($r^2 = 0.9958$).

Acceptability of linearity data is judged by examining the coefficient of determination and the y – intercept as follows.

(d) The plot of concentration Vs absorbance (mean of three observations) for the linear working range is depicted in Table 2.22 for paracetamol in water, in Table 2.24 for paracetamol in 1% HCl, in Table 2.26 for paracetamol in 2% HCl, in Table 2.28 for paracetamol for 5% methanol and in Table 2.30 for paracetamol for 20% methanol. The plot shows that a linear relationship exists between concentration and absorbance in the range of concentration 20 – 100mg L^{-1} for paracetamol in water and 10 – 50mg L^{-1} for paracetamol in 1% HCl, 2% HCl, 5% methanol and 20% methanol obeying Beer's – Lambert's law for its determination in the respective solution.

a. The coefficient of determination i.e. 0.9989 for paracetamol in water, 1.0000 for paracetamol in 1% HCl, 0.9991 for paracetamol in 2% HCl, 0.9964 for paracetamol in 5% methanol and 0.9958 for paracetamol 20% methanol, means that almost 99.6% of variation in y i.e. the change in the response of the analyte can be explained by the change in x i.e. concentration of the analyte in the respective solutions. The correlation coefficient is a measure of goodness of the fit of the calculated line to the sample data.

b. The slope of the regression line is 0.0675 for paracetamol in Water, 0.0675 in 1% HCl, 0.067 for paracetamol in 2% HCl, 0.0717 for paracetamol in 5% methanol and 0.0745 for 20% methanol, this indicates that one unit increase in the concentration of Aspirin in solution will result in an increase in the absorbance value by 0.0675, 0.0455, 0.067, 0.0717 and 0.0745 units respectively.

For HPLC

The validity of chromatographic procedure was established through a study of linearity, sensitivity, repeatability. Linearity was established with a series of working standard solutions prepared by diluting the stock solution with mobile phase to the final concentrations. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve. The calibration graphs involved at least five experimental points for each compound and they are described by the regression equation. Limit of detection (LOD) and quantitation (LOQ) were

calculated from visual determination method of %RSD of area. The validity of the methods for the analysis of aspirin and paracetamol was examined.

Aspirin

Linearity experiment in the range of 5 – 15mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 2.32.

Table 2.32. Linearity experiment for aspirin - HPLC: Concentration Vs peak area

Observation No.	Concentration (mg L^{-1})	Peak Area
1.	5.0	53887.33
2.	7.5	80642.67
3.	10.0	107503.67
4.	12.5	134366.67
5.	15.0	163261.67

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.33. Plot of peak area Vs concentration for aspirin – HPLC is shown in Figure 2.13.

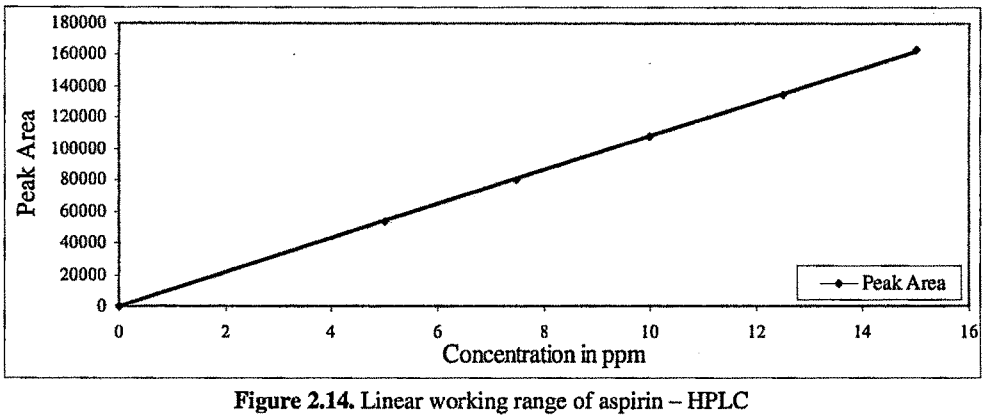

Figure 2.14. Linear working range of aspirin – HPLC

Table 2.33. Results of regression analysis: Aspirin – HPLC

Parameters	Aspirin – HPLC
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9998
Slope, a	10899
Intercept	1056.7
No. of observations	5

The calibration graph is described by the following equation: $y = 10899x + 1056.7$ ($r^2 = 0.9998$).

Paracetamol

Linearity experiment in the range of 5 – 15mg L^{-1} was conducted. The absorbance values with respective concentrations are tabulated in Table 2.34.

Table 2.34. Linearity experiment for paracetamol - HPLC: Concentration Vs peak area

Observation No.	Concentration (mg L^{-1})	Peak Area
1.	5.0	126957.33
2.	7.5	188941.00
3.	10.0	250672.67
4.	12.5	312605.33
5.	15.0	377811.67

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 2.35. Plot of peak area Vs concentration for paracetamol – HPLC is shown in Figure 2.15.

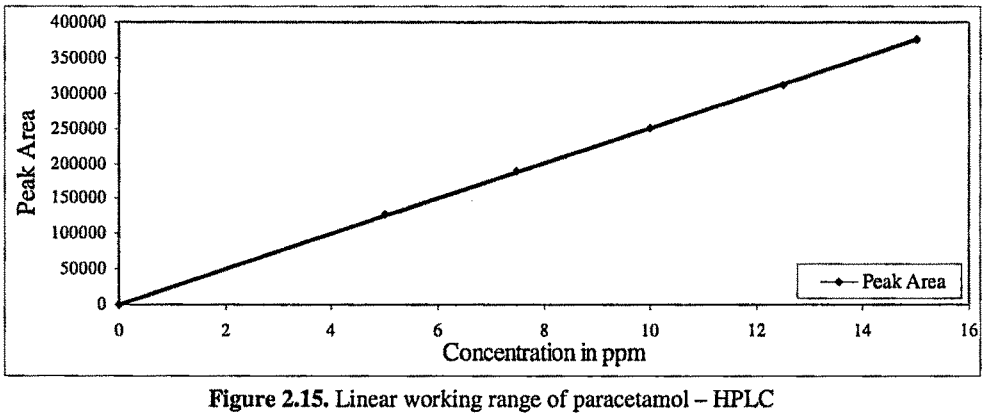

Figure 2.15. Linear working range of paracetamol – HPLC

Table 2.35. Results of regression analysis: Paracetamol – HPLC

Parameters	Paracetamol – HPLC
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9999
Slope, a	25015
Intercept	1248.4
No. of observations	5

The calibration graph is described by the following equation: $y = 25015x + 1248.4$ ($r^2 = 0.9999$)

Acceptability of linearity data is judged by examining the coefficient of determination and the y – intercept as follows.

a. The plot of concentration Vs peak area (mean of three observation) for the linear working range is depicted in Table 2.30 for aspirin and in Table 2.33 for paracetamol. The plot shows that a linear relationship exists between concentration and peak area in the range of concentration 5 – 15mg L^{-1}.

b. The coefficient of determination i.e. 0.9998 for aspirin and 0.9999 for paracetamol means that 99.9% of variation in y i.e. the change in the response of the analyte can be explained by the change in x i.e. concentration of the analyte. The correlation coefficient is a measure of goodness of the fit of the calculated line to the sample data.

c. The slope of the regression line is 10899 for aspirin, this indicates that one unit increase in the concentration of aspirin will result in an increase in the peak area value by 10899 units. Similarly one unit increase in concentration of paracetamol will result in an increase in the peak area value by 25015 units.

CONCLUSION

The method developed for pre – concentration of aqueous samples containing aspirin and paracetamol selected using UV – Spectrometer for quantification, is accurate, sensitive and reliable and enables the determination of the target pharmaceuticals in water samples at 0.025mg L^{-1} for aspirin and paracetamol. By using, easily available and less in cost macro – porous polymer of PSDVB with 8% cross – linking and anion – exchanger (Amberlite IRA – 93), in simple laboratory conditions an increase in concentration by a factor of 1.30 for aspirin and 0.76 for paracetamol can be obtained. Though the pre – concentration factors are not very encouraging, they produce a means of simultaneous solid phase extraction of two drugs for aqueous medium using materials available commercially in bulk with suitable modifications; these may be used as preparation aids for environmental water samples. Quantitative analysis of aspirin and paracetamol individual and together can

be done by UV – Visible spectrometer even at low levels. The environmental water sample collected from STP (Vadodara – India) after treatment does not show presence of aspirin and paracetamol up to the detection level of 0.03mg L^{-1}. This means either the concentration of these drugs is below the levels or STP removes these drugs effectively.

CHAPTER 3

PRE – CONCENTRATION AND QUANTITATIVE DETERMINATION OF ESOMEPRAZOL MAGNESIUM, FENOFIBRATE AND VENLAFAXINE HCl

ESOMEPRAZOLE MAGNESIUM TRIHYDRATE

Esomeprazole magnesium trihydrate, bis (5 – methoxy – 2 – [[(4- methoxy – 3, 5 – dimethyl – 2 – pyridinyl) methyl] sulfinyl] – 1 H – benzimidazole – 1 – yl) magnesium trihydrate (Figure 3.1), is a proton pump inhibitor (PPI) developed as an optical isomer (*S* – Esomeprazole) for the treatment of acid – related diseases (Lind *et al.* 2000).

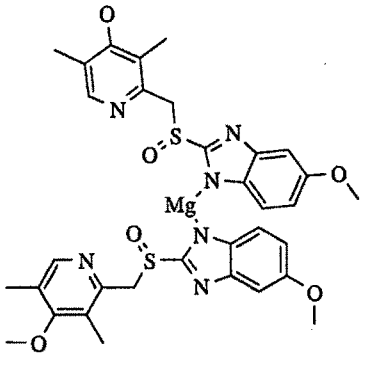

Figure 3.1. Chemical structure of Esomeprazole

Esomeprazole is a potent inhibitor of gastric acid secretion and accumulates in the acidic compartment of the parietal cells where molecule is transformed to its active sulfonamide form. Esomeprazole does not undergo chiral inversion in vivo (Andersson *et al.* 2001) and therefore Esomeprazole can be determined using the same methodology as for its racemate, Omeprazole.

The literature survey reveals that esomeprazole was analyzed in environmental water samples using solid phase extraction followed by LC – MS (Hernando *et al.* 2007; Castiglioni *et al.* 2005). Determination of esomeprazole in non – environmental samples has been widely reported. Esomeprazole has been determined in blood plasma by liquid chromatography with UV – detection (Lagerstrom and Persson 1984; Yeung *et al.* 1998; Yuen *et al.* 2001) and this technique has also been employed for simultaneous assay of the two major metabolites (Grundevik *et al.* 1986). Combination of liquid chromatography and mass spectrometry (LC – MS) has been used for omperazole and metabolites (Woolf and Matuszewski 1998; Kanazawa *et al.* 2002; Hoffman *et al.* 2006) and for esomeprazole and other PPIs (Shimizu *et al.* 2006; Oliveira *et al.* 2003). Enatioselective methods for detection of Esomeprazole by liquid

chromatography have also been presented which employ mass spectrometric (Wang *et al.* 2005) or UV – detection (Stenhoff *et al.* 1999). To our knowledge none of these methods have been applied for analysis of treated waste water for detection of esomeprazole using HPLC.

FENOFIBRATE

Fenofibrate, Isopropyl 2[4 – 4 – chlorobenzoyl) phenoxy] – 2 – methylpropinoate (Figure 3.2), is fibric acid derivative, used for regulating plasma lipids and treatment of hyperlipoproteinaemias (Sweetman 2002).

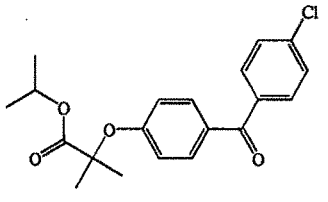

Figure 3.2. Chemical structure of Fenofibrate

Presence of fenofibrate in aquatic environment has been reported recently (Hernando *et al.* 2006; Reddersen and Heberer 2003). Several studies have reported presence of personal care and pharma products in aquatic systems (Buchberger 2003; Jones *et al.* 2003; Kanda *et al.* 2003).

The literature survey reveals that fenofibrate has been analyzed in environmental water samples using solid phase extraction followed by GC – MS (Reddersen and Heberer 2003; Sacher *et al.* 2001) or HPLC – ESI – MS – MS (Sacher *et al.* 2001). HPLC was used for determination of fenofibrate (British Pharmacopeia 2007; Romanyshyn and Tiller 2001), its metabolites (Streel *et al.* 2000; Masnatta *et al.* 1996; Ramusino and Carozzi 1986) and related impurities in non – environmental samples (Rao and Nagaraju 2003). Other HPLC methods for assay and purity of fenofibrate and NMR method for related compounds in fenofibrate raw materials are also reported (Lacroix *et al.* 1998). The reported HPLC methods resolved 11 known and six unknown impurities from fenofibrate. To our knowledge none of the reported HPLC methods have been applied for analysis of treated waste water for detection of fenofibrate.

VENLAFAXINE HCl

Venlafaxine, 1 – [2 – (dimethylamino) –1– (4 – methoxyphenyl) ethyl] cyclohexanol hydrochloride (Figure 3.3), is a novel, non – tricyclic antidepressant. Venlafaxine HCl imparts its antidepressant effects by inhibiting the neuronal uptake of norepinephrine, serotonin and to a lesser extent, dopamine (Rudorfer and Potter 1989; Haskins *et al.* 1985; Muth *et al.* 1986).

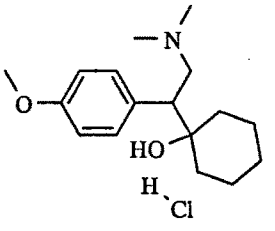

Figure 3.3. Chemical structure of Venlafaxine HCl

There are various HPLC (Hicks *et al.* 1994; Helmeste *et al.* 1997) and LC – MS (Schultz and Furlong 2008) methods reported in literature for quantitation of venlafaxine HCl for different purposes. To our knowledge none of the reported HPLC methods have been applied for analysis of treated sewage water for detection of venlafaxine HCl.

Fate and Environmental Significance

Esomeprazole is excreted in an unaltered form in the low proportion and its presence in aquatic environment has been reported (Hernando *et al.* 2007). Presence of fenofibrate in aquatic environment has been reported recently (Hernando *et al.* 2006; Reddersen and Heberer 2003). Venlafaxine HCl is soluble in water, which suggests that significant amount of active unused venlafaxine HCl may reach municipal sewage treatment plants through toilets and drains. Number of reports on the occurrence of a wide variety of antidepressants in the aquatic environment have been increasing steadily in recent times, (Weigel *et al.* 2004) especially venlafaxine (Schultz and Furlong 2008).

Understanding the fate of these drugs in water could lead to better waste water treatment options that would lead to more complete removal of such compounds. To

aid in this understanding, an analytical method that accurately measures low concentrations of esomeprazole, fenofibrate and venlafaxine HCl in water is an essential tool.

This chapter details the analytical method for quantitation of esomeprazole, fenofibrate and venlafaxine HCl in water sample obtained from waste water treatment plant using a new relatively simple and yet sensitive SPE method in combination with HPLC. Pre – concentration of esomeprazole, fenofibrate and venlafaxine HCl individually from aqueous solution was carried out using PSDVB beads. After adsorption the drugs were recovered from the solid phase using suitable solvents. The resultant solutions were subjected to quantitation using HPLC method based upon conditions described in reported methods and optimized for esomeprazole (British Pharmacopoeia 2007), fenofibrate (El – Gindy *et al.* 2005) and venlafaxine HCl (Lindsey *et al.* 2001). After optimizing the pre – concentration methods, they were applied to treated waste water sample collected from a local Sewage Treatment Plant (STP).

EXPERIMENTS

Chemicals and Reagents

Esomeprazole, Fenofibrate and Venlafaxine HCl were obtained from local drug industry. All other solvents and reagents were purchased from Qualigens and were of analytical or HPLC grade. These were used as obtained and Milli – Q water was prepared with Millipore Elix® -3.

Instrumentation

The chromatograph system comprised of Shimadzu LC – 10 AS equipped with Rheodyne injector (20µL capacity) and UV – Vis detector (SPD – 10A). Data integration was done using a software package (LC – 10). The column used for Esomeprazole and Venlafaxine HCl was BDS Hypersil C8 (4.6 x 250mm, 5µ) and for Fenofibrate it was Knauer C18 (250 x 4.6mm I.D., 5 µ).

Pre – concentration using SPE was carried out using same procedure as mentioned in chapter 2 (*Page No. 71*).

Stock solutions

Esomeprazole

Stock solution of 1000mg L^{-1} esomeprazole was prepared by dissolving 100mg of drug in 100mL milli – Q water. Working standard solution was prepared by diluting stock solution with milli – Q water to obtain the concentration of 500mgL^{-1}. To obtain standard curve, solutions of different concentration were prepared by diluting appropriate volumes of working standard solution with milli – Q water. Similarly stock solution of esomeprazole in methanol solvent was also prepared.

Fenofibrate

Stock solution of 1000mg L^{-1} fenofibrate was prepared by dissolving 100mg of the drug in 100mL milli – Q water: acetonitrile (60:40) (v/v). Working standard solution was prepared by diluting stock solution with milli – Q water: acetonitrile (60:40) (v/v) to obtain the concentration of 500mg L^{-1}. To obtain standard curve, solutions of different concentration were prepared by diluting appropriate volumes of working standard solution with milli – Q water: acetonitrile (60:40) (v/v). Similarly stock solutions of fenofibrate in methanol and acetonitrile solvent were also prepared.

Venlafaxine HCl

Stock solution of 1000mg L^{-1} venlafaxine HCl was prepared by dissolving 100mg of drug in 100mL milli – Q water. Working standard solution was prepared by diluting a suitable volume of stock solution with milli – Q water to obtain the concentration of 500mg L^{-1}. To obtain standard curve, solutions of different concentration were prepared by diluting appropriate volumes of working standard solution with milli – Q water. Similarly stock solution of venlafaxine HCl in methanol solvent was also prepared.

The stock solutions were refrigerated and were consumed within three days.

Treatment to PSDVB beads

PSDVB beads were treated using same procedure as mentioned in chapter 2 (*Page No. 73*).

Chromatography procedure

Esomeprazole

Synthetic samples of known concentration of esomeprazole were analysed by HPLC using mobile phase which was prepared by mixing buffer and acetonitrile in a ratio of 70:30 v/v. Buffer was prepared by mixing 5mL of triethyl amine and 995mL Milli – Q water containing 1.2g ammonium dihydrogen orthophospahe. The flow rate was 1.0mL min^{-1}. Detection was carried out at wavelength 302nm. All determinations were performed at room temperature. The injection volume was 20μL. Under these conditions the retention time of Esomeprazole prepared in methanol and water was in the range of 15.0 to 15.3min. Chromatogram showing peak of esomeprazole for 100mg L^{-1} is shown in Figure 3.4.

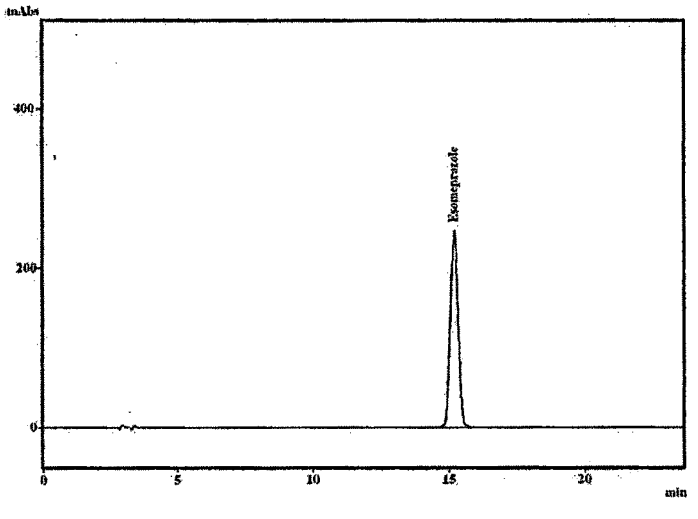

Figure 3.4. Chromatogram showing peak of esomeprazole for 100mg L^{-1} solution

Under these chromatographic conditions Limit of Quantification (LOQ) and Limit of Detection (LOD) for esomeprazole were determined as 0.19mg L^{-1} and

0.09mg L^{-1} respectively. Chromatograms showing peak of esomeprazole for 0.19mg L^{-1} (LOQ) and 0.09mg L^{-1} (LOD) are given in Figures 3.5 and Figure 3.6 respectively.

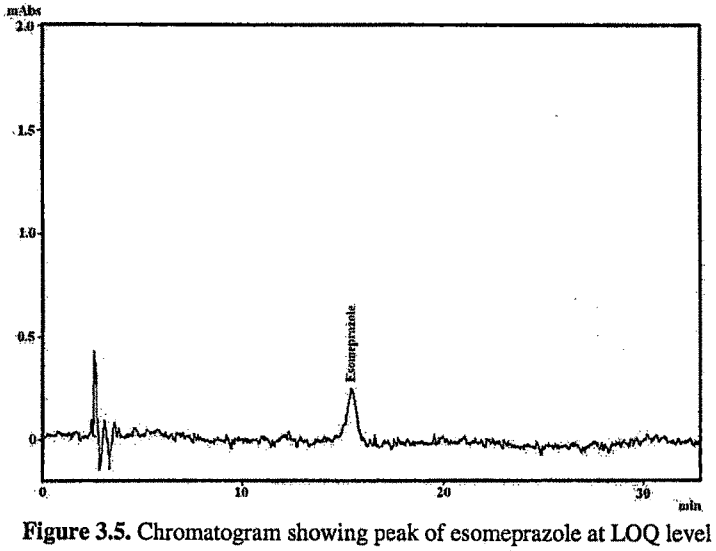

Figure 3.5. Chromatogram showing peak of esomeprazole at LOQ level

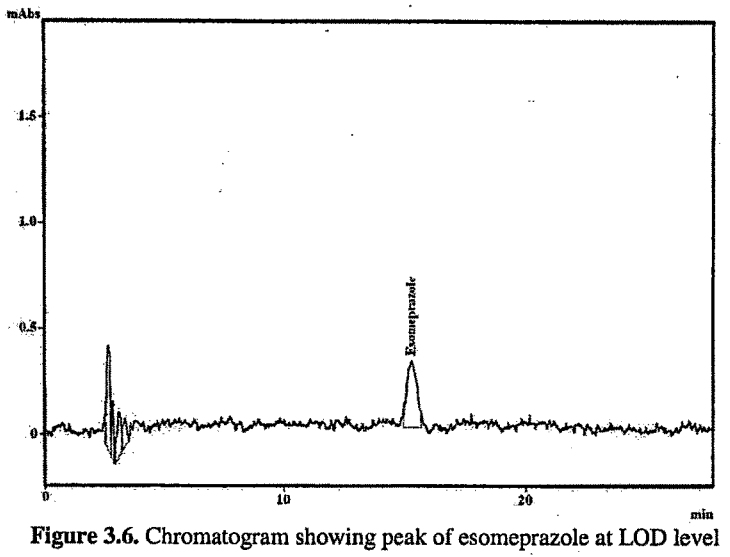

Figure 3.6. Chromatogram showing peak of esomeprazole at LOD level

Fenofibrate

Synthetic samples of known concentration of fenofibrate were analysed by HPLC using mobile phase which was prepared by mixing acetonitrile and milli – Q water in a ratio of 80:20 v/v and adjusted to pH 4.0 using phosphoric acid. The flow rate was 1.5mL min^{-1}. Detection was carried out at wavelength 287nm. All determinations were performed at room temperature. The injection volume was 20µL.

Under these conditions the retention time of fenofibrate prepared in methanol, water and acetonitrile was in the range of 6.0 to 6.3min. Chromatogram showing peak of Fenofibrate for 125mg L^{-1} solution is given in Figure 3.7.

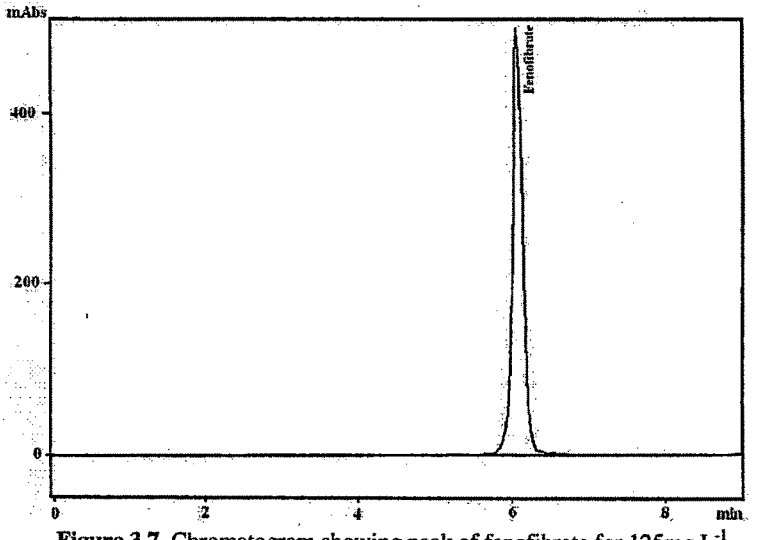

Figure 3.7. Chromatogram showing peak of fenofibrate for 125mg L^{-1}

Under these chromatographic conditions Limit of Quantification (LOQ) and Limit of Detection (LOD) for Fenofibrate were determined as 0.48mg L^{-1} and 0.06mg L^{-1} respectively. Chromatogram with peak of Fenofibrate for 0.48mg L^{-1} (LOQ) and 0.06mg L^{-1} (LOD) concentrations is given in Figure 3.8 and Figure 3.9 respectively.

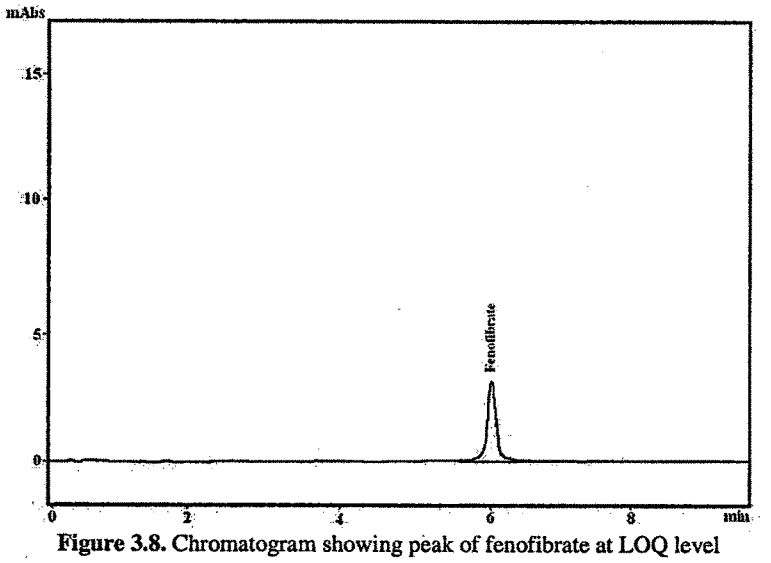

Figure 3.8. Chromatogram showing peak of fenofibrate at LOQ level

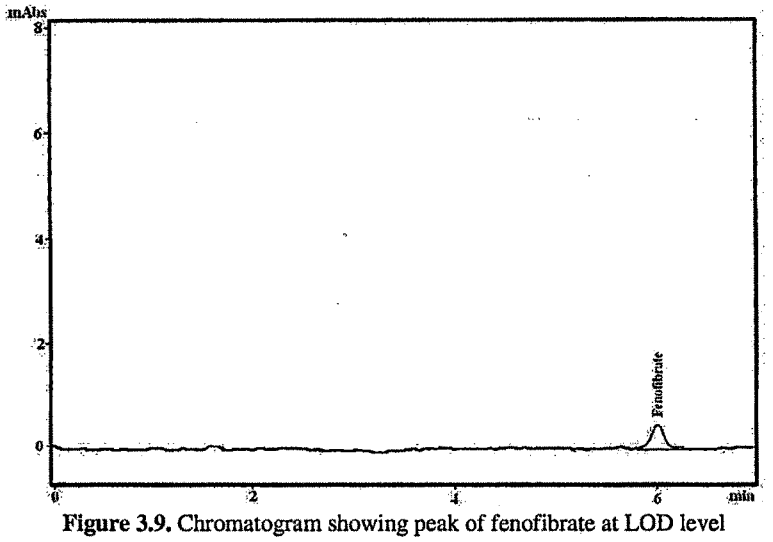

Figure 3.9. Chromatogram showing peak of fenofibrate at LOD level

Venlafaxine HCl

Synthetic samples of known concentration of venlafaxine HCl were analysed by HPLC using mobile phase consisting of acetnonitrile: sodium dihydrogen orthophosphate [0.04 M], pH 6.8 (75:25) at a flow rate of 1.5mL min^{-1}. Detection was carried out at wavelength 224nm. Under these conditions the retention time of Venlafaxine HCl prepared in methanol and water was in the range of 2.7 to 2.9min. Chromatogram showing peak of Venlafaxine HCl for 250mg L^{-1} is given in Figure 3.10.

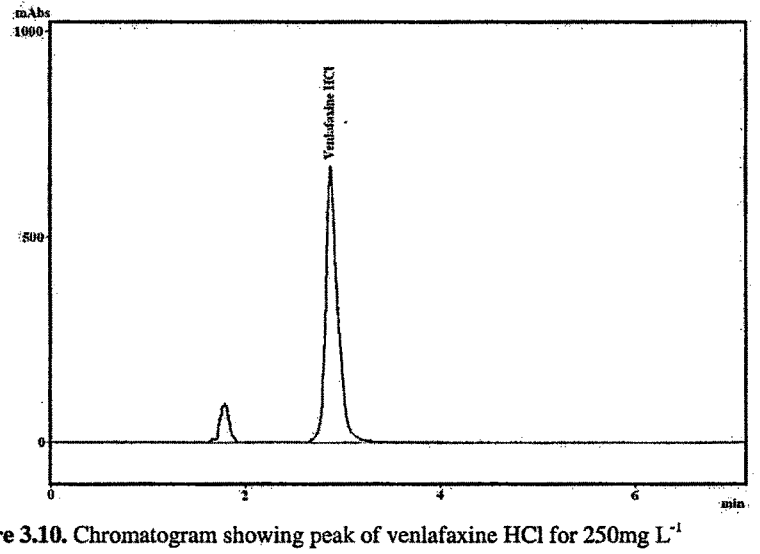

Figure 3.10. Chromatogram showing peak of venlafaxine HCl for 250mg L^{-1}

Under these chromatographic conditions Limit of Quantification (LOQ) and Limit of Detection (LOD) for venlafaxine HCl were determined as 0.48mg L^{-1} and 0.06mg L^{-1} respectively. Peak of venlafaxine HCl for 0.48mg L^{-1} (LOQ) and 0.06mg L^{-1} (LOD) is given in Figure 3.11 and Figure 3.12.

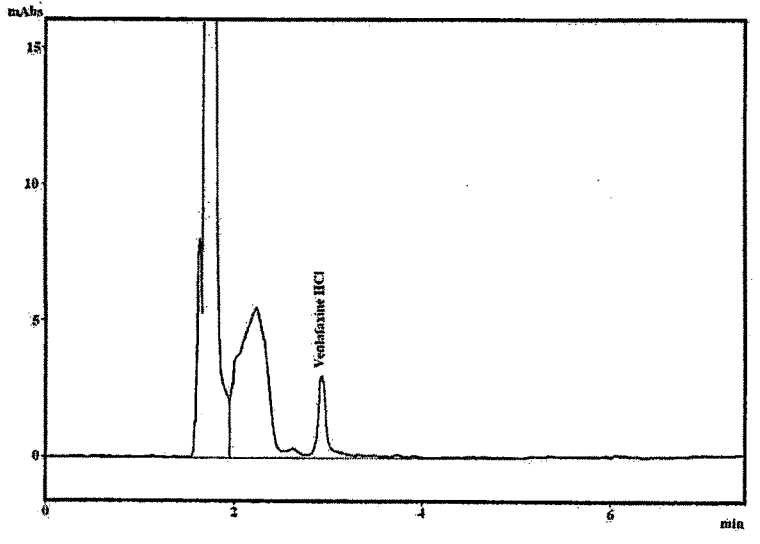

Figure 3.11. Chromatogram showing peak of venlafaxine HCl at LOQ level

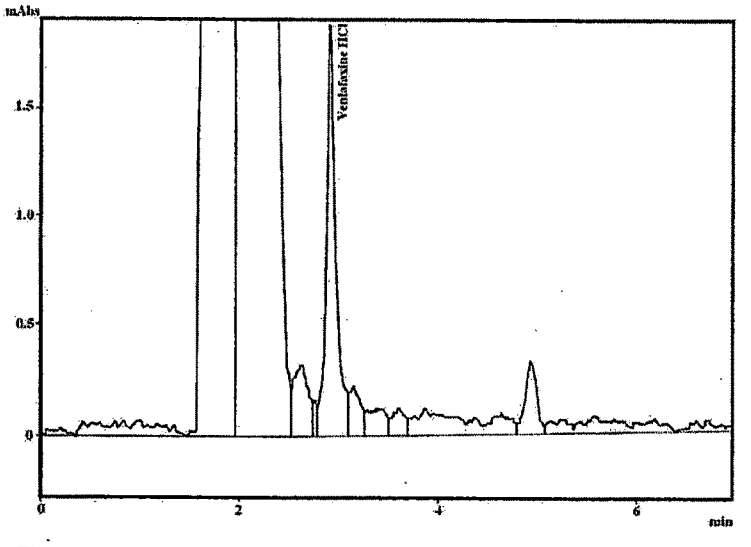

Figure 3.12. Chromatogram showing peak of venlafaxine HCl at LOD level

Pre – concentration studies

Esomeprazole

Based on the previous data collected for SPE of aspirin and paracetamol preliminary studies were conducted for optimum adsorption of esomeprazole and its recovery. A typical experiment was performed using synthetic sample of Esomeprazole. A sample of 100mg L^{-1} esomeprazole aqueous solution of 100mL volume was prepared by diluting an appropriate aliquot of stock solution.

The column packed with 1.0g of the adsorbent material (PSDVB beads) was activated by passing 5mL acetonitrile through it followed by 5mL of acetonitrile: water (80:20) (v/v) and then by 5mL of water. The aqueous drug sample was passed through the activated column at the rate of 0.66mL min^{-1}. The adsorbed drug was eluted with 10mL of methanol. Amounts of drug adsorbed and recovered in methanol were determined by HPLC analysis. To optimize the experimental conditions for pre – concentration of esomeprazole different experimental parameters where changed one – by – one, keeping other factors constant.

Fenofibrate

Preliminary studies were conducted to work out the experimental conditions for the optimum adsorption of fenofibrate on polymer beads and its recovery. A typical experiment was performed using synthetic sample of fenofibrate. A sample of 50mg L^{-1} fenofibrate aqueous solution of 50mL volume was prepared by diluting an appropriate aliquot of stock solution.

The column packed with 1.0g of the adsorbent material (PSDVB beads) was activated as mentioned for esomeprazole. The aqueous drug sample was passed through this activated column at the flow rate of 0.66mL min^{-1}. The adsorbed drug was recovered with 10mL of methanol. Amounts of drug adsorbed and recovered in methanol were determined by HPLC analyses. The experimental conditions for pre – concentration of fenofibrate were optimized.

Venlafaxine HCl

Preliminary studies were conducted to work out the experimental conditions for the optimum adsorption of venlafaxine HCl on polymer beads and its recovery. In a typical experiment, a sample of 50mg L^{-1} venlafaxine HCl aqueous solution of 50mL volume was prepared by diluting an appropriate aliquot of stock solution.

The column packed with 1.0g of the adsorbent material (PSDVB beads) was activated following the earlier method. The aqueous sample was passed through the column at a rate of 0.66mL min^{-1}. The adsorbed drug was recovered with 10mL of methanol. Amount of drug adsorbed and the amount of drug recovered in methanol was determined by HPLC analysis. The experimental conditions for pre – concentration of venlafaxine HCl were optimized.

Analysis of Environmental Sample

Environmental water samples were treated using same procedure as mentioned in chapter 2. (*Page No.74.*) An aliquot of the sample was then subjected to HPLC analysis as such whereas other was subjected to optimized pre – concentration step, the adsorbed drug recovered by methanol and this was also analysed using LC – MS.

RESULTS AND DISCUSSION

Pre – concentration studies of esomeprazole

The optimized conditions of pre – concentration studies for aspirin and paracetamol on different adsorbents from aqueous solution viz. 1.0g adsorbent, 100mL aqueous solution of drug and 5mL solvent for recovery were used as starting set of conditions for the present work.

Initially effect of flow rate of aqueous esomeprazole solution on adsorption was studied. Table 3.1., shows that with increase in flow rate adsorption of drug on adsorbent decreases.

Table 3.1. Pre – concentration studies of esomeprazole: Effect of flow rate

Sr. No.	Flow rate	Before		Percentage of Drug adsorbed	Recovered				PF
		Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
	mL min^{-1}	mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	1.4	5.10	51.01	67.06	10	2.84	284.39	83.04	5.57
2.	0.66	5.10	51.01	70.39	10	3.14	314.38	87.46	6.16

PF – Pre – concentration Factor, Type of solvent for recovery – Methanol, Initial drug solution – 100mL.

For subsequent experiments the 0.66mL min^{-1} flow rate was maintained, which resulted into a maximum drug adsorption of up to 70%.

Effect of changing volume of aqueous solution containing the drug, effect of changing amount of adsorbent while keeping volume and concentration of drug solution constant were studied and optimized for esomeprazole. Studies show (Table 3.2, Sr. No. 1) that more than 70% esomeprazole adsorbs on 1g of PSDVB macro – porous beads when 100mL of its aqueous solution is passed through column. Table 3.2., Sr. No. 2, show with 50mL of initial drug solution volume the percentage of drug adsorption increases to 85.11.

Table 3.2. Pre – concentration studies of esomeprazole: Effect of initial drug volume

Sr. No.	Before			Percentage of Drug adsorbed	Recovered				PF
	Initial Volume of drug solution	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
	mL	mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	100	5.10	51.01	70.39	10	3.14	314.38	87.46	6.16
2.	50	1.41	28.19	85.11	10	1.40	140.00	100.00	4.97

PF – Pre – concentration Factor, Type of solvent for recovered – Methanol, Flow rate – 0.66mL min^{-1}.

With this percentage of adsorption, methanol was used for recovery. The percentage of recovery was studied with four different volumes of methanol. Data in Table 3.3, show that maximum drug recovery of up to 102.91% was observed with 10mL of methanol resulting in pre concentration factor of 6.05.

Table 3.3. Pre – concentration studies of esomeprazole: Effect of volume of solvent for recovery

Sr. No.	Before		Percentage of Drug adsorbed	Recovered				PF
	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
					Weight	Conc.	Percentage	
	mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	1.41	28.19	85.11	10	1.40	140.00	100.00	4.97
2.	1.66	33.28	83.73	7	1.41	201.46	102.91	6.05
3.	1.66	33.28	87.95	5	1.47	294.23	100.68	8.84
4.	1.66	33.28	83.73	3	1.05	351.56	75.5	10.56

Initial volume of drug solution – 50mL, PF – Pre – concentration Factor, Type of solvent for recovered – Methanol, Flow rate – 0.66mL min^{-1}.

With the decrease in volume of methanol for recovery the percentage of drug recovered decreases though the pre – concentration factor increases. Therefore, for subsequent studies, volume of solvent for recovery was set to 5mL. With 5mL methanol 100.68% drug is recovered with pre – concentration factor 8.84.

With these optimized conditions for recovery, pre – concentration experiments were performed taking higher volumes of aqueous drug solutions keeping the amount of drug same. Table 3.4, shows that with increase in volume of initial aqueous drug solution, the percentage of drug adsorbed remains almost same but after recovery with 5mL methanol, pre – concentration factor for respective experiments increases.

Table 3.4. Pre – concentration studies of esomeprazole: Effect of initial drug volume

Sr. No.	Before			After		Recovered			PF
	Initial Volume of drug solution	Amount of drug present in solution	Conc. of drug solution	Drug Adsorbed		Amount of drug			
				Percentage of Drug adsorbed	Amount of drug adsorbed	Weight	Conc.	Percentage	
	mL	mg	mg L^{-1}	%	mg	mg	mg L^{-1}	%	
1.	50	1.66	33.28	87.95	1.46	1.47	100.68	100.68	8.84
2.	100	1.82	18.2	87.36	1.59	1.46	92.99	92.99	16.04
3.	150	1.82	12.13	86.26	1.57	1.48	94.27	94.27	24.35
4.	250	1.72	6.89	96.51	1.66	1.49	89.76	89.76	43.35
5.	500	1.72	3.44	94.19	1.62	1.50	92.59	92.59	87.02

PF – Pre – concentration Factor, Flow rate – 0.66mL min^{-1}, Type of solvent for recovered – Methanol, Volume of methanol – 5mL

However this trend changes with higher volume of initial drug solution. Table 3.4, Sr. No. 5, shows with 500mL initial drug solution, maximum of 96.51% drug gets adsorbed. Result (Table 3.4, Sr. No. 1) also shows that 5mL methanol can recover 100% drug even at lower amount of the adsorbed drug, viz, up to 1.47 mg. The

conditions in Table 3.4, Sr. No. 4 were selected as optimized conditions for maximum adsorption and recovery with better pre – concentration factor for esomeprazole from aqueous solution.

The optimized conditions for maximum adsorption of drug and its recovery with better per – concentration factor for esomeprazole are: 250mL of initial aqueous drug solution passed through 1g PSDVB beads with flow rate 0.66mL min^{-1} followed by 5mL methanol used for recovery of drug adsorbed.

Accuracy of the Pre – concentration method

The optimized conditions were used to determine accuracy of the pre – concentration method by fortifying known amounts of esomeprazole to the synthesized aqueous solution at concentration range of 30 times less than LOQ level of HPLC method. Esomeprazole was fortified 30 times less than LOQ level of HPLC method to achieve the target concentration range in synthetic aqueous sample for the designed level of pre – concentration. Thus, 0.006mg L^{-1}, 0.014mg L^{-1}, 0.026mg L^{-1}, 0.053mg L^{-1}, 0.105mg L^{-1} and 0.211mg L^{-1} aqueous solutions of esomeprazole were pre – concentrated using the optimized conditions to achieve LOQ level of HPLC method.

Table 3.5. Study of accuracy of the pre - concentration method for esomeprazole: Synthetic aqueous solution

Sr. No.	Before		Recovered		PF
	Amount of drug spiked in solution	Conc. of drug solution	Amount of drug		
			Weight	Conc.	
	mg	mg L^{-1}	mg	mg L^{-1}	
1.	0.0015	0.006	0.00095	0.191	31.83
2.	0.0035	0.014	0.00211	0.422	30.14
3.	0.0065	0.026	0.00397	0.793	30.50
4.	0.0133	0.053	0.00832	1.664	31.40
5.	0.0263	0.105	0.01593	3.186	30.34
6.	0.0528	0.211	0.03526	6.512	30.86

Initial volume of drug solution – 250mL, PF – Pre – concentration Factor, Flow rate – 0.66mL min^{-1}, Amount of adsorbent – 1.0g, Type of solvent for recovered – Methanol, Volume of methanol – 5mL.

Thus as shown in Table 3.5, 0.006mg L^{-1}, 0.014 mg L^{-1}, 0.026mg L^{-1}, 0.053mg L^{-1}, 0.105mg L^{-1} and 0.211mg L^{-1} aqueous solutions of esomeprazole can be pre – concentrated to 0.191mg L^{-1}, 0.422mg L^{-1}, 0.793mg L^{-1}, 1.664mg L^{-1},

3.186mg L^{-1} and 6.512mg L^{-1} respectively with pre – concentration factor more than 30, confirming that the designed level of pre – concentration is achieved in the target concentration range in synthetic aqueous sample. Figure 3.13 shows the relation between concentration of esomeprazole before and after pre – concentration in synthetic water sample.

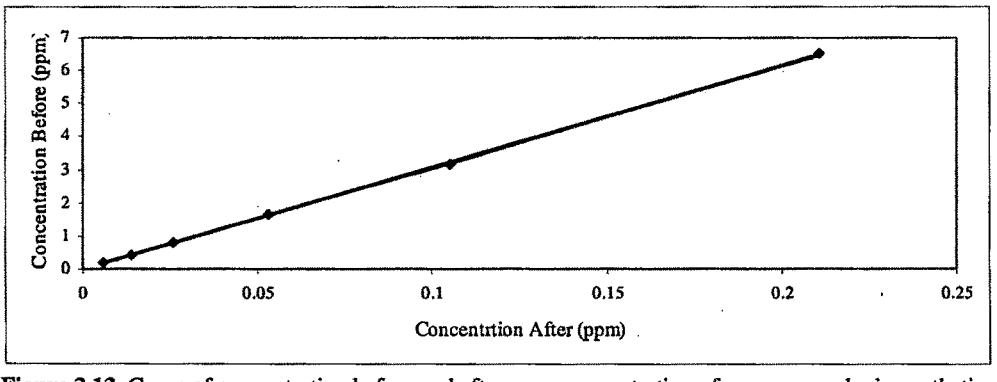

Figure 3.13. Curve of concentration before and after pre – concentration of esomeprazole in synthetic aqueous solution

The curve of concentration before pre – concentration verses after pre – concentration was linear in the range of 0.006 to 0.211mg L^{-1} with equation $y = 30.803x - 0.0025$ ($r^2 = 0.9999$).

Similarly, the developed optimized conditions were used to determine matrix effect by fortifying a known amount of esomeprazole to the environmental water sample (collected from STP and treated as described on Chapter 2 *Page No. 74.)* at concentration range of 30 times less than LOQ level of HPLC method.

Table 3.6. Study of accuracy of the pre - concentration method for esomeprazole: Environmental water sample

Sr. No.	Before		Recovered		PF
	Amount of drug spiked in solution	Conc. of drug solution	Amount of drug		
			Weight	Conc.	
	mg	mg L^{-1}	mg	mg L^{-1}	
1.	0.00175	0.007	0.00108	0.216	30.86
2.	0.00400	0.016	0.00245	0.491	30.69
3.	0.00750	0.030	0.00461	0.921	30.70
4.	0.01525	0.061	0.00929	1.857	30.44
5.	0.03000	0.120	0.01827	3.654	30.45
6.	0.06025	0.241	0.03640	7.283	30.22

Initial volume of drug solution – 250mL, PF – Pre – concentration Factor, Flow rate – 0.66mL min^{-1}, Amount of adsorbent – 1.0g, Type of solvent for recovered – Methanol, Volume of methanol – 5mL.

As shown in Table 3.6, 0.007mg L^{-1}, 0.016mg L^{-1}, 0.030mg L^{-1}, 0.061mg L^{-1}, 0.120mg L^{-1} and 0.241mg L^{-1} esomeprazole could be pre – concentrated to 0.216mg L^{-1}, 0.491mg L^{-1}, 0.921mg L^{-1}, 1.857mg L^{-1}, 3.654mg L^{-1} and 7.283mg L^{-1} respectively with pre – concentration factor more 30, confirming that the designed level of pre – concentration is achieved in the target concentration range in environmental water samples. Figure 3.14 shows the relation between concentration of esomeprazole before and after pre – concentration in environmental water sample.

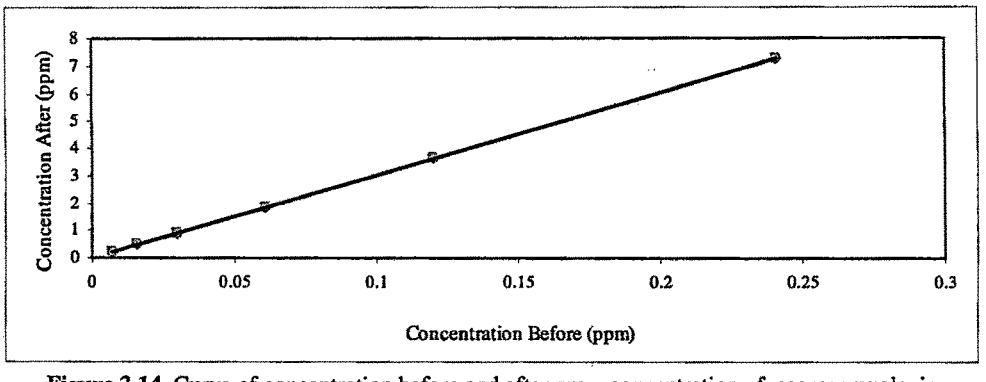

Figure 3.14. Curve of concentration before and after pre – concentration of esomeprazole in environmental water samples

The curve of concentrations before pre – concentration verses after pre – concentration was linear in the range of 0.007 to 0.241mg L^{-1} with equation $y = 30.204x + 0.0125$ ($r^2 = 1.0000$).

Developed optimized method for pre – concentration of esomeprazole was applied to environmental water sample collected from STP. Before pre – concentration the sample was also analysed by HPLC. In this case, no peaks were observed in the chromatogram for esomeprazole. The samples were spiked with a known amount of drug (1mg L^{-1}) and analyzed but the signal enhancement for 1mg L^{-1} added drug was not seen. Results indicate no presence of esomeprazole at these concentration levels in the sample collected from the STP which was confirmed separately by LC – MS method.

HPLC environmental water sample with PDA detector did not show any peaks corresponding to the three drugs when the sample was analysed without pre –

concentration. Also LC – MS for environmental water samples without pre – concentration did not show presence of these drugs.

Analytical performance characteristics

The validity of chromatographic procedure was established through a study of linearity, sensitivity, repeatability. Linearity was established with a series of working standard solutions prepared by diluting the stock solution with both water and methanol individually to the final concentrations. This was required because amount of the drug before and after pre – concentration was determined each time in aqueous solution whereas adsorbed drug was recovered in methanol and amount of drug determined in methanol. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve.

Esomeprazole in water

Linearity experiment in the range of 0.195 – 50mg L^{-1} was carried out. The peak area values with respective concentrations are shown in Table 3.7.

Table 3.7. Linearity experiment for esomeprazole in water: Concentration Vs Peak area

Observation No.	Concentration (mg L^{-1})	Peak Area
1.	0.195	11740.4
2.	0.391	18839
3.	0.781	38959.33
4.	1.563	70252.33
5.	3.125	137487
6.	6.25	273647.7
7.	12.5	545801.3
8.	25.0	1083160
9.	50.0	2165581

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 3.8. Plot of peak area Vs concentration for esomeprazole in water is Figure 3.15.

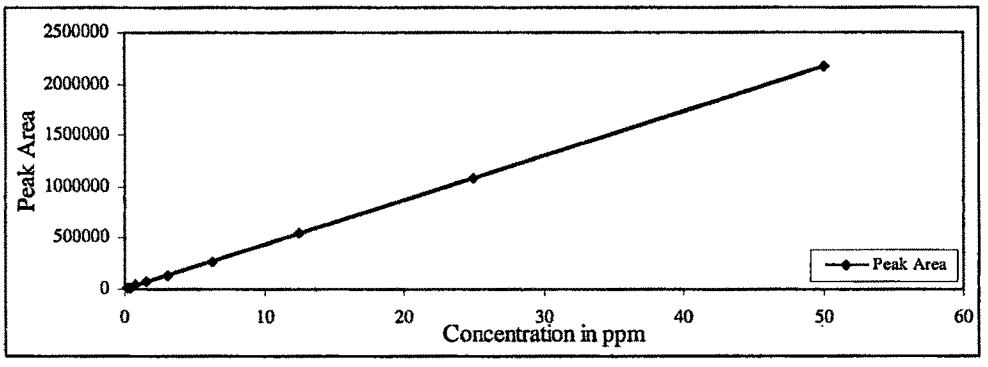

Figure 3.15. Linear working range of esomeprazole in water

Table 3.8. Results of regression analysis: esomeprazole in water

Parameters	Esomeprazole in water
Regression Equation (y)	
Correlation Coefficient (r^2)	1.0000
Slope, a	43243
Intercept	3294.5
No. of observations	9
Limit of Quantification (mg L^{-1})	0.19
Limit of Detection (mg L^{-1})	0.09

The calibration graphs is described by the following equation: $y = 43243x + 3294.5$ ($r^2 = 1.0000$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area.

Esomeprazole in Methanol

Linearity experiment in the concentration range of 0.195 – 50mg L^{-1} was carried out. The peak area values with respective concentrations are tabulated below:

Table 3.9. Linearity experiment for esomeprazole in methanol: Concentration Vs Peak area

Observation No.	Concentration (mg L^{-1})	Peak Area
1.	0.195	10990
2.	0.391	13307
3.	0.781	38882
4.	1.563	70341
5.	3.125	138734
6.	6.25	273133
7.	12.5	548125
8.	25.0	1081063
9.	50.0	2165523

The calibration data was subjected to regression analysis. The result of the regression analysis for calibration data is given in Table 3.10. Plot of peak area Vs concentration for esomeprazole in methanol is Figure 3.16.

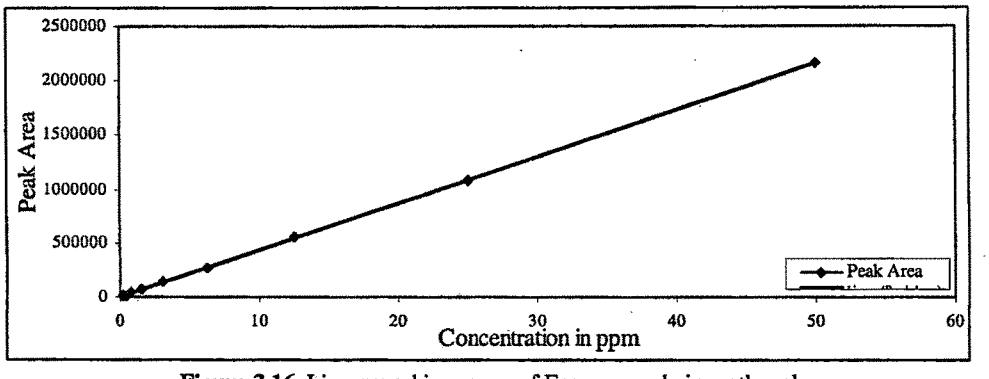

Figure 3.16. Linear working range of Esomeprazole in methanol

Table 3.10. Results of regression analysis: Esomeprazole in methanol

Parameters	Esomeprazole in methanol
Regression Equation (y)	
Correlation Coefficient (r^2)	1.0000
Slope, a	43257
Intercept	2539.5
No. of observations	9
Limit of Quantification (mg L^{-1})	0.19
Limit of Detection (mg L^{-1})	0.09

The calibration is described by the following equation: $y = 43257x + 2539.5$ ($R^2 = 1.0000$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area.

Acceptability of linearity data is judged by examining the coefficient of determination and the y – intercept as follows.

a. The plot of concentration Vs peak area for the linear working range is depicted in Table 3.15 for esomeprazole in water and in Table 3.17 for Esomeprazole in methanol. The plot shows that a linear relationship exists between concentration and peak area in the range of concentration 0.195 – 50mg L^{-1}.

b. The coefficient of determination i.e. 1.0000 for esomeprazole both in water and methanol means that 100% of variation in y i.e. the change in the response of the analyte can be explained by the change in x i.e. concentration of the analyte. The

correlation coefficient is a measure of goodness of the fit of the calculated line to the sample data.

c. The slope of the regression line is 43243 for esomeprazole in water, this indicates that one unit increase in the concentration of Esomeprazole in water will result in an increase in the peak area value by 43243 units. Similarly one unit increase in concentration of esomeprazole in methanol will result in an increase in the peak area value by 43257 units.

Pre – concentration studies of fenofibrate

Optimization of the initial parameters for pre – concentration was carried out. Initially effect of flow rate of aqueous fenofibrate solution on adsorption was studied. Table 3.11, shows that with increase in flow rate adsorption of drug on adsorbent decreases.

Table 3.11. Pre – concentration studies of fenofibrate: Effect of flow rate

Sr. No.	Flow rate	Before		Percentage of Drug adsorbed	Recovered				PF
		Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
	$mL\ min^{-1}$	mg	$mg\ L^{-1}$	%	mL	mg	$mg\ L^{-1}$	%	
1.	1.4	2.935	58.69	99.80	10	2.242	224.18	76.54	3.82
2.	0.66	2.935	58.69	97.04	10	2.171	217.07	76.23	3.70

Initial volume of drug solution – 50mL, PF – Pre – concentration factor, Type of solvent for recovered – Acetonitrile.

For subsequent experiments the 0.66mL min^{-1} flow rate was maintained, which resulted into a maximum drug adsorption of up to 99.80%.

With this percentage of adsorption, methanol was used for recovery. The recovery of adsorbed drug from the adsorbent was less than 50%. To get better recovery, acetonitrile was used as recovery solvent instead of methanol. The percentage of recovery was studied with two different volumes of acetonitrile.

Data in Table 3.11, Sr. No. 1 and 2, show that maximum drug recovery of up to 76.23% was observed with 10mL of acetonitrile resulting in pre – concentration factor of 3.82. With the decrease in volume of acetonitrile to 5mL for recovery the

percentage of drug recovered decreases to 57.60% though the pre – concentration factor increases to 5.75.

Table 3.12. Pre – concentration studies of fenofibrate: Effect of volume of solvent for recovery

Sr. No.	Before		Percentage of Drug adsorbed	Recovered				PF
	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
					Weight	Conc.	Percentage	
	mg	mg L^{-1}	%	mL	mg	mg L^{-1}	%	
1.	2.935	58.69	99.80	10	2.242	224.18	76.54	3.82
2.	2.935	58.69	99.80	5	1.687	337.36	57.60	5.75

PF – Pre – concentration Factor, Flow rate – 0.66mL min^{-1}, Type of solvent for recovered – Acetonitrile, Initial volume of drug solution – 50mL.

Considering this trend, the condition for recovery of drug adsorbed on 1.0g of adsorbent was optimized to 5mL of acetonitrile. With these optimized conditions for recovery, pre – concentration experiments were performed taking higher volumes of aqueous drug solutions keeping the amount of drug same.

Table 3.13. show that with increase in volume of initial aqueous drug solution, the percentage of amount of drug adsorbed decreases but after their recovery with 5mL acetonitrile pre – concentration factor for respective experiments increases.

Table 3.13. Pre – concentration studies of fenofibrate: Effect of initial volume of drug solution

Sr. No.	Before			After		Recovered			PF
	Initial Volume of drug solution	Amount of drug present in solution	Conc. of drug solution	Drug Adsorbed		Amount of drug			
				Percentage of Drug adsorbed	Amount of drug adsorbed	Weight	Conc.	Percentage	
	mL	mg	mg L^{-1}	%	mg	mg	mg L^{-1}	%	
1.	50	2.935	58.69	99.80	2.929	1.687	337.36	57.60	5.75
2.	100	2.541	25.41	97.40	2.475	2.012	402.48	88.94	15.84
3.	150	2.541	16.94	95.63	2.430	1.835	367.01	75.51	21.67
4.	250	2.541	10.16	89.18	2.266	1.264	252.81	55.78	24.88
5.	500	2.541	5.08	61.83	1.571	1.784	356.85	113.56	70.25

PF – Pre – Concentration factor, Flow rate – 0.66mL min^{-1}, Type of solvent for recovered – Acetonitrile, Volume of Acetonitrile – 5mL

Result (Table 3.13, Sr. No. 5) also shows that 5mL acetonitrile can recover almost 100% drug even at lower amount of the adsorbed drug, viz, up to 1.571mg. The conditions in Table 3.13, Sr. No. 4 were selected as optimized conditions for maximum adsorption and recovery with better pre – concentration factor for fenofibrate from aqueous solution.

The optimized conditions for maximum adsorption of drug and its recovery with better per – concentration factor for Fenofibrate are: 250mL of initial aqueous drug solution passed through 1.0g PSDVB beads with flow rate 0.66mL min^{-1}, followed by 5mL acetonitrile used for recovery of drug adsorbed.

Accuracy of the Pre – concentration method

The developed optimized conditions were used to determine accuracy of the pre – concentration method by fortifying known amounts of fenofibrate to the synthesized aqueous solution at concentration range of 20 times less than LOQ level of HPLC method. Thus, 0.046mg L^{-1}, 0.23 mg L^{-1}, 0.46 mg L^{-1} and 0.69 mg L^{-1} aqueous solution of fenofibrate were pre – concentrated using the optimized conditions to achieve LOQ level of HPLC method.

Table 3.14. Study of accuracy of the pre - concentration method for fenofibrate: Synthetic aqueous solution

Sr. No.	Before		Recovered		PF
	Amount of drug present in solution	Conc. of drug solution	Amount of drug		
			Weight	Conc.	
	mg	mg L^{-1}	mg	mg L^{-1}	
1.	0.0115	0.046	0.005	0.99	21.52
2.	0.0575	0.230	0.027	5.49	23.87
3.	0.1150	0.460	0.051	10.19	22.15
4.	0.1725	0.690	0.070	14.03	20.33

Initial volume of drug solution – 250mL, PF – Pre – concentration Factor, Flow rate – 0.66mL min^{-1}, Amount of adsorbent – 1.0 g, Type of solvent for recovered – Acetonitrile, Volume of acetonitrile – 5mL.

Table 3.14, shows that 0.046mg L^{-1}, 0.230mg L^{-1}, 0.460mg L^{-1} and 0.690mg L^{-1} aqueous solutions of Fenofibrate can be pre – concentrated to 0.99mg L^{-1}, 5.49mg L^{-1}, 10.19mg L^{-1} and 14.03mg L^{-1} respectively with pre – concentration factor more than 20, confirming that the designed level of pre – concentration is achieved in the target concentration range in synthetic aqueous sample. Figure 3.17 shows the relation between concentration of fenofibrate before and after pre – concentration in synthetic aqueous sample.

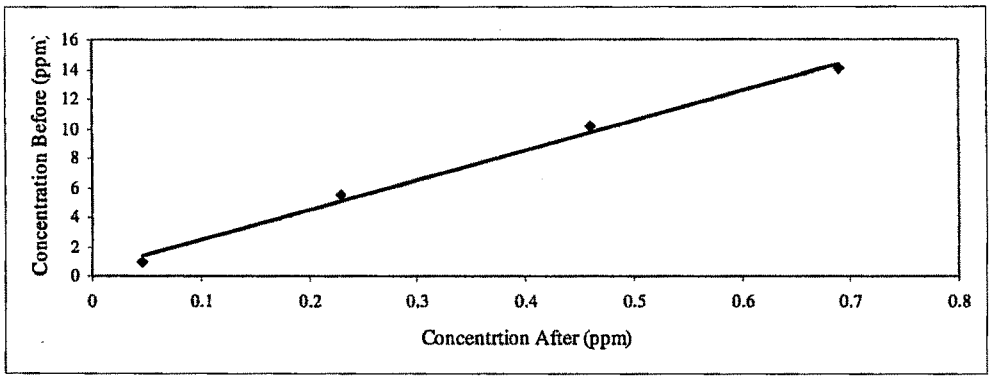

Figure 3.17. Curve of concentration before and after pre – concentration of fenofibrate in synthetic aqueous solution

The curve of concentration before pre – concentration verses after pre – concentration was linear in the range of 0.046mg L^{-1}, 0.23mg L^{-1}, 0.46mg L^{-1} and 0.69mg L^{-1} with equation $y = 20.191x + 0.4768$ ($r^2 = 0.9934$).

Similarly, the developed optimized conditions were used to determine matrix effect by fortifying a known amount of fenofibrate to the environmental water sample at concentration range of 20 times less than LOQ level of HPLC method.

Thus, 0.058mg L^{-1}, 0.29mg L^{-1}, 0.58mg L^{-1} and 0.87mg L^{-1} aqueous solution of fenofibrate were pre – concentrated using the optimized conditions to achieve LOQ level of HPLC method.

Table 3.15. Study of accuracy of the pre - concentration method for fenofibrate: Environmental water samples

Sr. No.	Before		Recovered		PF
	Amount of drug present in solution	Conc. of drug solution	Amount of drug		
			Weight	Conc.	
	mg	mg L^{-1}	mg	mg L^{-1}	
1.	0.0145	0.058	0.007	1.43	24.65
2.	0.0725	0.290	0.028	5.67	19.55
3.	0.1450	0.580	0.059	11.89	20.5
4.	0.2175	0.870	0.086	17.28	19.86

Initial volume of drug solution – 250mL, PF – Pre – concentration Factor, Flow rate – 0.66mL min^{-1}, Amount of adsorbent – 1.0g, Type of solvent for recovered – Acetonitrile, Volume of acetonitrile – 5mL.

Table 3.15 shows 0.058mg L^{-1}, 0.290mg L^{-1}, 0.580mg L^{-1} and 0.870mg L^{-1} aqueous solution fenofibrate can be pre – concentrated to 1.43mg L^{-1}, 5.67mg L^{-1}, 11.89mg L^{-1} and 17.28mg L^{-1} respectively with pre – concentrated factor more than

20. Figure 3.18 shows the relation between concentration of fenofibrate before and after pre – concentration in environmental water sample.

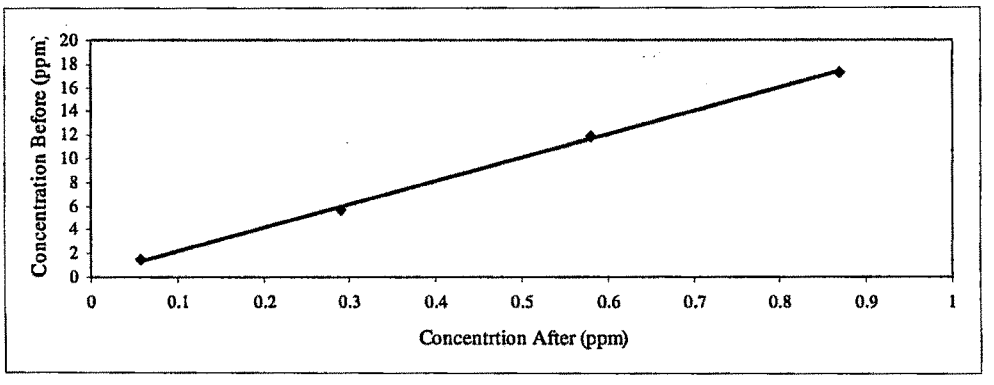

Figure 3.18. Curve of concentration before and after pre – concentration of fenofibrate in environmental water samples

The curve of concentration before pre – concentration verses after pre – concentration was linear in the range of 0.058mg L^{-1}, 0.29mg L^{-1}, 0.58mg L^{-1} and 0.87mg L^{-1} with equation $y = 19.738x + 0.1952$ ($r^2 = 0.999$).

Developed optimized method for pre – concentration of fenofibrate was applied to environmental water sample collected from STP. Before and after pre – concentration samples were analysed by HPLC. No peaks were observed in the chromatogram for fenofibrate in both cases. The samples were spiked with a known amount of drug (1mg L^{-1}) and analyzed but the signal enhancement was not seen. Results indicate no presence of fenofibrate in the sample collected from the STP which was confirmed by a LC – MS method.

Analytical performance characteristics

The validity of chromatographic procedure was established through a study of linearity, sensitivity and repeatability. Linearity was established with a series of working standard solutions prepared by diluting the stock solution with both milli – Q water: acetonitrile (60:40) (v/v) and acetonitrile individually to the final concentrations. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve.

Fenofibrate in water: acetonitrile (60:40)

Linearity experiment in the range of 0.061 – 62.5mg L^{-1} was carried out. The peak area values with respective concentrations are tabulated in Table 3.16.

Table 3.16. Linearity experiment for fenofibrate in water: acetonitrile (60: 40): Concentration Vs Peak area

Observation No.	Concentration (mg L^{-1})	Peak Area
1.	0.061	4930.667
2.	0.122	8717
3.	0.244	15297.33
4.	0.488	29614.33
5.	0.977	49649.33
6.	1.953	93729.67
7.	3.906	182813
8.	7.813	339577.7
9.	15.63	671469
10.	31.25	1293026
11.	62.5	2449331

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 3.17. Plot of peak area Vs concentration for fenofibrate in water: acetonitrile (60:40) is in Figure 3.19.

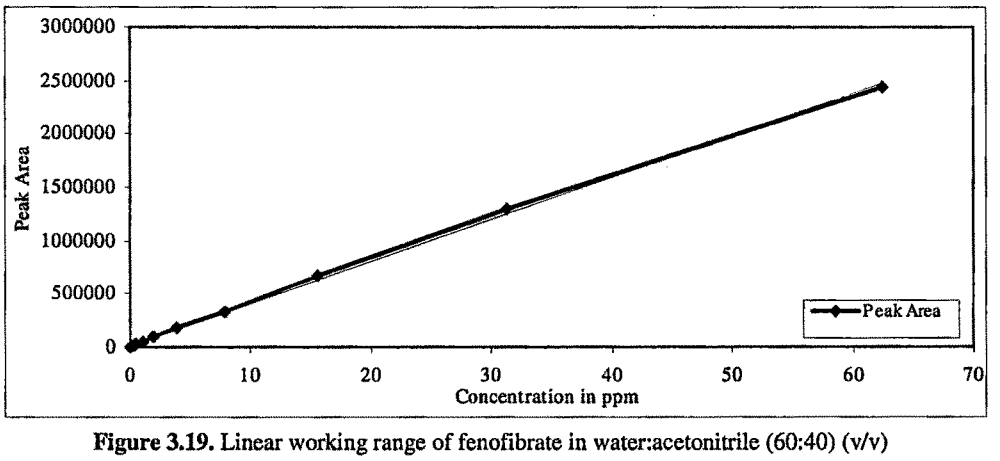

Figure 3.19. Linear working range of fenofibrate in water:acetonitrile (60:40) (v/v)

Table 3.17. Results of regression analysis : fenofibrate in water : acetonitrile (60 : 40)

Parameters	Fenofibrate in water : acetonitrile (60:40)
Regression Equation (y)	
Correlation Coefficient (r^2)	0.999
Slope, a	39385
Intercept	19766
No. of observations	11
Limit of Quantification (mg L^{-1})	0.488
Limit of Detection (mg L^{-1})	0.06

The calibration graphs is described by the following equation: $y = 39385x + 19766$ ($r^2 = 0.9991$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area.

Fenofibrate in acetonitrile

Linearity experiment in the range of 0.061 – 125mg L^{-1} was conducted. The peak area values with respective concentrations are shown in Table 3.18.

Table 3.18. Linearity experiment for fenofibrate in acetonitrile: Concentration Vs Peak area

Observation No.	Concentration (mg L^{-1})	Peak Area
1.	0.061	2670.667
2.	0.122	5703.667
3.	0.244	11245
4.	0.488	20970.67
5.	0.977	42492
6.	1.953	83683
7.	3.906	165787
8.	7.813	321934.3
9.	15.63	617220
10.	31.25	1193726
11.	62.5	2400815
12.	125.0	4953004

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 3.19 Plot of peak area Vs concentration for fenofibrate in acetonitrile is in Figure 3.20.

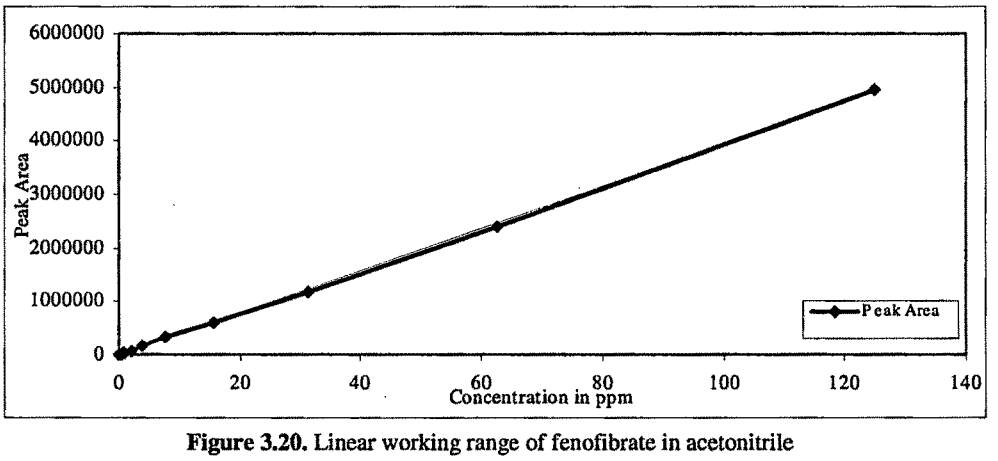

Figure 3.20. Linear working range of fenofibrate in acetonitrile

Table 3.19. Results of regression analysis: Fenofibrate in acetonitrile

Parameters	Fenofibrate in acetonitrile
Regression Equation (y)	
Correlation Coefficient (r^2)	0.999
Slope, a	39353
Intercept	- 1379
No. of observations	12
Limit of Quantification (mg L^{-1})	0.488
Limit of Detection (mg L^{-1})	0.06

The calibration graphs is described by the following equation $y = 39353x - 1379.7$ ($r^2 = 0.9997$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area.

Acceptability of linearity data is judged by examining the coefficient of determination and the y – intercept as follows.

(a) The plot of concentration verses peak area for the linear working range is depicted in Table 3.16 for fenofibrate in water: acetonitrile (60:40) (v/v) and in Table 3.18 for fenofibrate in acetonitrile. The plot shows that a linear relationship exists between concentration and peak area in the range of concentration 0.061 – 62.5mg L^{-1} for fenofibrate in water: acetonitrile (60:40) (v/v) and 0.061 – 125mg L^{-1} for fenofibrate in acetonitrile.

(b) The coefficient of determination i.e. 0.999 for fenofibrate both in water: acetonitrile (60:40) (v/v) and acetonitrile means that 99.9% of variation in y i.e. the change in the response of the analyte can be explained by the change in x i.e. concentration of the analyte. The correlation coefficient is a measure of goodness of the fit of the calculated line to the sample data.

(c) The slope of the regression line is 39385 for fenofibrate in water: acetonitrile (60:40) (v/v), this indicates that one unit increase in the concentration of fenofibrate in water: acetonitrile (60:40) (v/v) will result in an increase in the peak area value by 39385 units. Similarly one unit increase in concentration of fenofibrate in acetonitrile will result in an increase in the peak area value by 39353 units.

Pre – concentration studies of venlafaxine HCl

Optimization of initial parameters for pre – concentration was carried out. Effect of changing volume of aqueous solution containing the drug, effect of changing amount of adsorbent while keeping volume and concentration of drug solution constant was studied and optimized in present work for venlafaxine HCl.

Initially effect of flow rate of aqueous venlafaxine HCl solution on adsorption was studied. Table 3.20, show that with increase in flow rate adsorption of drug on adsorbent decreases.

Table 3.20. Pre – concentration studies of venlafaxine HCl: Effect of flow rate

Sr. No.	Flow rate	Before		Percentage of Drug adsorbed	Recovered				PF
		Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
						Weight	Conc.	Percentage	
	$mL\ min^{-1}$	mg	$mg\ L^{-1}$	%	mL	mg	$mg\ L^{-1}$	%	
1.	1.4	2.498	49.95	50.71	10	1.266	126.6	99.96	2.53
2.	0.66	2.498	49.95	51.91	10	1.296	129.6	99.92	2.59

Initial drug solution – 50mL, PF – Pre – concentration factor, Type of solvent for recovered – Methanol.

For subsequent experiments the $0.66mL\ min^{-1}$ flow rate was maintained, which resulted into a maximum drug adsorption of up to 51.91%.

With this percentage of adsorption, methanol was used for recovery. The percentage of recovery was studied with four different volumes of methanol. Data in Table 3.21, show that maximum drug recovery of up to 99.92% was observed with 10mL of methanol resulting in pre – concentration factor of 2.59.

Table 3.21. Pre – concentration studies of venlafaxine HCl: Effect of volume of solvent for recovery

Sr. No.	Before		Percentage of Drug adsorbed	Recovered				PF
	Amount of drug present in solution	Conc. of drug solution		Volume of solvent	Amount of drug			
					Weight	Conc.	Percentage	
	mg	$mg\ L^{-1}$	%	mL	mg	$mg\ L^{-1}$	%	
1.	49.95	2.498	51.91	10	1.296	129.6	99.92	2.59
2.	49.95	2.498	51.91	5	1.293	184.65	99.73	3.70
3.	49.95	2.498	51.93	7	1.146	229.21	88.36	4.59
4.	49.95	2.498	51.75	3	0.798	265.95	61.74	5.32

Initial volume of drug solution – 50mL, PF – Pre – concentration Factor, Type of solvent for recovered – Methanol, Flow rate – $0.66mL\ min^{-1}$.

With the decrease in volume of methanol for recovery the percentage of drug recovered decreases though the pre – concentration factor increases respectively. Considering this trend, the condition for recovery of drug adsorbed on 1g of adsorbent was optimized to 5mL of methanol. With 5mL methanol 88.36% drug is recovered with pre – concentration factor 4.59. With these optimized conditions for recovery, pre – concentration experiments were performed taking higher volumes of aqueous drug solutions keeping the amount of drug same.

Table 3.22, shows that with increase in volume of initial aqueous drug solution, the percentage of amount of drug adsorbed decreases but after their recovery with 5mL methanol pre – concentration factor for respective experiments increases.

Table 3.22. Pre – concentration studies of venlafaxine HCl: Effect of volume of initial drug solution

Sr. No.	Before			After		Recovered			PF
	Initial Volume of drug solution	Amount of drug present in solution	Conc. of drug solution	Drug Adsorbed		Amount of drug			
				Percentage of Drug adsorbed	Amount of drug adsorbed	Weight	Conc.	Percentage	
	mL	mg	mg L^{-1}	%	mg	mg	mg L^{-1}	%	
1.	50	49.95	2.498	51.93	1.297	1.146	229.21	88.36	4.59
2.	100	39.83	3.983	41.15	1.639	1.139	227.84	69.51	5.72
3.	150	26.55	3.983	31.16	1.241	0.964	192.74	77.66	7.26
4.	250	15.93	3.983	20.60	0.821	0.821	164.2	100.0	10.31
5.	500	7.97	3.983	18.40	0.733	0.723	144.85	98.62	18.1

PF – Pre – concentration Factor, Flow rate – 0.66mL min^{-1}, Type of solvent for recovered – Methanol, Volume of methanol – 5mL

Table 3.22, shows that with 50mL initial drug solution, maximum of 51.93% drug gets adsorbed. Result also shows that 5mL methanol can recover 100% drug at lower amount of the adsorbed drug, viz, 0.821mg. The conditions in Table 3.22, Sr. No. 1 were selected as optimized conditions for maximum adsorption and recovery with better pre – concentration factor for venlafaxine HCl from aqueous solution.

The optimized conditions for maximum adsorption of drug and its recovery with better per – concentration factor for venlafaxine HCl are: 50mL of initial aqueous drug solution passed through 1.0g PSDVB beads with flow rate 0.66mL min^{-1}, followed by 5mL acetonitrile used for recovery of drug adsorbed.

Accuracy of the Pre – concentration Method

The developed optimized conditions were used to determine accuracy of the pre – concentration method by fortifying known amounts of venlafaxine HCl to the synthesized aqueous solution at concentration range of 10 times less than LOQ level of HPLC method. Thus, 0.03mg L^{-1}, 0.06mg L^{-1}, 0.12mg L^{-1}, 0.24mg L^{-1}, 0.47mg L^{-1} and 0.95mg L^{-1} aqueous solution of venlafaxine HCl were pre – concentrated using the optimized conditions to achieve LOQ level of HPLC method.

Table 3.22. Study of accuracy of the pre - concentration method for venlafaxine HCl: Synthetic aqueous solution

Sr. No.	Before		Recovered		PF
	Amount of drug present in solution	Conc. of drug solution	Amount of drug		
			Weight	Conc.	
	mg	mg L^{-1}	mg	mg L^{-1}	
1.	0.0015	0.03	0.002	0.31	10.33
2.	0.0030	0.06	0.003	0.61	10.17
3.	0.0060	0.12	0.006	1.23	10.25
4.	0.0120	0.24	0.012	2.37	9.88
5.	0.0235	0.47	0.023	4.58	9.74
6.	0.0475	0.95	0.046	9.26	9.75

Initial volume of drug solution – 50mL, PF – Pre – concentration Factor, Flow rate – 0.66mg L^{-1}, Amount of adsorbent – 1.0g, Type of solvent for recovered – Methanol, Volume of methanol – 5mL.

Table 3.23, shows 0.03mg L^{-1}, 0.06mg L^{-1}, 0.12mg L^{-1}, 0.24mg L^{-1}, 0.47mg L^{-1} and 0.95mg L^{-1} aqueous solution of venlafaxine HCl can be pre – concentrated to 0.31mg L^{-1}, 0.61mg L^{-1}, 1.23mg L^{-1}, 2.37mg L^{-1}, 4.58mg L^{-1} and 9.26mg L^{-1} respectively with pre – concentration factor more than 10, confirming that the designed level of pre – concentration is achieved in the target concentration range in synthetic aqueous sample. Figure 3.21 shows the relation between concentration of venlafaxine HCl before and after pre – concentration in synthetic aqueous sample.

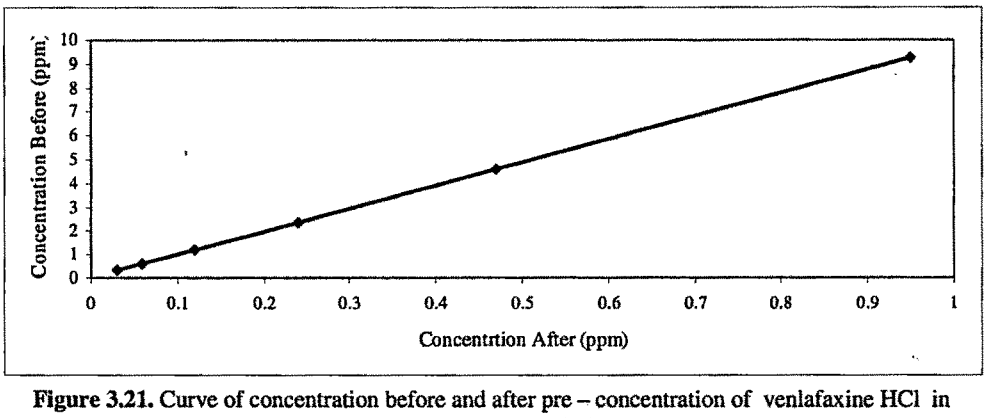

Figure 3.21. Curve of concentration before and after pre – concentration of venlafaxine HCl in synthetic aqueous solution

The curve of concentration before pre – concentration verses after pre – concentration was linear in the range of 0.03 to 0.95mg L^{-1} with equation $y = 9.7065x + 0.0348$ ($r^2 = 1.0000$).

Similarly, the developed optimized conditions were used to determine matrix effect by fortifying a known amount of venlafaxine HCl to the environmental water sample at concentration range of 10 times less than LOQ level of HPLC method.

Thus, 00.03mg L^{-1}, 0.06mg L^{-1}, 0.12mg L^{-1}, 0.24mg L^{-1}, 0.47mg L^{-1} and 0.95mg L^{-1} environmental water samples of venlafaxine HCl were pre – concentrated using the optimized conditions to achieve LOQ level of HPLC method.

Table 3.24. Study of accuracy of the pre - concentration method for venlafaxine HCl: Environmental water sample

Sr. No.	Before		Recovered		PF
	Amount of drug present in solution	Conc. of drug solution	Amount of drug		
			Weight	Conc.	
	mg	mg L^{-1}	mg	mg L^{-1}	
1.	0.0015	0.03	0.002	0.31	10.33
2.	0.0030	0.06	0.003	0.6	10.0
3.	0.0060	0.12	0.006	1.21	10.08
4.	0.0120	0.24	0.012	2.3	9.58
5.	0.0235	0.47	0.022	4.49	9.55
6.	0.0475	0.95	0.046	9.1	9.58

Initial volume of drug solution – 50mL, PF – Pre – concentration Factor, Flow rate – 0.66mL min^{-1}, Amount of adsorbent – 1.0g, Type of solvent for recovered – Methanol, Volume of methanol – 5 mL.

Table 3.24 shows 0.03mg L^{-1}, 0.06mg L^{-1}, 0.12mg L^{-1}, 0.24mg L^{-1}, 0.47mg L^{-1} and 0.95mg L^{-1} environmental water samples of venlafaxine HCl can be pre – concentrated to 0.31mg L^{-1}, 0.6mg L^{-1}, 1.21mg L^{-1}, 2.3mg L^{-1}, 4.49mg L^{-1} and 9.1mg L^{-1} respectively with pre – concentration factor more than 10. Figure 3.22 shows the relation between concentration of venlafaxine HCl before and after pre – concentration in environmental water sample.

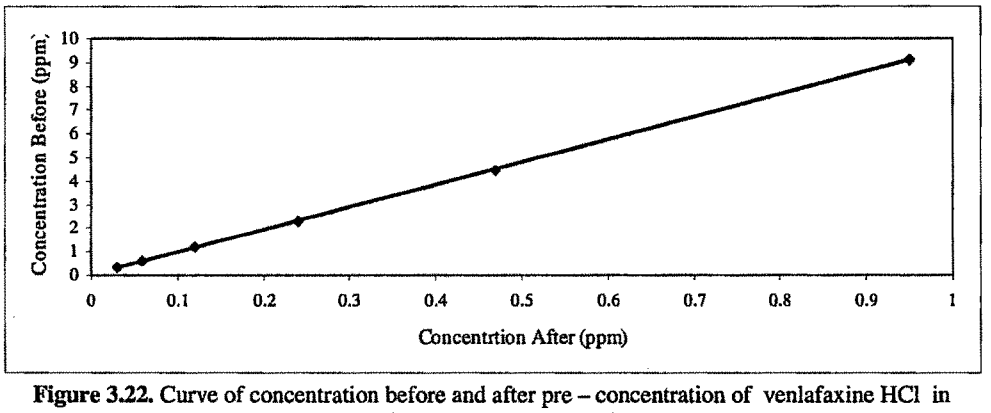

Figure 3.22. Curve of concentration before and after pre – concentration of venlafaxine HCl in environmental water sample

The curve of concentration before pre – concentration verses after pre – concentration was linear in the range of 0.03 to 0.95mg L^{-1} with equation $y = 9.5363x + 0.0295$ ($r^2 = 1.0000$).

Developed optimized method for pre – concentration of venlafaxine HCl was applied to environmental water sample collected from STP. Before and after pre – concentration samples were analysed by HPLC. No peaks were observed in the chromatogram for venlafaxine HCl in both cases. The samples were spiked with a known amount of drug (1mg L^{-1}) and analyzed but the signal enhancement was not seen. Results indicate no presence of venlafaxine HCl in the sample collected from the STP which was confirmed by a LC – MS method. Details of results for LC – MS experiment for venlafaxine HCl along with other drugs are being processed as separate paper.

Analytical performance characteristics

The validity of chromatographic procedure was established through a study of linearity, sensitivity, repeatability. Linearity was established with a series of working standard solutions prepared by diluting the stock solution with both water and methanol individually to the final concentrations. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve.

Venlafaxine HCl in water

Linearity experiment in the range of 0.244 – 500mg L^{-1} was carried out. The peak area values with respective concentrations are tabulated in Table 3.25.

Table 3.25. Linearity experiment for venlafaxine HCl in water: Concentration Vs Peak area

Observation No.	Concentration (mg L^{-1})	Peak Area
1.	0.244	5374
2.	0.488	10849
3.	0.977	21890
4.	1.953	43797.7883
5.	3.906	87594.977
6.	7.813	175290
7.	15.63	350379.91
8.	31.25	701759
9.	62.5	1402520
10.	125	2803139
11.	250.0	5605076
12.	500.0	11212157

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 3.26. Plot of peak area Vs concentration for venlafaxine HCl in water is in Figure 3.23.

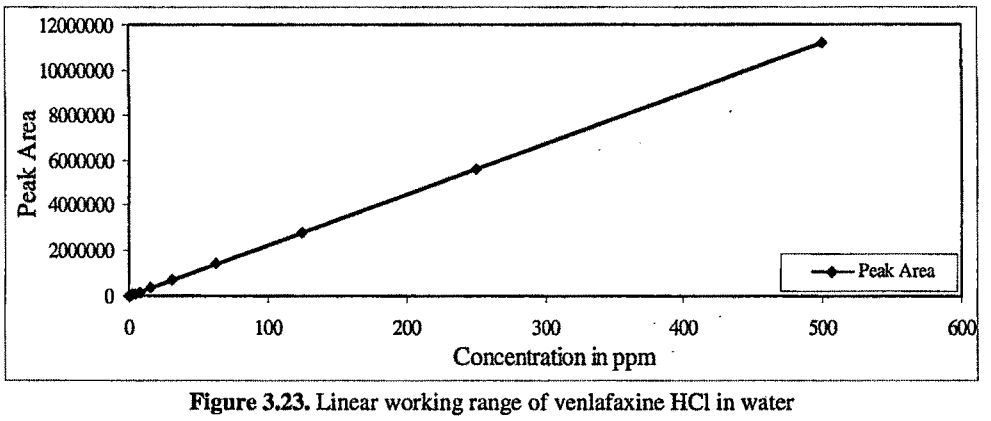

Figure 3.23. Linear working range of venlafaxine HCl in water

Table 3.26. Results of regression analysis : Venlafaxine HCl in water

Parameters	Venlafaxine HCl in water
Regression Equation (y)	
Correlation Coefficient (r^2)	1.0000
Slope, a	22423
Intercept	157.57
No. of observations	12
Limit of Quantification (mg L^{-1})	0.488
Limit of Detection (mg L^{-1})	0.06

The calibration graphs is described by the following equation: $y = 22423x + 157.57$ ($r^2 = 1.0000$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area.

Venlafaxine HCl in Methanol

Linearity experiment in the range of 0.244 – 500mg L^{-1} was carried out. The peak area values with respective concentrations are tabulated in Table 3.27.

Table 3.27. Linearity experiment for venlafaxine HCl in methanol: Concentration Vs Peak area

Observation No.	Concentration (mg L^{-1})	Peak Area
1.	0.244	5844.698
2.	0.488	11689.4
3.	0.977	24378
4.	1.953	47757
5.	3.906	94515.17
6.	7.813	187430.3
7.	15.63	374260
8.	31.25	748521
9.	62.5	1496243
10.	125.0	2982486
11.	250.0	5984971

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 3.28. Plot of peak area Vs concentration for venlafaxine HCl in methanol is in Figure 3.24.

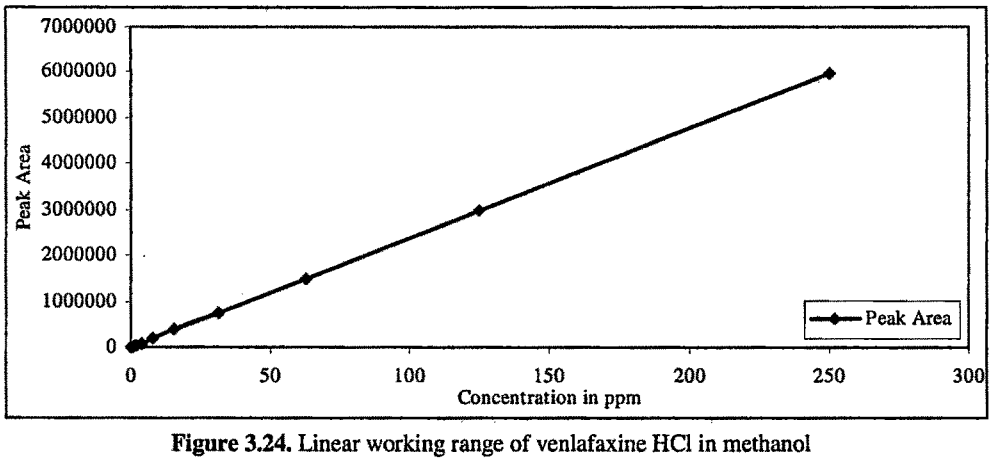

Figure 3.24. Linear working range of venlafaxine HCl in methanol

Table 3.28. Results of regression analysis: Venlafaxine HCl in methanol

Parameters	Venlafaxine HCl in methanol
Regression Equation (y)	
Correlation Coefficient (r^2)	1.0000
Slope, a	23924
Intercept,	167.52
No. of observations	12
Limit of Quantification (mg L^{-1})	0.488
Limit of Detection (mg L^{-1})	0.06

The calibration graphs is described by the following equation: $y = 23924 x + 167.52$ ($r^2 = 1.0000$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area.

Acceptability of linearity data is judged by examining the coefficient of determination and the y – intercept as follows.

(a) The plot of concentration Vs peak area for the linear working range is depicted in Table 3.25 for venlafaxine HCl in water and in Table 3.27 for venlafaxine HCl in methanol. The plot shows that a linear relationship exists between concentration and peak area in the range of concentration 0.244 – 500mg L^{-1} for venlafaxine HCl in water and 0.244 – 250mg L^{-1} for venlafaxine HCl in methanol.

(b) The coefficient of determination i.e. 1.0000 for venlafaxine HCl both in water and methanol means that 100% of variation in y i.e. the change in the response of the analyte can be explained by the change in x i.e. concentration of the analyte. The correlation coefficient is a measure of goodness of the fit of the calculated line to the sample data.

(c) The slope of the regression line is 22423 for venlafaxine HCl in water; this indicates that one unit increase in the concentration of venlafaxine HCl in water will result in an increase in the peak area value by 22423 units. Similarly one unit increase in concentration of venlafaxine HCl in methanol will result in an increase in the peak area value by 23924units.

CONCLUSION

Pre – concentration of Esomeprazole Magnesium

The method developed for pre – concentration of aqueous solutions containing esomeprazole using a HPLC method for quantification, is accurate, sensitive and reliable and enables the determination of the target drug in water sample at 0.0044mg L^{-1}. In simple laboratory conditions aqueous solution of Esomeprazole can be pre-concentrated by a factor of 30 by using, commercially available macro – porous polymer of PSDVB with 8% cross – linking.

The water sample collected from STP (Vadodara- India) after treatment does not show presence of Esomeprazole up to the detection level of 0.002mg L^{-1} considering the pre – concentration factor in optimized conditions. This means

concentration of this drug is below this level or STP is efficient in removing the drug effectively.

Pre – concentration of Fenofibrate

The new method developed for pre – concentration followed by quantitation using HPLC for aqueous solutions containing fenofibrate is accurate, sensitive and reliable and enables the determination of the target drug in water sample at 0.046mg L^{-1}. In simple laboratory conditions aqueous solution of fenofibrate can be pre-concentrated by a factor of 20.00 by using commercially available macro – porous polymer PSDVB with 8% cross – linking at lower concentration in the range of 0.046mg L^{-1} to 0.69mg L^{-1}.

The water sample collected from STP (Vadodara- India) after treatment does not show presence of fenofibrate up to the detection level of 0.046mg L^{-1} considering the pre – concentration factor in optimized conditions. This means concentration of this drug is below this level or STP is efficient in removing the drug effectively.

Pre – concentration of Venlafaxine HCl

The method new developed for pre – concentration followed by quantitation using HPLC for aqueous solutions containing venlafaxine HCl is accurate, sensitive and reliable and enables the determination of the target drug in water sample at 0.024mg L^{-1}. In simple laboratory conditions aqueous solution of venlafaxine HCl can be pre – concentrated by a factor of 10 by using commercially available macro – porous polymer PSDVB with 8% cross – linking.

The water samples collected from STP (Vadodara – India) after treatment does not show presence of venlafaxine HCl up to the detection level of 0.003mg L^{-1} considering the pre – concentration factor in optimized conditions. This means concentration of this drug is below this level or STP is efficient in removing the drug effectively.

CHAPTER 4

SIMULTANEOUS DETERMINATION AND VALIDATION OF HPLC METHOD FOR ESOMEPRAZOL MAGNESIUM, FENOFIBRATE AND VENLAFAXINE HCl

VALIDATION OF AN ANALYTICAL METHOD

The principal purpose of analytical method validation is to ensure that a selected analytical procedure will give reproducible and reliable results that are adequate for the intended purpose. It is thus necessary to define properly, both the conditions in which the procedure is to be used and the purpose for which it is intended.

CHARACTERISTICS OF ANALYTICAL PROCEDURES

Important characteristics that need to be specific for analytical procedures are listed below and defined, with an indication of how they may be determined. Not all the characteristics are applicable to every test procedure or to every material. Much depends on the purpose for which the procedure is required.

Accuracy

The accuracy of the procedure is the closeness of the results obtained by the procedure to the true value. Accuracy may be determined by applying the procedure to samples of the material to be examined that have been prepared with quantitative accuracy. Wherever possible, these samples should contain all the components of the material including the analyte. Samples in which the analyte has been incorporated in quantities some 10% above the expected range of the values should be prepared. Accuracy may also be determined by comparing the results with those obtained using an alternative procedure that has already been validated.

Precision

The precision of the procedure is the degree of agreement among individual test results. It is measured by scatter of individual results from the mean and it is usually expressed as the standard deviation or as the coefficient of variation (relative standard deviation) when the complete procedure is applied repeatedly to separate, identical samples drawn from the same homogenous batch of material.

Repeatability

This is the precision of the procedure when repeated by an analyst under the same set of conditions (same reagents, equipments, settings, and laboratory) and within a short interval of time. The repeatability of a procedure is assessed by carrying out complete separate determinations on separate identical samples of the same homogenous batch of material and thus provides a measure of the precision of the procedure under normal operating conditions.

Reproducibility

This is the precision of the procedure when it is carried out under different conditions usually in different laboratories, on separate, identical samples taken form the same homogenous batch of material. Comparisons of results obtained by different analyst, by the use of different equipment, or by carrying out the analysis at different times can also provide valuable information.

Linear Dynamic Range

The detector response is said to be linear if the difference in response for two concentrations of a given compound is proportional to the difference in concentration of the two samples. Such response appears as a straight line in the calibration curve. The linear dynamic range is that concentration over which the detector output is linearly related to the solute concentration. The linear dynamic range extends from the minimum detectable to that concentration where the response index is greater or less than the defined linearity limits.

Selectivity

The selectivity of a procedure is its ability to measure an analyte in a manner that is free from interference from other components in the sample being examined. Selectively may be expressed in terms of the bias of the assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other components.

Limit of detection (LOD)

The limit of detection is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. Such a limit is usually expressed in terms of concentration of analyte in the sample. Where the final measurement is based on an instrumental reading due account will be needed to be taken of the background response (the signal – to – noise characteristics of the response observed). In several cases, visual inspections of the results is also used for determining LOD.

Limit of quantification (LOQ)

The limit of quantification is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. It is measured by analysing samples containing diminishing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable. Where the final assessment is based on an instrumental reading the magnitude of background response (the signal – to – noise ratio) may be needed to be assessed and taken into account. In many cases the limit of quantification is approximately twice the limit of detection.

METHOD DEVELOPMENT FOR DETERMINATION OF ESOMEPRAZOLE MAGNESIUM, VENLAFAXINE HCl AND FENOFIBRATE IN MIXUTRE

According to the information collected from literature there is no reported method for simultaneous determination of esomeprazole, venlafaxine HCl and fenofibrate using HPLC which can be applied for detection of these drugs present in water at low concentrations. In the present work we report development and validation of a new HPLC method for simultaneous determination of esomeprazole, venlafaxine HCl and fenofibrate in a synthetic mixture. For recovery studies, treated sewage water collected from a Sewage Treatment Plant (STP), Vadodara, India was used. The new method is simple and sensitive HPLC method with total run time less than twenty minutes for the simultaneous determination of esomeprazole, venlafaxine

HCl and fenofibrate. The method has been validated and can be applied to quality control and for other analytical purposes.

EXPERIMENTS

Materials and Reagents

Same as mentioned in Chapter 3 except A.R grade formic acid and ammonium acetate were purchased from Qualigens and used as such.

Instrumentation

For HPLC *(Validation method)*

The LC system used was a Shimadzu LC 2010 C_{HT} series 200 binary pump equipped with auto sampler and UV detector. The output signal was monitored and processed using Empower software.

For MS *(Identification of API)*

Water – Micro Mass Quattro Detectors.

For LC – MS *(Identification of the target drugs environmental water sample)*

Water Alliance 2695 with PDA (996) Detector. Waters Micro Mass ZQ Mass Detector.

Conditions

For HPLC *(Validation method)*

Separation was carried out on a C18 column (150cm x 4.6mm, 3.5µm particle size), from Agilent. Mobile phase **A** contained a mixture of buffer and acetonitrile in the ratio 75:25 (*v/v*). Mobile phase **B** consisted of buffer and acetonitrile in the ratio of 30:70 (*v/v*). The buffer consists of 0.3% formic acid. The mobile phase was premixed, filtered through a 0.45µm nylon filter and degassed. The flow rate was kept at 1.1mL min^{-1} throughout. The LC gradient was time (min) / mobile phase: 0.00 / **A**,

6.01 / **B** and 15.01 / **A**. The detection was monitored at 230nm. The injection volume was 10µL.

For MS *(Identification of API)*

Mass range: 110 – 1000amu. Mode: Direct Injection with Electro Spray Ionisation (+ve ion mode). Diluent: Water: Acetonitrile (30:70).

For LC – MS *(Identification of environmental water sample)*

BDS Hypersil C8 column (250 x 4.6mm, 5µ particle size) using a mixture of acetonitrile: buffer (0.13% formic acid, 15.50% 0.1 mol L^{-1} ammonium acetate) in the ratio 25:75 (v/v) (pH 3.8) as mobile phase **A** and acetonitrile as mobile phase **B** with flow rate 1.0mL min^{-1} Gradient time table is given in Table 4.1. Mass range: 110 – 1000amu. Mode: Electro Spray Ionisation (+ve ion mode) through HPLC.

Table4.1. Validation: LC – MS gradient

Time	Mobile Phase A%	Mobile Phase B%
0	100.0	0.0
15	100.0	0.0
35	30.0	70.0
40	30.0	70.0
41	100.0	0.0
45	100.0	0.0

Environmental Sample Preparation

Environmental water samples treated as mentioned in *Chapter 2*.

Preparation of standard stock solution

Preparation of esomeprazole standard stock solution

A quantity of 41.68mg esomeprazole standard was weighed into a volumetric flask of 10mL capacity, dissolved in 5mL methanol and the volume was made upto the mark with methanol. Solution concentration was 4147.16mg L^{-1} **(Stock Solution A)**. A volume of 2.5mL **Stock Solution A** was transferred into a volumetric flask of 10mL capacity and the volume was made upto the mark with acetonitrile. Solution

concentration was 103.79mg L^{-1} **(Solution A1).** A volume of 1.0mL **Standard Solution A1** was transferred into a volumetric flask of 10mL capacity and the volume was made upto the mark with acetonitrile. The actual concentration of solution was 103.68mg L^{-1} **(Standard Solution A2).**

Preparation of venlafaxine HCl standard stock solution

A quantity of 40.92mg venlafaxine HCl standard was weighed into a volumetric flask of 10mL capacity, dissolved in 5mL methanol and the volume was made upto the mark with methanol. Solution concentration was 4017.54mg L^{-1} **(Stock Solution B).** A volume of 2.5mL **Stock Solution B** was transferred into a volumetric flask of 10mL capacity and the volume was made upto the mark with acetonitrile. Solution concentration was 1017.89mg L^{-1} **(Standard solution B1).** A volume of 1.0mL **Standard Solution B1** was transferred into a volumetric flask of 10mL capacity and the volume was made upto the mark with acetonitrile. Solution concentration was 101.79mg L^{-1} **(Standard solution B2).**

Preparation of fenofibrate standard stock solution

A quantity of 42.0mg fenofibrate standard was weighed into a volumetric flask of 10mL capacity, dissolved in 5mL methanol and the volume was made upto the mark with methanol. Solution concentration was 40179mg L^{-1} **(Stock Solution C).** A volume of 2.5mL **Stock Solution C** was transferred into a volumetric flask of 10mL capacity and the volume was made upto the mark with acetonitrile. Solution concentration was 1044.75 **(Standard Solution C1).** A volume of 1.0mL standard solution was transferred into a volumetric flask of 10mL capacity and the volume was made upto the mark with acetonitrile. Solution concentration was 104.48mg L^{-1} **(Standard Solution C2).**

Preparation of Mixture standard solution

A quantity of 2.5mL each of above three **Stock Solutions A, B and C,** into a volumetric flask of 10mL capacity and volume was made upto the mark with acetonitrile. The solution is called **Standard Solution (ABC).** Solution concentration

was 1036.79, 1017.89 and 1044.75mg L^{-1} for esomeprazole, venlafaxine and fenofibrate respectively.

Preparation of standard solutions for LDR

A volume of 2.5, 1.0, 0.5, 0.5 and 0.1mL **Standard Solution (ABC)** was transferred into separate volumetric flasks of 5, 5, 5, 10 and 10mL capacity individually and the volume of each flask was made upto the mark with acetonitrile.

Solutions concentration were 518.40, 207.63, 103.68, 51.84 and 10.37mg L^{-1} **(Solution D, E, F, G and H)**, respectively for esomeprazole.

Solutions concentration were 508.94, 203.58, 101.79, 50.89 and 10.18mg L^{-1} **(Solution D, E, F, G and H)**, respectively for venlafaxine.

Solutions concentration were 522.38, 208.95, 104.48, 52.24 and 10.45mg L^{-1} **(Solution D, E, F, G and H)**, respectively for fenofibrate.

Preparation of standard solution for LOD / LOQ

A volume of 1.0mL and 0.5mL **Standard Solution (ABC)** was transferred into separate volumetric flasks of 10mL capacity and the volume of each flask was made upto the mark with acetonitrile. Solution concentration were 103.68, 101.79 and 104.48mg L^{-1} for esomeprazole, venlafaxine and fenofibrate **(Solution I)** and 51.84, 50.89 and 52.24mg L^{-1} for esomeprazole, venlafaxine and fenofibrate **(Solution J)** respectively.

A volume of 0.5mL and 1.0mL of **Standard Solution (J)** were transferred into separate volumetric flasks of 25mL and 10mL capacity and the volume of each flask was made upto the mark with acetonitrile. Solutions were called **Solution (K), Solution (L)**. Solution concentrations were 1.02, 1.02 and 1.05mg L^{-1} for esomeprazole, venlafaxine and fenofibrate **(K)** and 5.18, 5.09 and 5.22mg L^{-1} for esomeprazole, venlafaxine and fenofibrate **(L)**.

Preparation of solution for precision and accuracy (fortification in environmental water sample)

Precision: Six replicates of solution **(E)** and **(H)** were injected in to HPLC and %RSD was calculated.

Esomeprazole: A quantity of 10.42mg references substances was weighed into a volumetric flask of 10mL capacity and dissolved in to methanol, sonicated for two minutes and the volume was made upto the mark with methanol. **[Stock solution (RE1), 1036.79mg L^{-1}]**

Venlafaxine: A quantity of 10.23mg reference substance was weighed into a volumetric flask of 10mL capacity and dissolved in to methanol, sonicated for two minutes and the volume was made upto the mark with methanol. **[Stock solution (RE2), 1017.885mg L^{-1}]**

Fenofibrate: A quantity of 10.50mg reference substance was weighted into a volumetric flask of 10mL capacity and dissolved in to methanol, sonicated for two minutes and the volume was made upto the mark with methanol. **[Stock solution (RE3), 1044.75mg L^{-1}]**

Thereafter a volume of 1.0mL each of Solution **(RE1, RE2, RE3)** was transferred into a volumetric flask of 10mL capacity and the volume was made upto the mark with acetonitrile **[Solution (RE4)**, concentration 103.68, 101.79, 104.48mg L^{-1} for esomeprazole, venlafaxine and fenofibrate respectively].

Fortification and preparation of sample solution

Fortification was performed at two levels, 5mg L^{-1} and 50mg L^{-1}.

5mg L^{-1} level: A volume of 0.5mL of each of **Solution (RE4)** was transferred into a volumetric flask of 10mL capacity and mixed into environmental water sample, sonicated for two minutes and the volume was made upto the mark with environmental water sample. The solution concentrations were 5.18, 5.09 and 5.22mg $L^{-1}$for esomeprazole, venlafaxine and fenofibrate respectively. **[Solution (RW1)]**.

50mg L^{-1} level : A volume of 2.5mL of **Solution (RE4)** was transferred into a volumetric flask of 5mL capacity and dissolved into water, sonicated for two minutes and the volume was made upto the mark with environmental water sample. The solution concentration were 51.84, 50.89 and 52.24mg L^{-1} for esomeprazole, venlafaxine and fenofibrate respectively. **[Solution (RW2)]**

Preparation of system suitability solution

A volume of 1.0mL **Standard Solution (ABC)** was transferred into separate volumetric flasks of 10mL capacity and the volume of each flask was made upto the mark with acetoitrile. Solution concentration was 103.68mg L^{-1} for esomeprazole, 101.79mg L^{-1} for venlafaxine, 104.48mg L^{-1} for fenofibrate **[Solution (SS)]** respectively.

Analytical Method Validation

The method was validated for specificity, precision, LOD, LOQ, Linearity dynamic range, accuracy, robustness and system suitability. The validated analytical method satisfies International Conference on Harmonisation guideline. (ICH Topic Q2 R1)

Specificity

The specificity of the method for esomeprazole, venlafaxine and fenofibrate was studied by injecting acetonitrile (solvent used for standard and sample solutions preparation), mobile phase, methanol, esomeprazole standard, venlafaxine standard and fenofibrate standard.

System Suitability

The solution (SS) was injected on to HPLC in six replication and %RSD was calculated for retention time and peak area of esomeprazole, venlafaxine and fenofibrate separately.

Linear dynamic range (LDR)

The **Standard Solutions (D, E, F, G and H)** were injected onto the HPLC in two replications and the mean areas were plotted against concentration (mg L^{-1}). The correlation co – efficient (r), slope (b) and intercept (a) were calculated.

Limit of detection (LOD) and Limit of Quantification (LOQ)

The solution **(K and L)** were injected onto HPLC in three replications to determine limit of detection and limit of Quantification. The minimum concentration, which could be detected by the HPLC with S/N ratio of 3 ± 0.5, was calculated as limit of detection (LOD). The minimum concentration, which could be quantified by the HPLC with S/N ratio between 5 to 10, was calculated as limit of quantification (LOQ).

Limit of Detection and Limit of Quantification

For calculating the LOD and LOQ values, solutions with known decreasing concentrations of analytes were injected into the HPLC system. The limit of detection (LOD) and quantification (LOQ) were then measured by calculating the minimum level at which the analytes can be readily detected (signal to noise ratio of 3:1) and quantified (signal to noise ratio of 10:1) with accuracy, respectively.

Precision

Precision of the developed method was determined at two levels, 10mg L^{-1} and 200mg L^{-1} of three drugs. For evaluating the within-day precision, results of six replicate analyses of two different concentrations of samples were used on a single day. The between – day precision was calculated from results obtained from the same samples analyzed on five different days.

Accuracy

Method accuracy was determined by fortifying known amounts of esomeprazole, venlafaxine HCl and fenofibrate to the pre – analysed environmental

water sample at the LOQ level (5.0mg L^{-1}) and 10 times LOQ level (50mg L^{-1}) and then comparing the added concentration with the found concentration. The concentration of three drugs in each replicate were calculated using the following formula:

$$\text{Concentration (ppm)} = \frac{Y - a}{b} \times D$$

where,

Y = Peak area of the sample
a = Constant
b = Regression coefficient for Y on X
D = Dilution factor

The %RSD was calculated using the following formula:

$$\text{Precision (\% RSD)} = \frac{\text{Standard Deviation}}{\text{Mean Concentration}} \times 100$$

The accuracy (%Recovery) was calculated using the following formula:

$$\text{\% Recovery} = \frac{\text{Recovered concentration}}{\text{Fortified concentration}} \times 100$$

Identification of Esomeprazole, Venlafaxine HCl and Fenofibrate by LC – MS

Esomeprazole, Venlafaxine HCl and Fenofibrate were identified by MS. Environmental water samples were analysed by LC – MS.

RESULTS AND DISCUSSION

To develop the method different stationary phases (C18, C8), mobile phases containing buffers like formic acid, ammonium acetate and organic modifiers like acetonitrile in the mobile phase were used.

At the beginning of method development a chromatographic condition was set for the separation of esomeprazole, venlafaxine HCl and fenofibrate individually by BDS Hypersil C8 column (250 x 4.6mm, 5µ particle size) using a mixture of acetonitrile: buffer (0.13% formic acid, 15.50% 0.1mol L^{-1} ammonium acetate) in the ratio 25:75 (v/v) (pH 3.8) as mobile phase A and acetonitrile as mobile phase B at a wavelength of 302nm with flow rate 1.0mL min^{-1} with run time 45min. The gradient

LC conditions are mentioned in Table 4.1. *(Page No. 138.).*

To reduce the run time chromatographic conditions were changed. This was achieved on a C18 (150cm x 4.6mm, 3.5µm particle size) column and mixture of acetonitrile: buffer (0.3% formic acid) in the ratio 25:75 (v/v) as mobile phase A and in the ratio 30:70 (v/v) as mobile phase B. At the wavelength of 230nm all the three drugs gave a good response. Under these conditions, sharp peaks that belong to Esomeprazole, Venlafaxine HCl and Fenofibrate were obtained at retention time 3.25, 4.77 and 13.12 minutes respectively as shown in Figure 4.1.

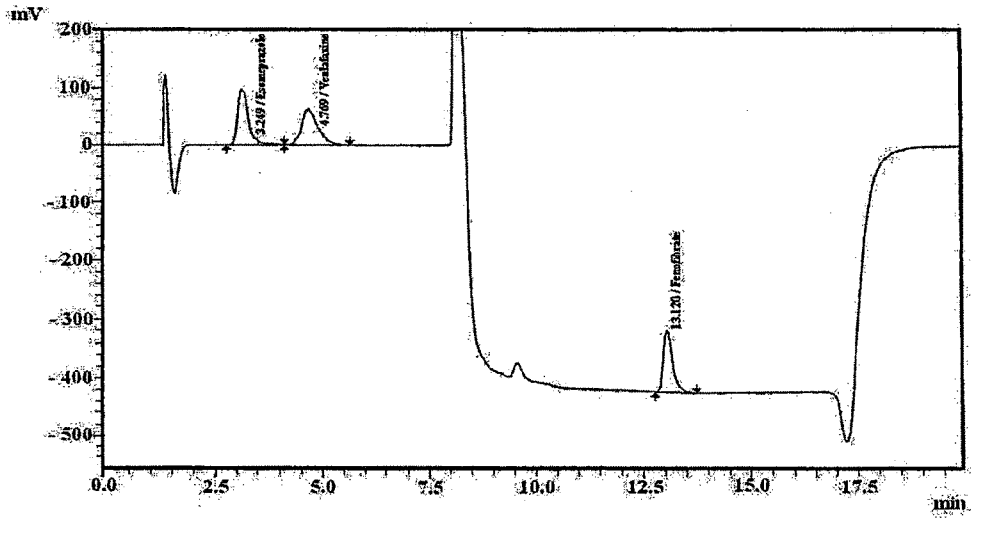

Figure 4.1 Chromatogram for esomeprazole, venlafaxine HCl and fenofibrate

The tailing factor for esomeprazole, venlafaxine HCl and fenofibrate was 1.288, 1.478 and 1.290 respectively.

Method Validation

Specificity

Since there was no interference of peaks of esomeprazole, venlafaxine HCl and fenofibrate standard, in to each other, as well as no interfering peaks appeared at retention time of above compounds, the method was considered to be specific for the each of analyte. The representative chromatograms of 100mg L^{-1} esomeprazole,

100mg L^{-1} venlafaxine HCl, 100mg L^{-1} fenofibrate, methanol, acetonitrile and mobile phase obtained for the specificity study are given in Figure 4.2, 4.3, 4.5, 4.6, 4.7 and 4.8 respectively.

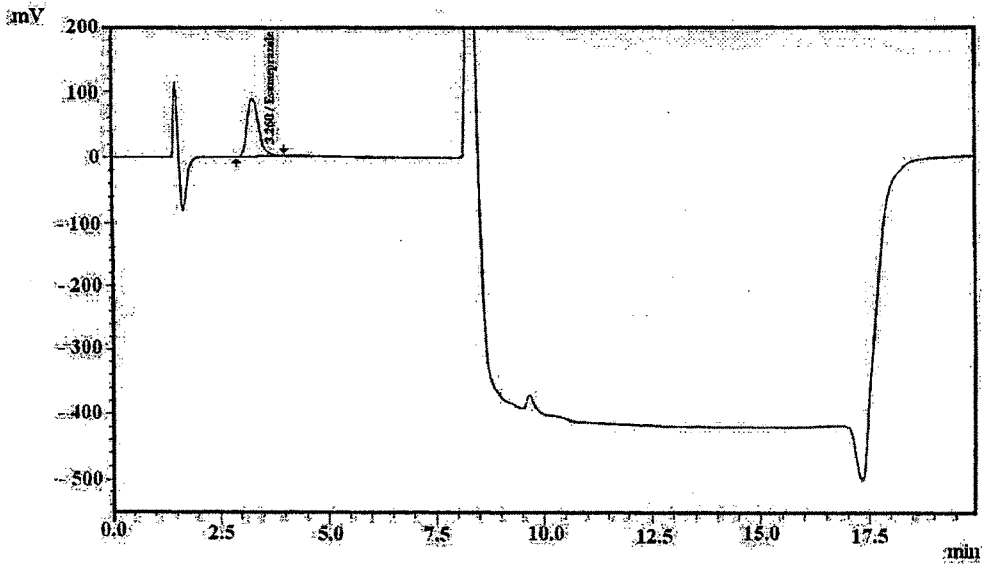

Figure 4.2. Specificity – Chromatogram for 100mg L^{-1} esomeprazole

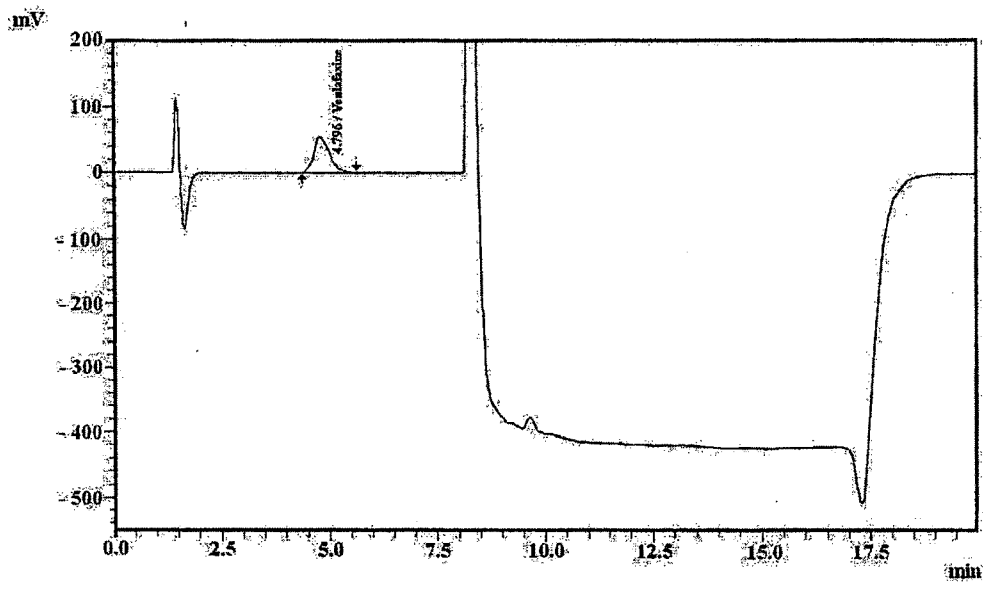

Figure 4.3. Specificity – Chromatogram for 100mg L^{-1} venlafaxine HCl

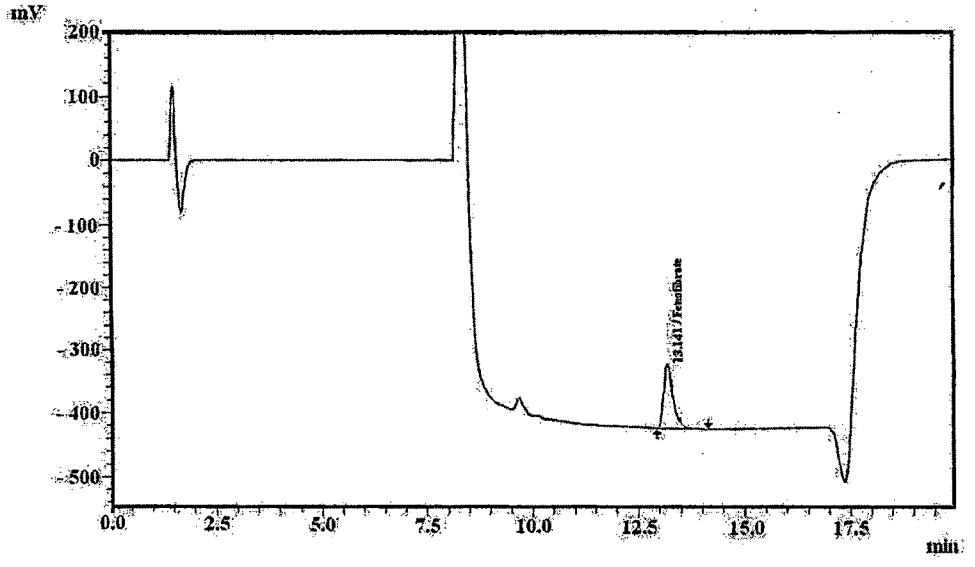

Figure 4.4. Specificity – Chromatogram for 100 mg L^{-1} fenofibrate

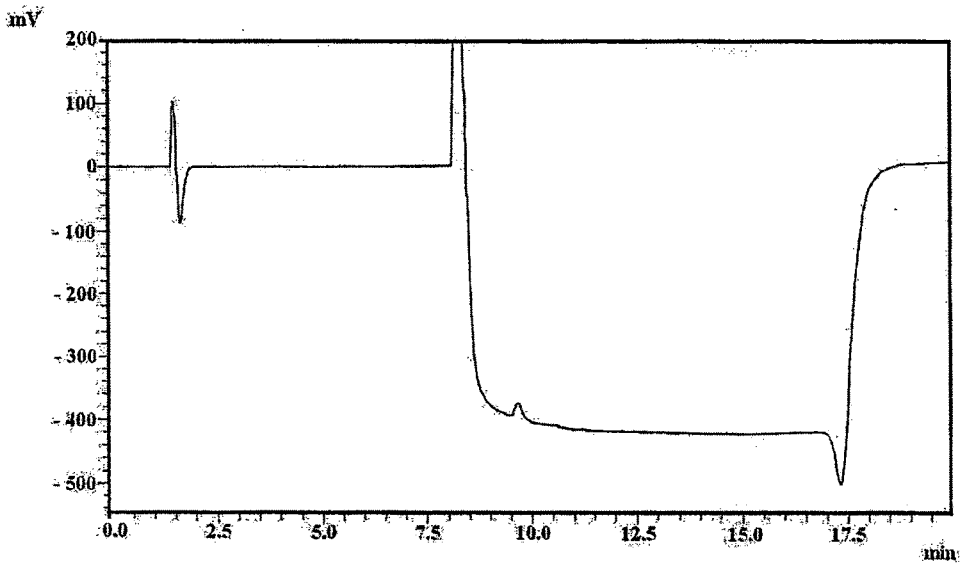

Figure 4.5. Specificity – Chromatogram for acetonitrile

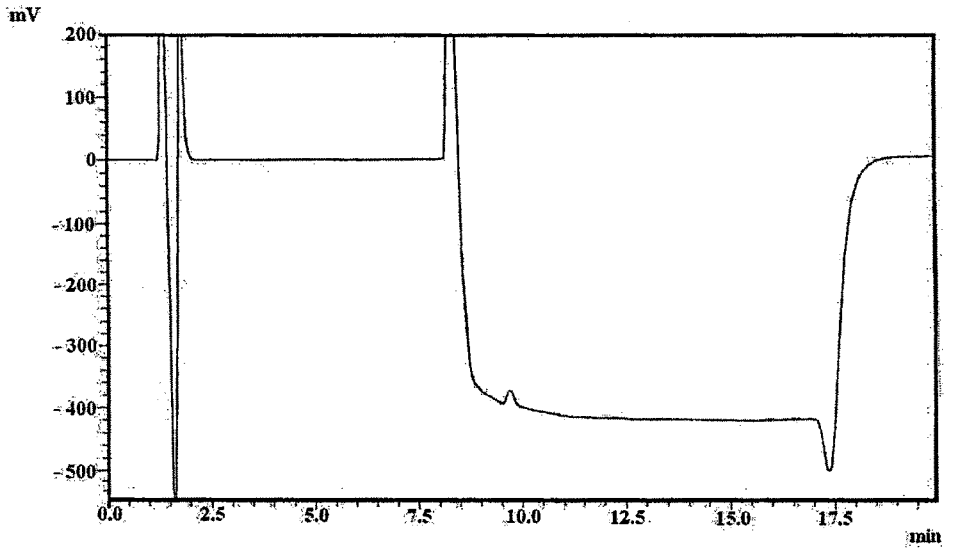

Figure 4.6. Specificity – Chromatogram for methanol

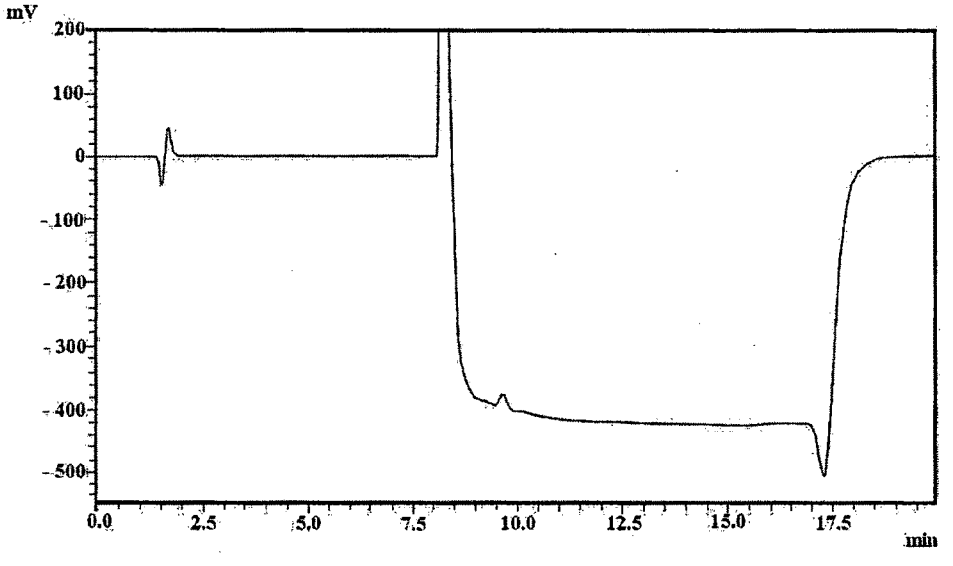

Figure 4.7. Specificity – Chromatogram for mobile phase

System Suitability

The %RSD for retention times were 0.03, 0.02 and 0.08 for esomeprazole, venlafaxine HCl and fenofibrate respectively. The %RSD for peak area were 1.16, 1.16 and 0.88 for esomeprazole, venlafaxine HCl and fenofibrate respectively. The results are shown in Table 4.2, 4.3 and 4.4.

The representative chromatograms of 100mg L^{-1} R1, R2, R3, R4, R5 and R6 obtained for the system suitability study are given in Figure 4.8, 4.9, 4.10, 4.11, 4.12 and 4.13 respectively.

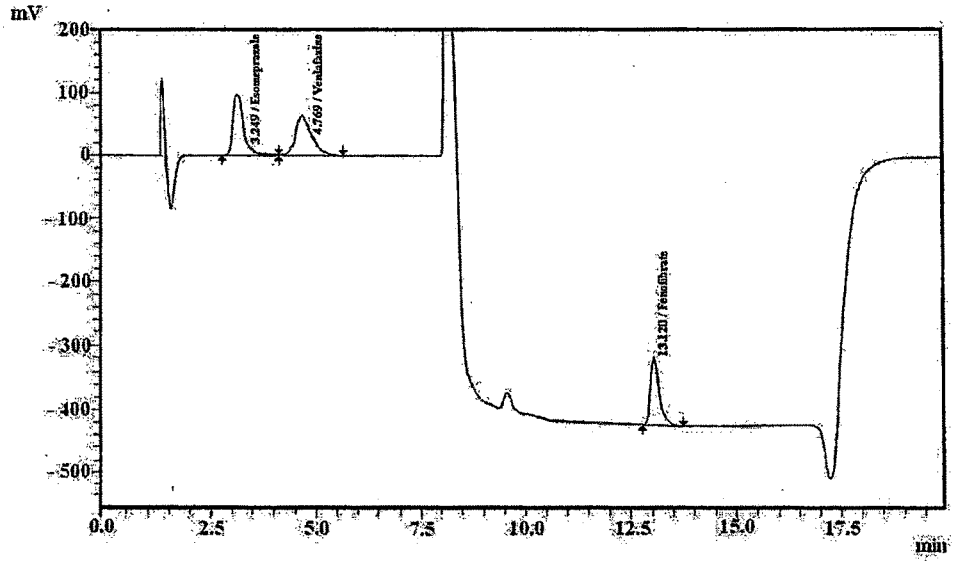

Figure 4.8. System suitability – Chromatogram for 100mg L^{-1} R1

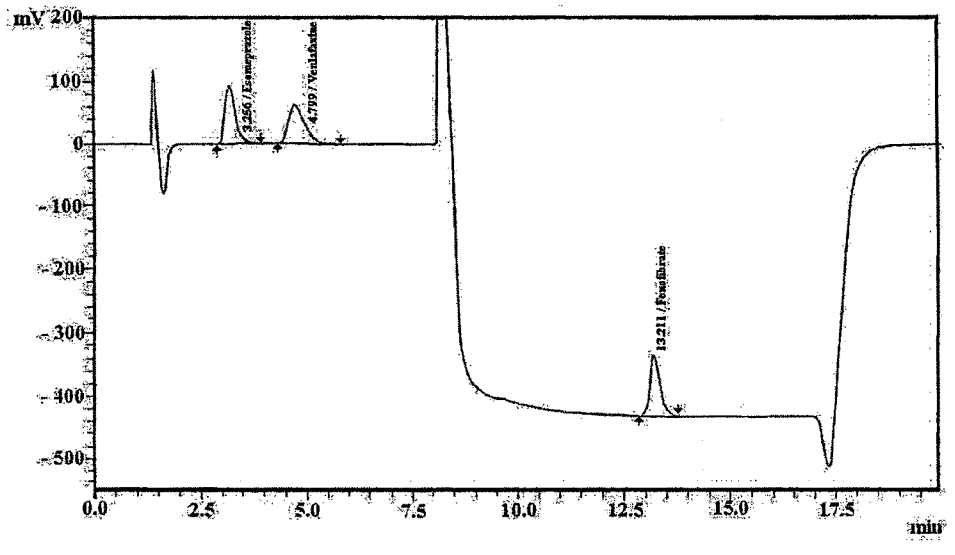

Figure 4.9. System suitability – Chromatogram for 100mg L^{-1}R2

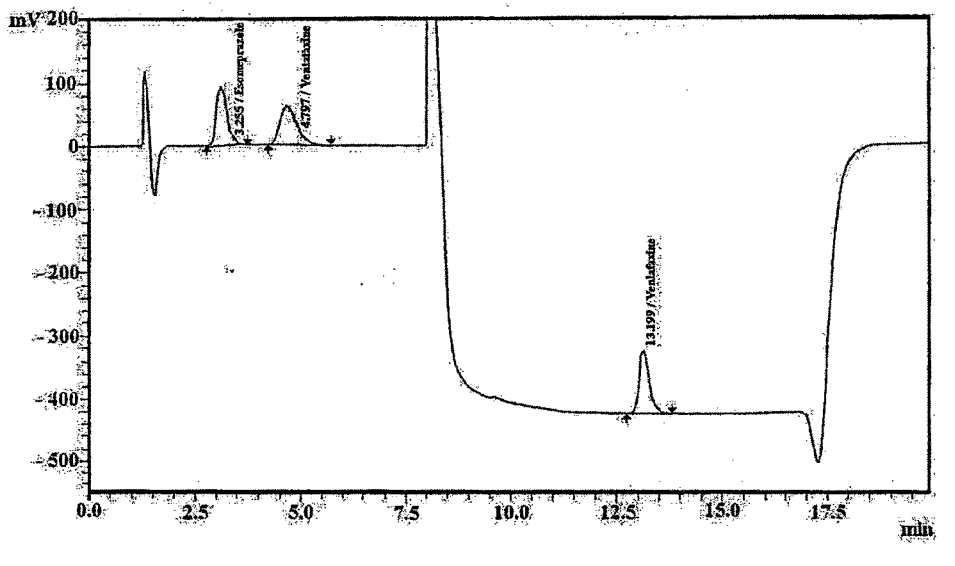

Figure 4.10. System suitability – Chromatogram for 100mg L^{-1} R3

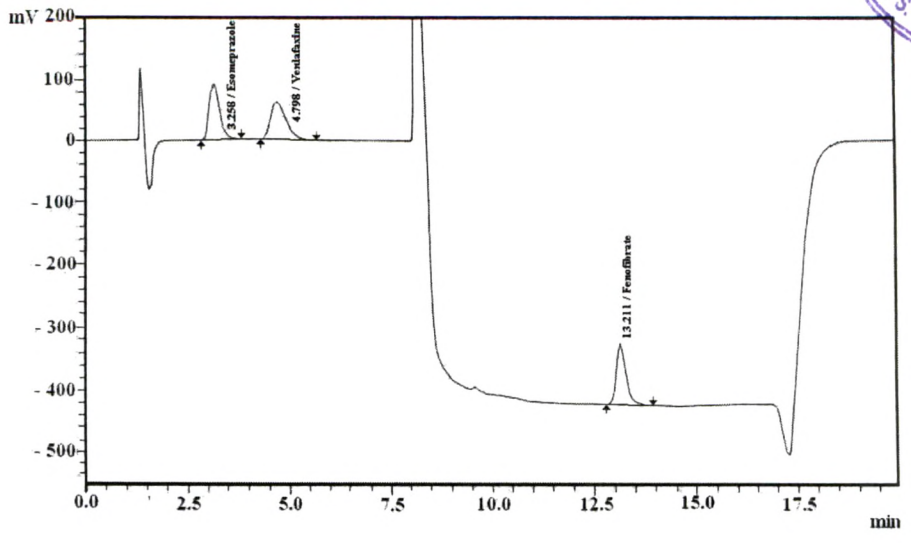

Figure 4.11. System suitability – Chromatogram for 100mg L^{-1} R4

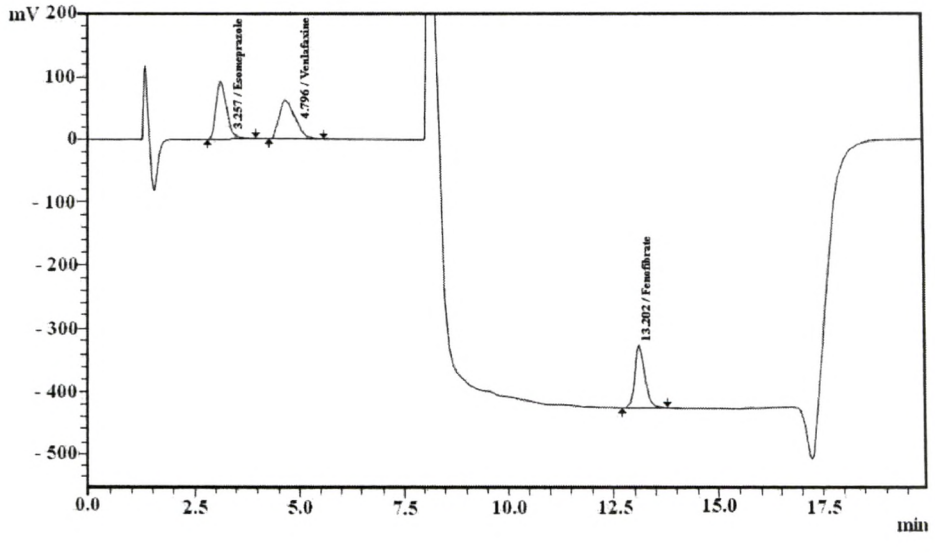

Figure 4.12. System suitability – Chromatogram for 100mg L^{-1} R5

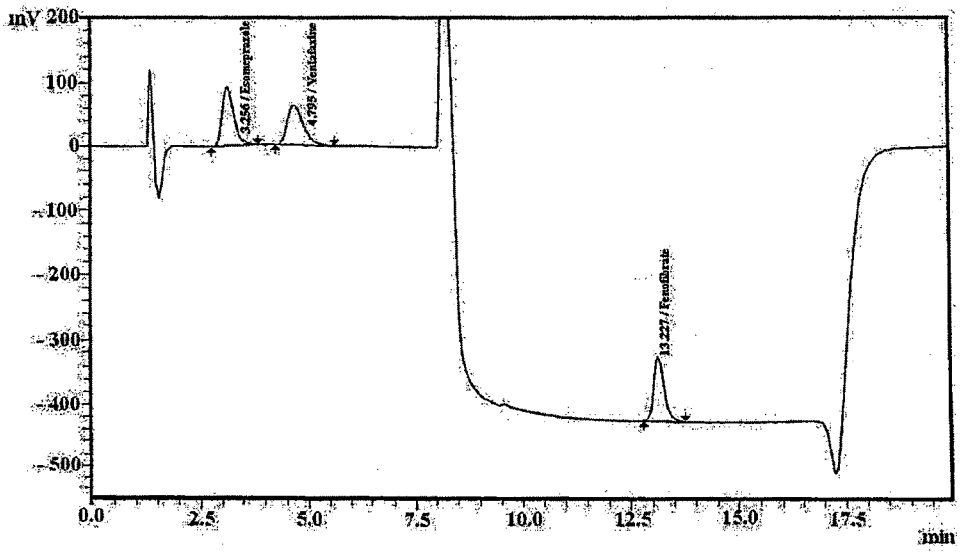

Figure 4.13. System suitability – Chromatogram for 100mg L^{-1} R6

Table 4.2. System suitability study for esomeprazole.

Esomeprazole (103.68mg L^{-1})		
Replication	Retention Time	Peak Area
R1	3.255	1627480
R2	3.256	1619394
R3	3.255	1589943
R4	3.258	1622639
R5	3.257	1648607
R6	3.256	1623318
Mean	3.256	1621897
SD	0.001	18839
%RSD	0.03	1.16

Table 4.3. System suitability study for venlafaxine HCl.

Venlafaxine HCl (101.79mg L^{-1})		
Replication	Retention Time	Peak Area
R1	4.798	1651785
R2	4.799	1640829
R3	4.797	1647049
R4	4.798	1613478
R5	4.796	1612408
R6	4.795	1611894
Mean	4.797	1629574
SD	0.001	18930
%RSD	0.02	1.16

Table 4.4. System suitability study for fenofibrate.

Fenofibrate (104.48mg L^{-1})		
Replication	Retention Time	Peak Area
R1	13.197	1641687
R2	13.211	1605120
R3	13.199	1629048
R4	13.211	1613823
R5	13.202	1623625
R6	13.227	1606558
Mean	13.208	1619977
SD	0.011	14182
%RSD	0.08	0.88

Linear Dynamic Range (LDR)

The computed equations of the calibration curve for the three drugs are: esomeprazole: $y = 16375.54x - 3513.49$, for venlafaxine HCl: $y = 15400.66x + 30904.46$, and for fenofibrate: $y = 15356.84x + 15485.60$. The results shown in Table 4.5, 4.6 and 4.7.

The results show that an excellent correlation existed between the peak area and concentration. The correlation coefficient (r^2) was 0.999, 0.999 and 0.999 for esomeprazole, venlafaxine HCl and fenofibrate respectively.

The representative chromatograms of 500mg L^{-1}, 200mg L^{-1}, 100mg L^{-1}, 50mg L^{-1} and 10mg L^{-1} of esomeprazole, venlafaxine HCl and fenofibrate respectively with two sets R1 and R2 are shown in Figures 4.14 to 4.23.

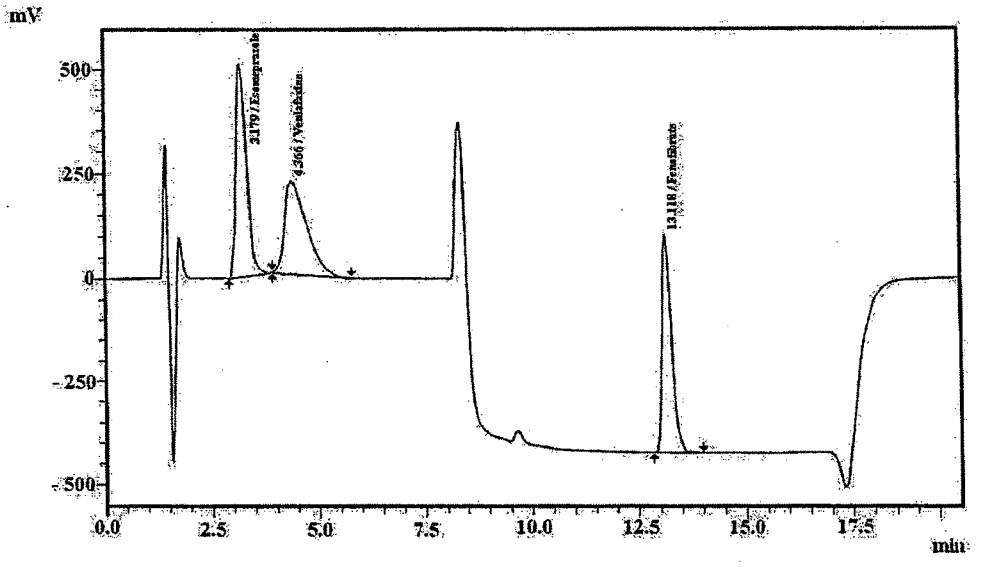

Figure 4.14. Linear dynamic range – Chromatogram for 500mg L^{-1}R1

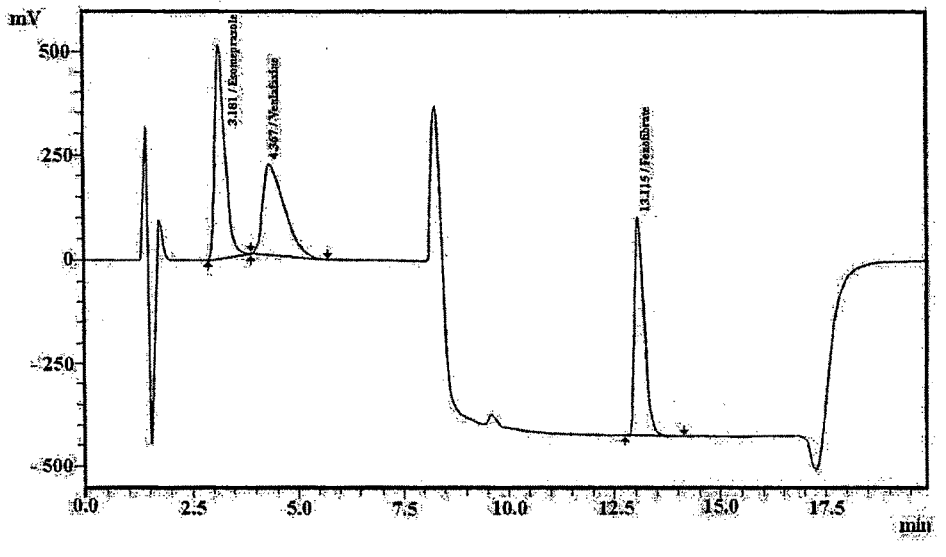

Figure 4.15. Linear dynamic range – Chromatogram for 500mg L^{-1}R2

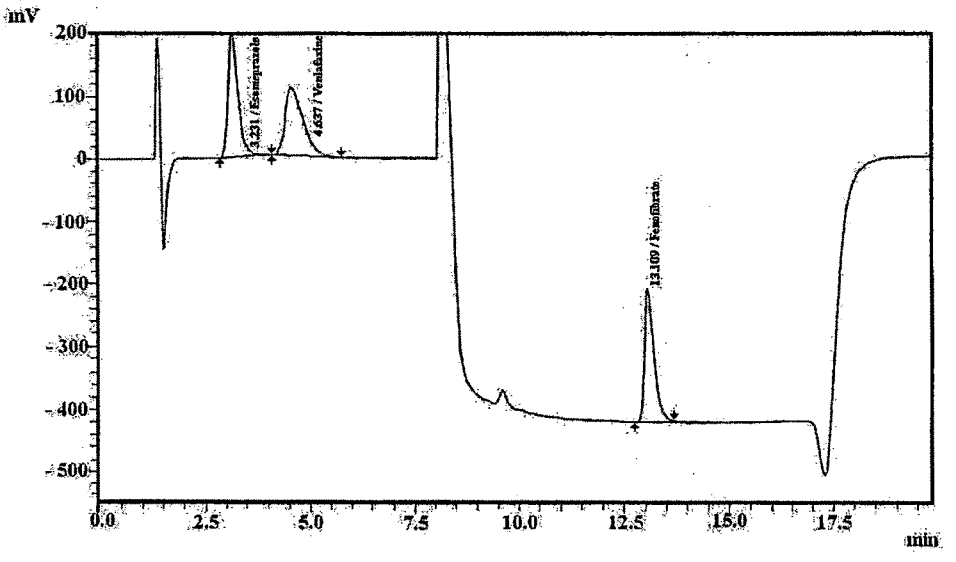

Figure 4.16. Linear dynamic range – Chromatogram for 200mg L^{-1}R1

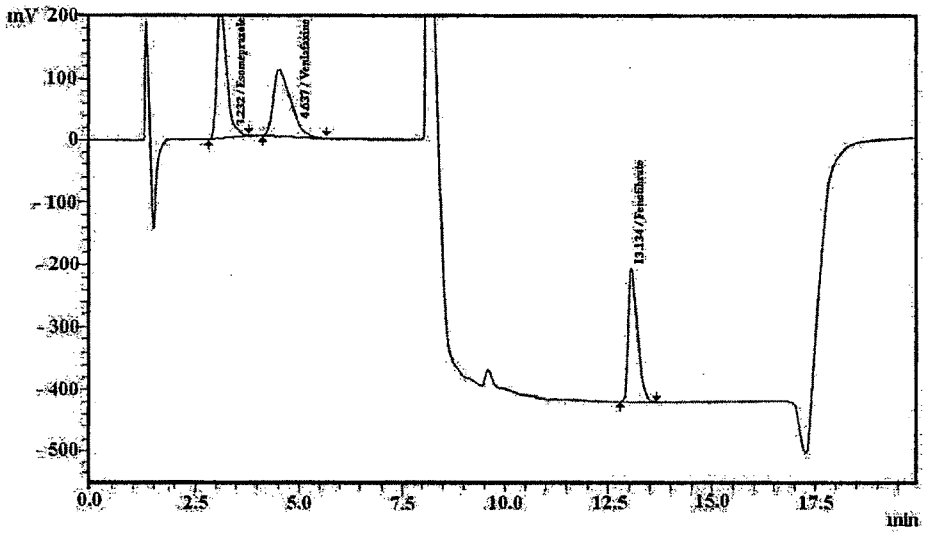

Figure 4.17. Linear dynamic range – Chromatogram for 200mg L^{-1}R2

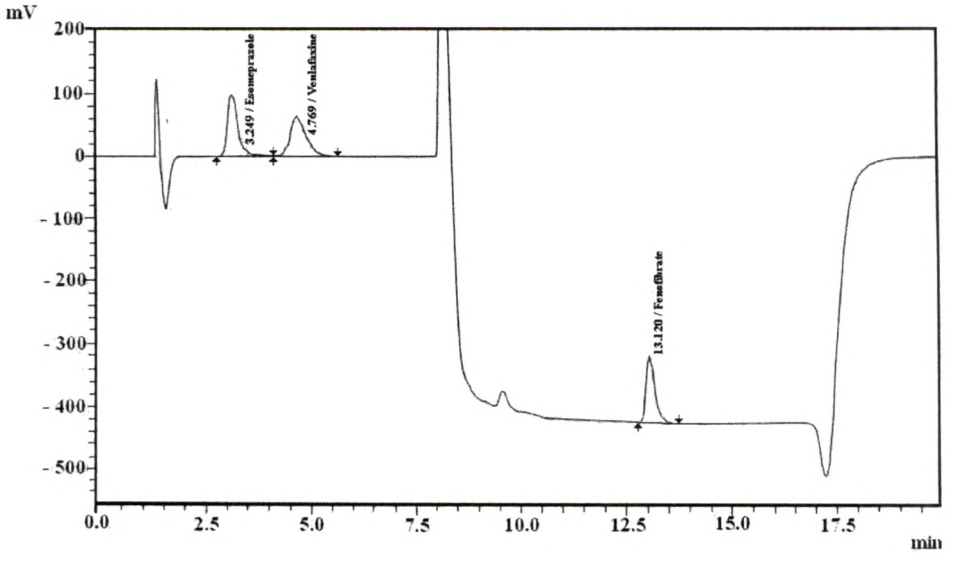

Figure 4.18. Linear dynamic range – Chromatogram for 100mg L^{-1}R1

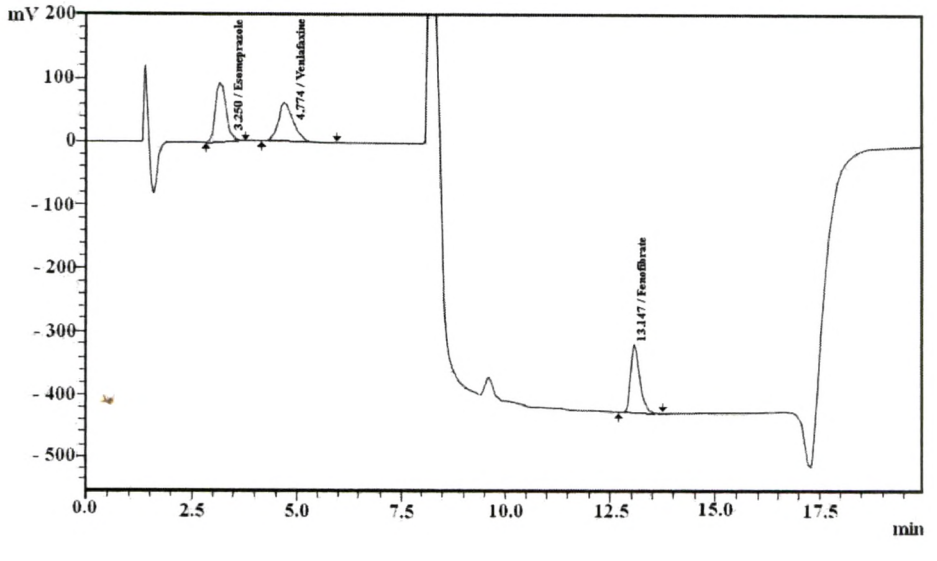

Figure 4.19. Linear dynamic range – Chromatogram for 100mg L^{-1}R2

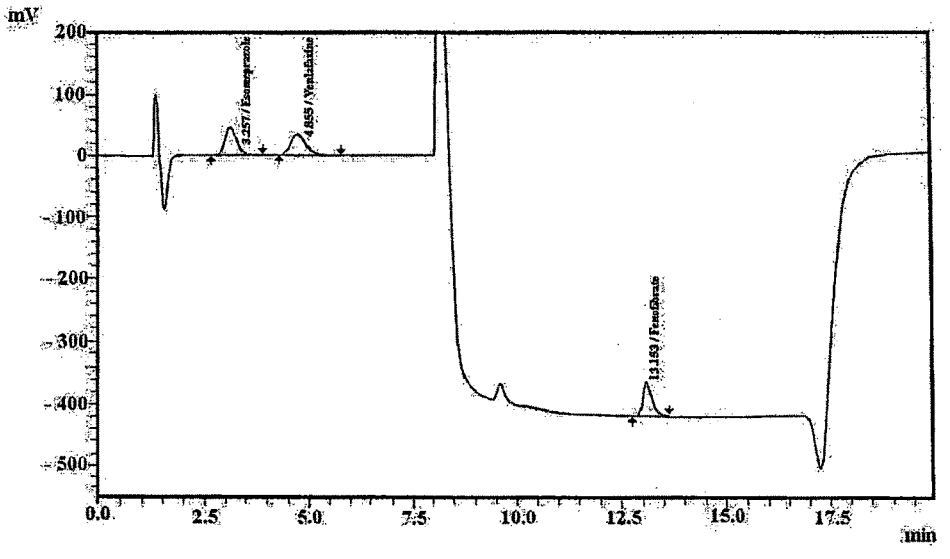

Figure 4.20. Linear dynamic range – Chromatogram for 50mg L^{-1}R1

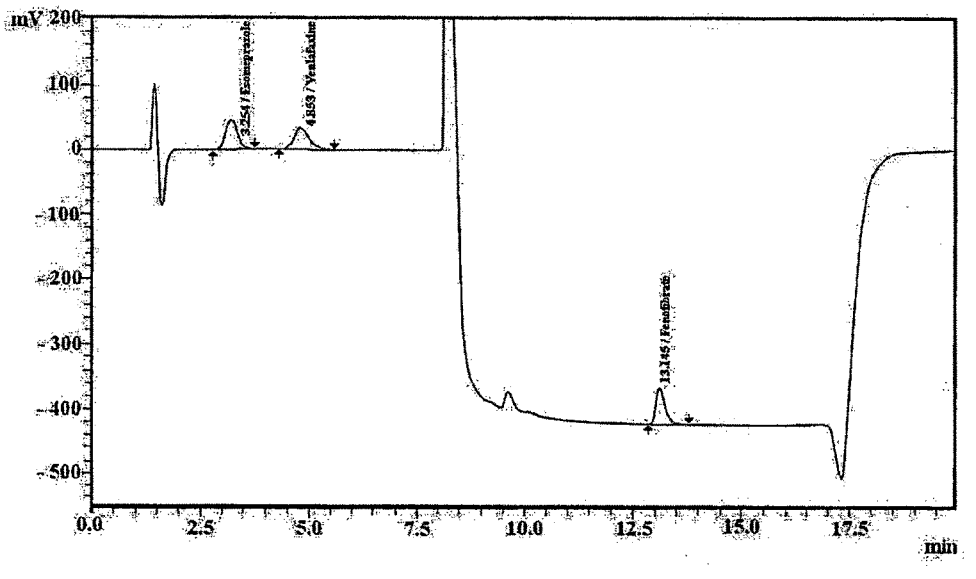

Figure 4.21. Linear dynamic range – Chromatogram for 50mg L^{-1}R2

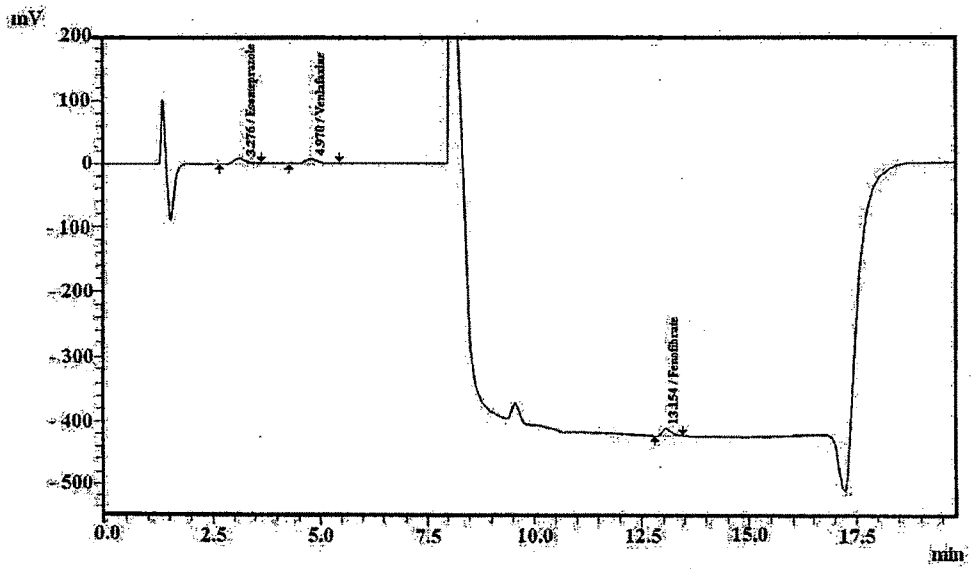

Figure 4.22. Linear dynamic range – Chromatogram for 10mg L^{-1}R1

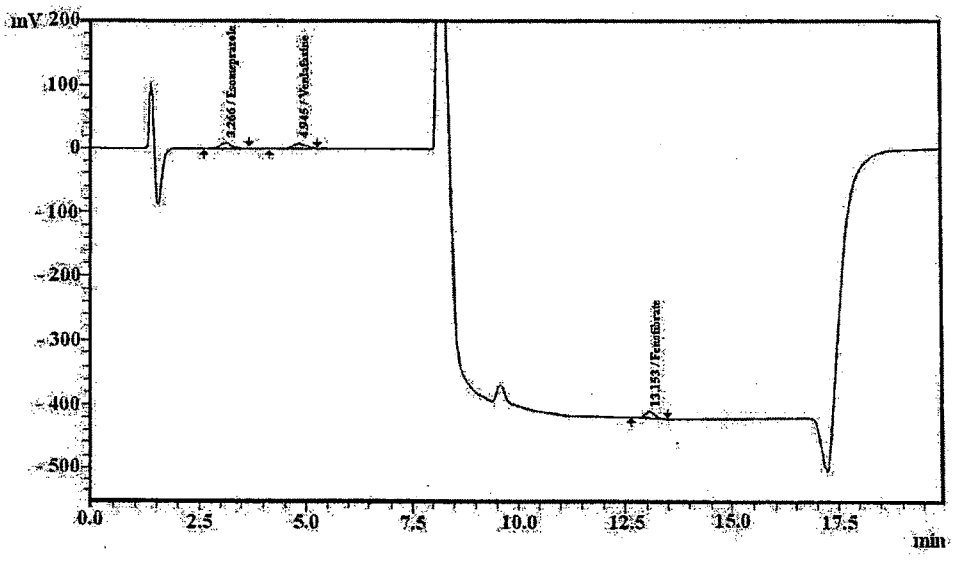

Figure 4.23. Linear dynamic range – Chromatogram for 10mg L^{-1}R2

Table 4.5 Linear Dynamic Range Data for Esomeprazole Standard

Concentration(mg L^{-1})	Replications	Peak Area Counts	Mean Peak Area Counts	%Variation
10.37	R1	181684	182442.50	- 0.83
	R2	183201		
51.84	R1	838509	829888.50	2.06
	R2	821268		
103.68	R1	1700148	1682775.00	2.04
	R2	1665402		
207.36	R1	3435061	3404385.00	1.79
	R2	3373709		
518.40	R1	8486354	8484196.00	0.05
	R2	8482038		
Typical Calculation				
$\%\ \text{Variation} = \frac{\text{Maximum Area} - \text{Minimum Area}}{\text{Maximum Area}} \times 100$			$= \frac{181684 - 183201}{183201} \times 100$	$= -0.83$

Table 4.6. Linear Dynamic Range Data for Venlafaxine HCl Standard

Concentration(mg L^{-1})	Replications	Peak Area Counts	Mean Peak Area Counts	%Variation
10.18	R1	164175	164956.5	- 0.95
	R2	165738		
50.89	R1	825901	823896.5	0.49
	R2	821892		
101.79	R1	1591977	1595884.5	- 0.49
	R2	1599792		
203.58	R1	3213848	3192950.5	1.3
	R2	3172053		
508.94	R1	7861435	7858265	0.08
	R2	7855095		
Typical Calculation				
$\%\ \text{Variation} = \frac{\text{Maximum Area} - \text{Minimum Area}}{\text{Maximum Area}} \times 100$			$= \frac{164175 - 165738}{165738} \times 100$	$= -0.95$

Table 4.7. Linear Dynamic Range Data for Fenofibrate Standard

Concentration(mg L^{-1})	Replications	Peak Area Counts	Mean Peak Area Counts	%Variation
10.45	R1	170748	170033.5	0.84
	R2	169319		
52.24	R1	823158	824412.5	- 0.3
	R2	825667		
104.48	R1	1586211	1603156	- 2.14
	R2	1620101		
208.95	R1	3257370	3246370.5	0.68
	R2	3235371		
522.38	R1	8017173	8031572.5	- 0.36
	R2	8045972		
Typical Calculation				
$\%\ \text{Variation} = \frac{\text{Maximum Area} - \text{Minimum Area}}{\text{Maximum Area}} \times 100$			$= \frac{170748 - 169319}{170748} \times 100$	$= 0.84$

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection of esomeprazole was 1.02mg L^{-1} with signal to noise ratio of 2.75. The lowest quantifiable concentration for esomeprazole with signal to noise ratio of 7.8 was 5.18mg L^{-1}. Results are shown in Table 4.8.

The limit of detection of venlafaxine was 1.02mg L^{-1} with signal to noise ratio of 3.46. The lowest quantifiable concentration for venlafaxine with signal to noise ratio of 8.34 was 5.09mg L^{-1}. Results are shown in Table 4.9.

The limit of detection of fenofibrate was 1.05mg L^{-1} with signal to noise ratio of 2.66. The lowest quantifiable concentration for fenofibrate with signal to noise ratio of 7.77 was 5.22mg L^{-1}. Results are shown in Table 4.10.

The representative chromatograms of LOD and LOQ studies for esomeprazole, venlafaxine HCl and fenofibrate are given in Figures 4.24 to 4.29.

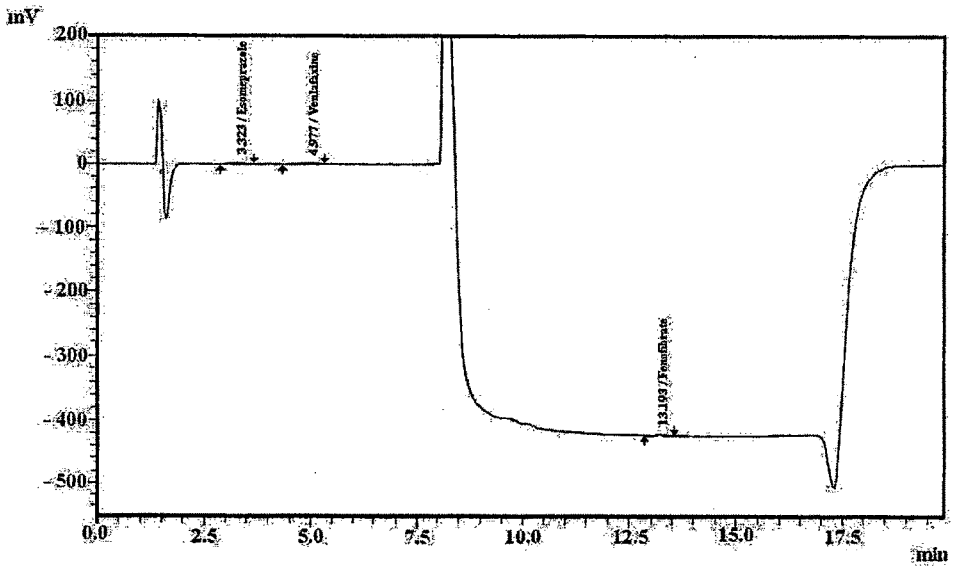

Figure 4.24. LOD – LOQ – Chromatogram for 1mg L^{-1} R1

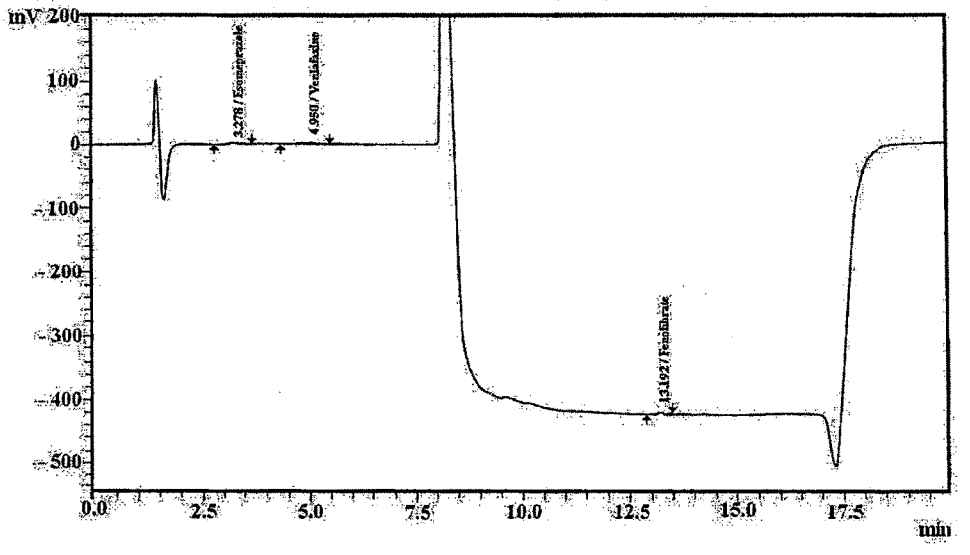

Figure 4.25. LOD – LOQ – Chromatogram for 1mg L^{-1} R2

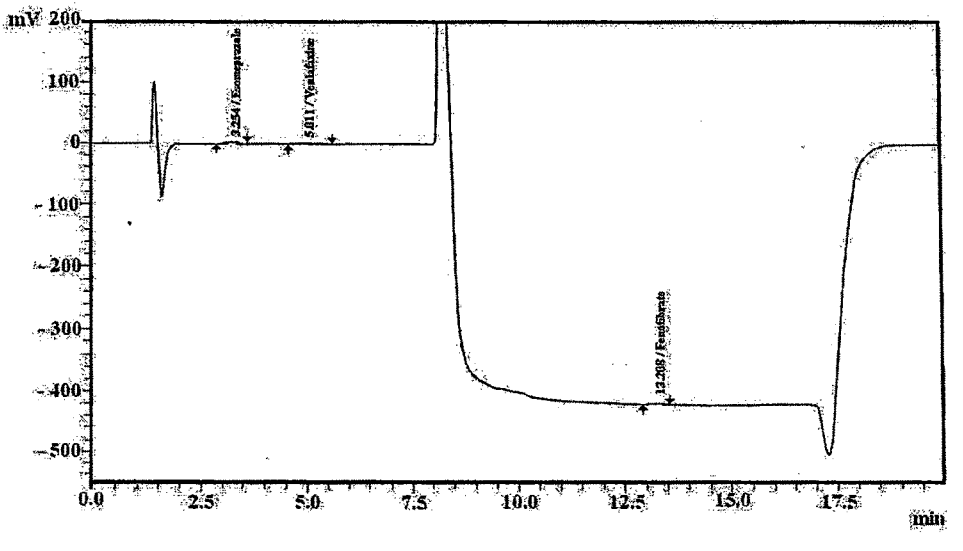

Figure 4.26. LOD – LOQ – Chromatogram for 1mg L^{-1}R3

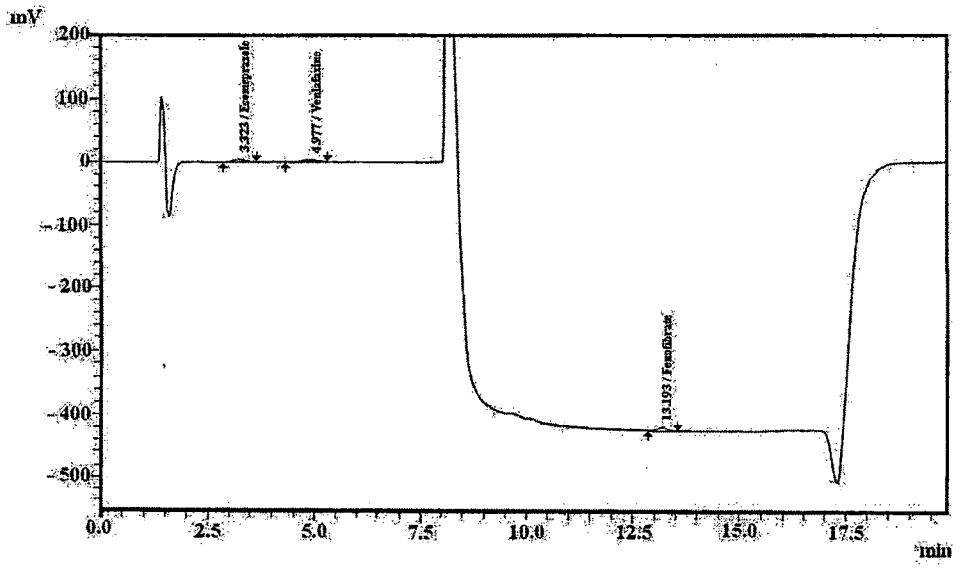

Figure 4.27. LOD – LOQ – Chromatogram for 5mg L^{-1} R1

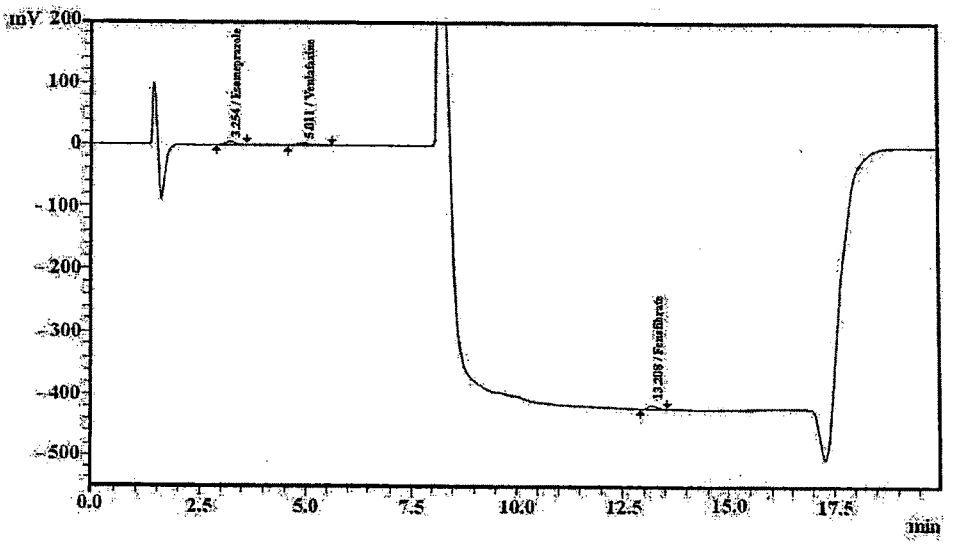

Figure 4.28. LOD – LOQ – Chromatogram for 5mg L^{-1} R2

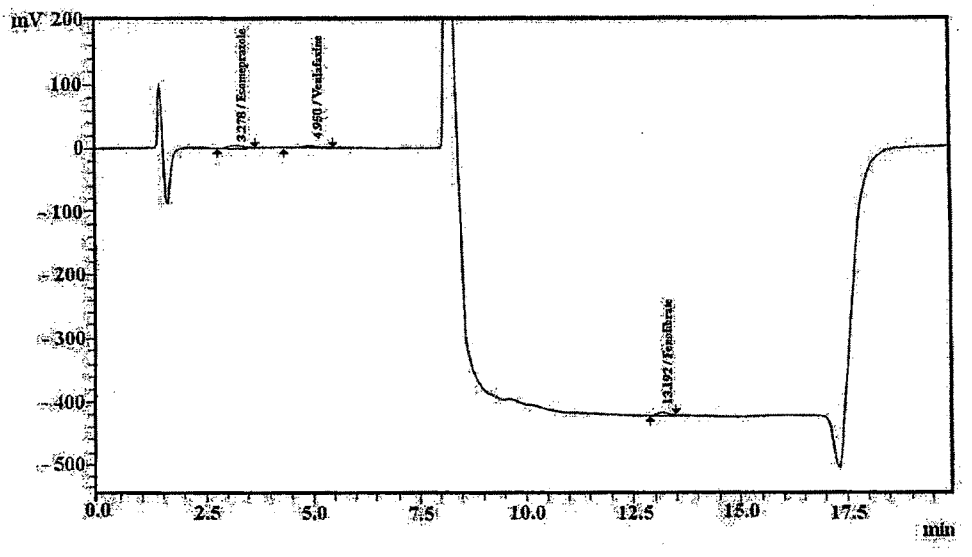

Figure 4.29. LOD – LOQ – Chromatogram for 5mg L^{-1} R3

Table 4.8. LOD and LOQ for esomeprazole

Solution Concentration (mg L^{-1})	Replication	Peak Area Count	Mean Peak Area	Mean Noise	Signal to Noise Ratio (S/N)	LOD	LOQ
1.02	R1	29558	29061	10531.83	2.75	LOD	
	R2	28082					
	R3	29543					
5.18	R1	89142	82213		7.8		LOQ
	R2	68945					
	R3	88552					

Replication	Total Peak Area of Noise in Blank (a)	N° of Noise Peak in Blank (b)	Average = a / b
I	31077	3	10359
II	32114	3	10704.66
Average Noise Peak Area of Blank			10531.83

Typical Calculation

$$\text{Signal to Noise Ratio} = \frac{\text{Mean Peak Area}}{\text{Average Noise Area}}$$

Limit of Detection	Limit of Quantification
$\frac{29061}{10531.83} = 2.75$	$\frac{82213}{10531.83} = 7.8$
1.02mg L^{-1}	5.18mg L^{-1}

Table 4.9. LOD and LOQ for venlafaxine HCl

Solution Concentration ($mg\ L^{-1}$)	Replication	Peak Area Count	Mean Peak Area	Mean Noise	Signal to Noise Ratio (S/N)	LOD	LOQ
1.02	R1	27842	28018	10531.83	2.66	LOD	
	R2	32113					
	R3	24099					
5.09	R1	87512	83625		8.34		LOQ
	R2	80588					
	R3	82776					

Replication	Total Peak Area of Noise in Blank (a)	N° of Noise Peak in Blank (b)	Average = a / b
I	31077	3	10359
II	32114	3	10704.66
Average Noise Peak Area of Blank			10531.83

Typical Calculation

$$\text{Signal to Noise Ratio} = \frac{\text{Mean Peak Area}}{\text{Average Noise Area}}$$

Limit of Detection	Limit of Quantification
$\frac{28018}{10531.83} = 2.66$	$\frac{83625}{10531.83} = 8.34$
$1.02 mg\ L^{-1}$	$5.09 mg\ L^{-1}$

Table 4.10. LOD and LOQ for fenofibrate

Solution Concentration ($mg\ L^{-1}$)	Replication	Peak Area Count	Mean Peak Area	Mean Noise	Signal to Noise Ratio (S/N)	LOD	LOQ
1.05	R1	36641	36475	10531.83	3.46	LOD	
	R2	44924					
	R3	28489					
5.22	R1	76593	76923		7.77		LOQ
	R2	81116					
	R3	73059					

Replication	Total Peak Area of Noise in Blank (a)	N° of Noise Peak in Blank (b)	Average = a / b
I	31077	3	10359
II	32114	3	10704.66
Average Noise Peak Area of Blank			10531.83

Typical Calculation

$$\text{Signal to Noise Ratio} = \frac{\text{Mean Peak Area}}{\text{Average Noise Area}}$$

Limit of Detection	Limit of Quantification
$\frac{36475}{10531.83} = 3.46$	$\frac{76923}{10531.83} = 7.77$
$1.05 mg\ L^{-1}$	$5.22 mg\ L^{-1}$

Precision (%RSD)

The precision (%RSD) of solutions of esomeprazole, venlafaxine HCl and fenofibrate at 10mg L^{-1} level were 0.79, 0.73 and 0.62% respectively. The corresponding precisions (%RSD) at 200mg L^{-1} level were 0.39, 0.91 and 0.35% respectively. Results are shown in Table 4.11. Representative chromatograms for esomeprazole, venlafaxine HCl and fenofibrate at 10mg L^{-1} is shown in Figure 4.27 and at 200mg L^{-1} is shown in Figure 4.28.

Table 4.11. Precision study at 10mg L^{-1} and 200mg L^{-1}

Precision (10mg L^{-1}level)			
Replication	Esomeprazole	Venlafaxine	Fenofibrate
	(10.37mg L^{-1})	(10.18mg L^{-1})	(10.45mg L^{-1})
R1	170272	162825	162418
R2	172358	160483	161302
R3	172587	162984	161535
R4	172709	161482	163487
R5	174530	160892	163801
R6	172875	160254	162163
Mean	172555	161487	162451
SD	1362.25	1175.89	1013.81
%RSD	0.79	0.73	0.62
Precision (200mg L^{-1})			
Replication	Esomeprazole	Venlafaxine	Fenofibrate
	(207.36 mg L^{-1})	(203.58mg L^{-1})	(208.95mg L^{-1})
R1	3429268	3224735	3245100
R2	3457973	3209076	3234179
R3	3426742	3160318	3251789
R4	3433912	3227045	3252902
R5	3418623	3172329	3227377
R6	3435213	3222352	3255675
Mean	3433622	3202643	3244504
SD	13318.02	29064.36	11390.2
%RSD	0.39	0.91	0.35

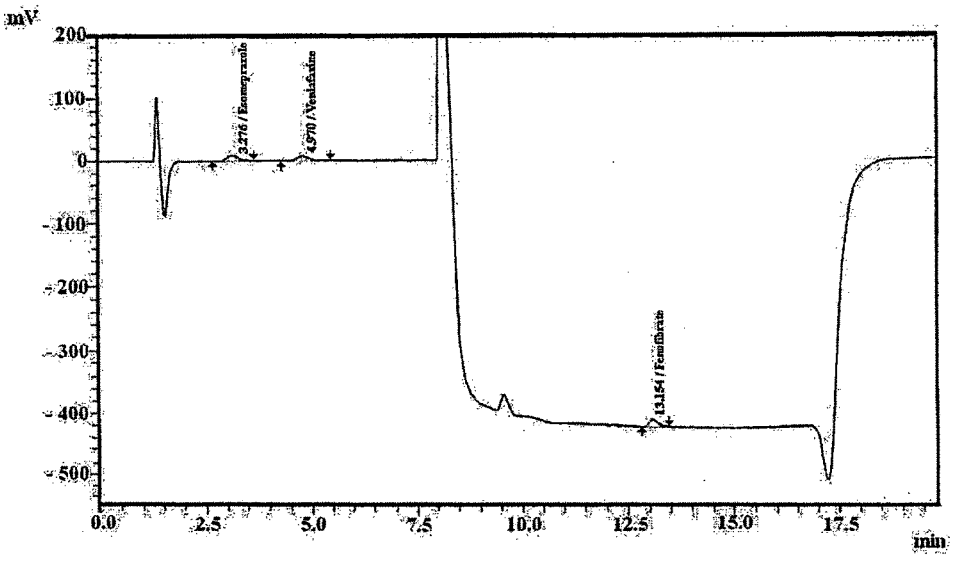

Figure 4.27. Precision – Chromatogram for 10mg L^{-1}

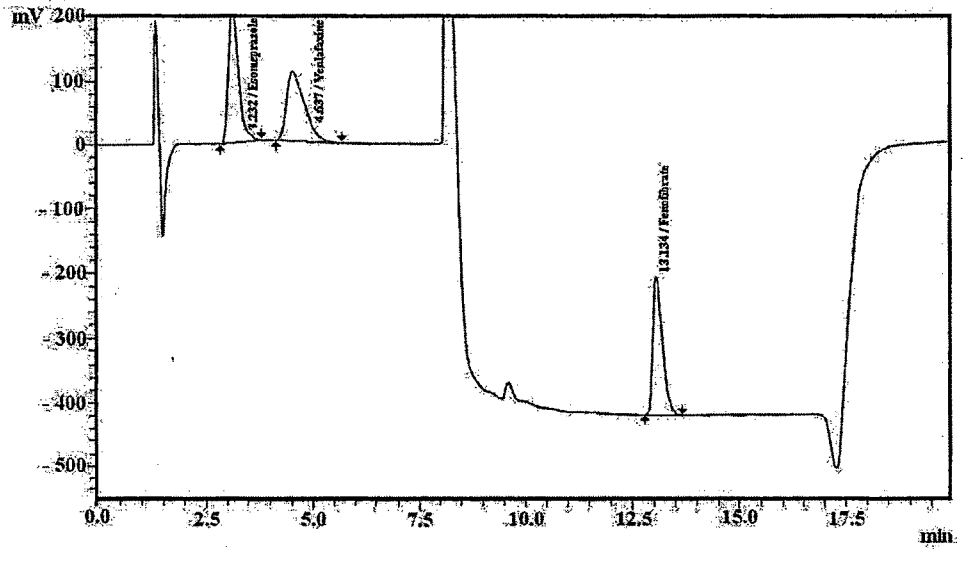

Figure 4.28. Precision – Chromatogram for 200mg L^{-1}

Accuracy (%Recovery)

The mean accuracies (%recovery) of esomeprazole, venlafaxine and fenofibrate in environmental water samples at LOQ level were 95.21, 73.28 and 71.07% respectively.

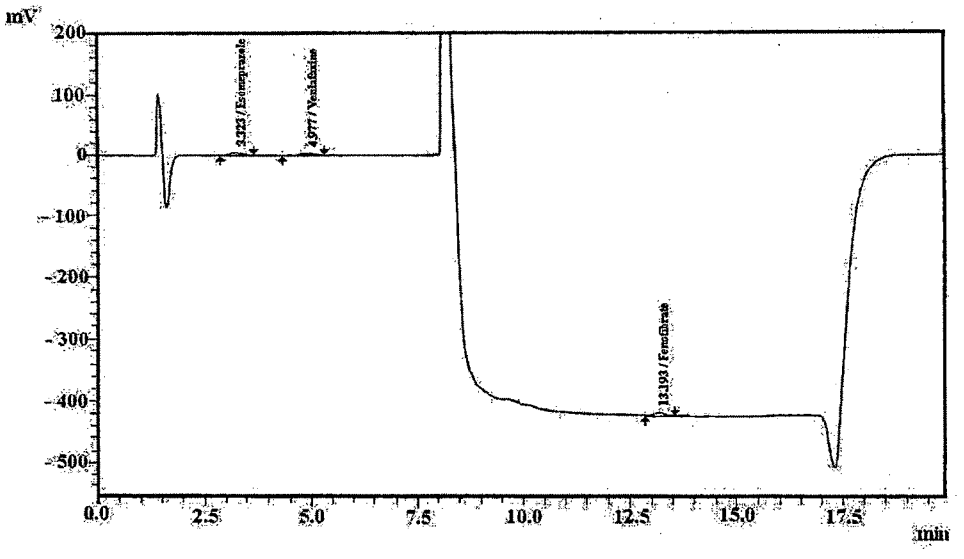

Figure 4.29. Accuracy – Chromatogram 5mg L^{-1} R1

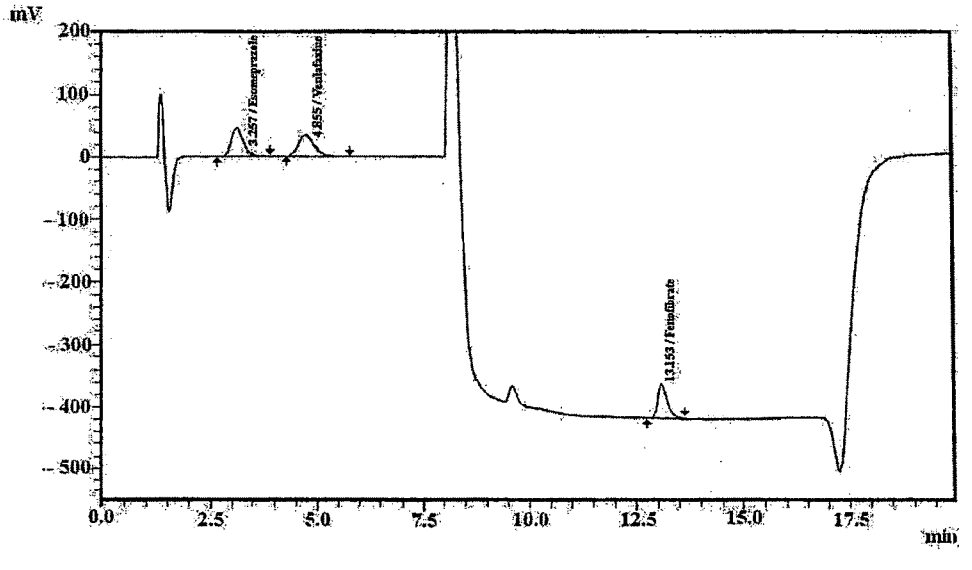

Figure 4.30. Accuracy – Chromatogram 50mg L^{-1} R1

The corresponding mean accuracies (%recovery) at 10 times LOQ level were 73.1, 75.36 and 73.72% for esomeprazole, venlafaxine and fenofibrate respectively. Results shown in Table 4.12, 4.13 and 4.14. The representative chromatograms of accuracy study are shown in Figure 4.29 to 4.30.

Table 4.12. Acuracy study for esomeprazole

Fortification Level (mg L^{-1})	Replication	Peak Area of Sample	Recovered (mg L^{-1})	Recovery (%)	Mean Conc.	Mean Recovery (%)	Standard Deviation	% RSD
Control	R1	ND	ND	-	-	-	-	-
	R2	ND	ND	-				
5.18	R1	80995	5.16	99.61	4.93	95.21	4.25	4.46
	R2	79294	5.06	97.68				
	R3	75890	4.85	93.63				
	R4	79891	5.09	98.26				
	R5	71206	4.56	88.03				
	R6	76291	4.87	94.02				
51.84	R1	599559	36.83	71.05	37.89	73.1	2.00	2.73
	R2	624209	38.33	73.94				
	R3	606541	37.25	71.86				
	R4	63611	39.06	75.35				
	R5	600478	36.88	71.14				
	R6	635240	39.01	75.25				

Typical Calculation

Intercept with y – axis (a)	- 3513.49	Slope of the line (b)	16375.54
Correlation of coefficient (r)	0.999	Dilution Factor (D)	-

Concentration (mg L^{-1})	Precision (%RSD)	%Recovery
$= \frac{Y - a}{b} \times D$	$= \frac{\text{Standard Deviation}}{\text{Mean Recovery}} \times 100$	$= \frac{\text{Quantity Recovered}}{\text{Quantity Fortified}} \times 100$
$= \frac{80995 - (-3513.49)}{16375.54}$	$= \frac{4.25}{95.21} \times 100$	$= \frac{5.16}{5.18} \times 100$
= 5.16mg L^{-1}	= 4.46%	= 99.61%

ND = Not detected

Table 4.13 Acuracy study for venlafaxine HCl

Fortification Level (mg L^{-1})	Replication	Peak Area of Sample	Recovered (mg L^{-1})	Recovery (%)	Mean Conc.	Mean Recovery (%)	Standard Deviation	% RSD
Control	R1	ND	ND	-	-	-	-	-
	R2	ND	ND	-				
5.09	R1	89470	3.8	74.66	3.73	73.28	7.00	9.56
	R2	96331	4.25	83.50				
	R3	91150	3.91	76.82				
	R4	80563	3.22	63.26				
	R5	84292	3.47	68.17				
	R6	88378	3.73	73.28				
50.89	R1	611302	37.69	74.06	38.35	75.36	1.68	2.23
	R2	623447	38.48	75.61				
	R3	640479	39.58	77.78				
	R4	628523	38.8	76.24				
	R5	602696	37.13	72.96				
	R6	622729	38.43	75.52				

Typical Calculation			
Intercept with y – axis (a)	30904.46	Slope of the line (b)	15400.66
Correlation of coefficient (r)	0.999	Dilution Factor (D)	-

Concentration (mg L^{-1})	Precision (%RSD)	%Recovery
$= \frac{Y - a}{b} \times D$	$= \frac{\text{Standard Deviation}}{\text{Mean Recovery}} \times 100$	$= \frac{\text{Quantity Recovered}}{\text{Quantity Fortified}} \times 100$
$= \frac{89470 - (30904.46)}{15400.66}$	$= \frac{7.00}{73.28} \times 100$	$= \frac{3.8}{5.09} \times 100$
= 3.8mg L^{-1}	= 9.56%	= 74.66%

ND = Not detected

Table 4.13 Acuracy study for fenofibrate

Fortification Level (mg L^{-1})	Replication	Peak Area of Sample	Recovered (mg L^{-1})	Recovery (%)	Mean Conc.	Mean Recovery (%)	Standard Deviation	% RSD
Control	R1	ND	ND	-	-	-	-	-
	R2	ND	ND	-				
5.22	R1	74970	3.87	74.14	3.71	71.07	5.62	7.9
	R2	80175	4.21	80.65				
	R3	69675	3.53	67.62				
	R4	70573	3.59	68.77				
	R5	67612	3.39	64.94				
	R6	71396	3.64	69.73				
52.24	R1	605787	38.44	73.58	38.51	73.72	1.50	2.03
	R2	595464	37.77	72.30				
	R3	601726	38.17	73.07				
	R4	608102	38.59	73.87				
	R5	629623	39.99	76.55				
	R6	60004	38.07	72.88				

Typical Calculation

Intercept with y – axis (a)	15485.60	Slope of the line (b)	15356.84
Correlation of coefficient (r)	0.999	Dilution Factor (D)	-

Concentration (mg L^{-1})	Precision (%RSD)	%Recovery
$= \frac{Y-a}{b} \times D$	$= \frac{\text{Standard Deviation}}{\text{Mean Recovery}} \times 100$	$= \frac{\text{Quantity Recovered}}{\text{Quantity Fortified}} \times 100$
$= \frac{74970-(15485.60)}{15356.84}$	$= \frac{5.62}{71.07} \times 100$	$= \frac{3.87}{5.22} \times 100$
= 3.87mg L^{-1}	= 7.9%	= 74.14%

ND = Not detected

Identification of Esomeprazole, Venlafaxine HCl and Fenofibrte by LC – MS

The MS spectrum of esomeprazole in methanol is shown in Figure 4.31.

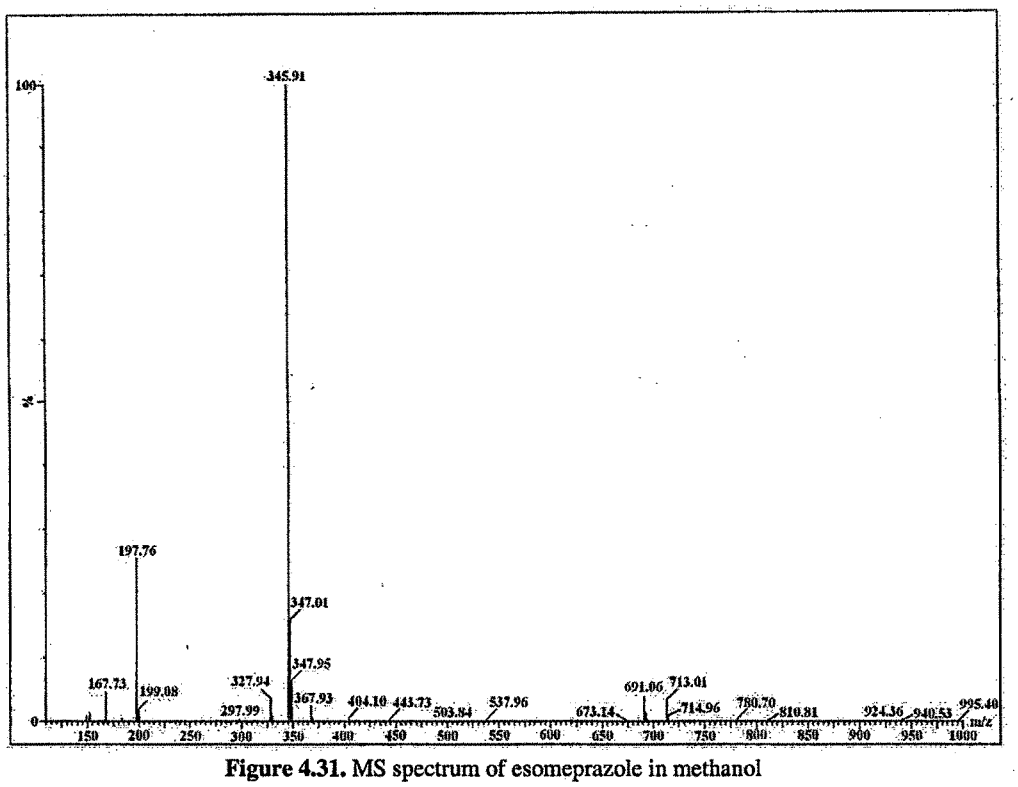

Figure 4.31. MS spectrum of esomeprazole in methanol

The MS spectrums of venlafaxine HCl and fenofibrate in methanol are shown in Figure 4.31 and Figure 4.32.

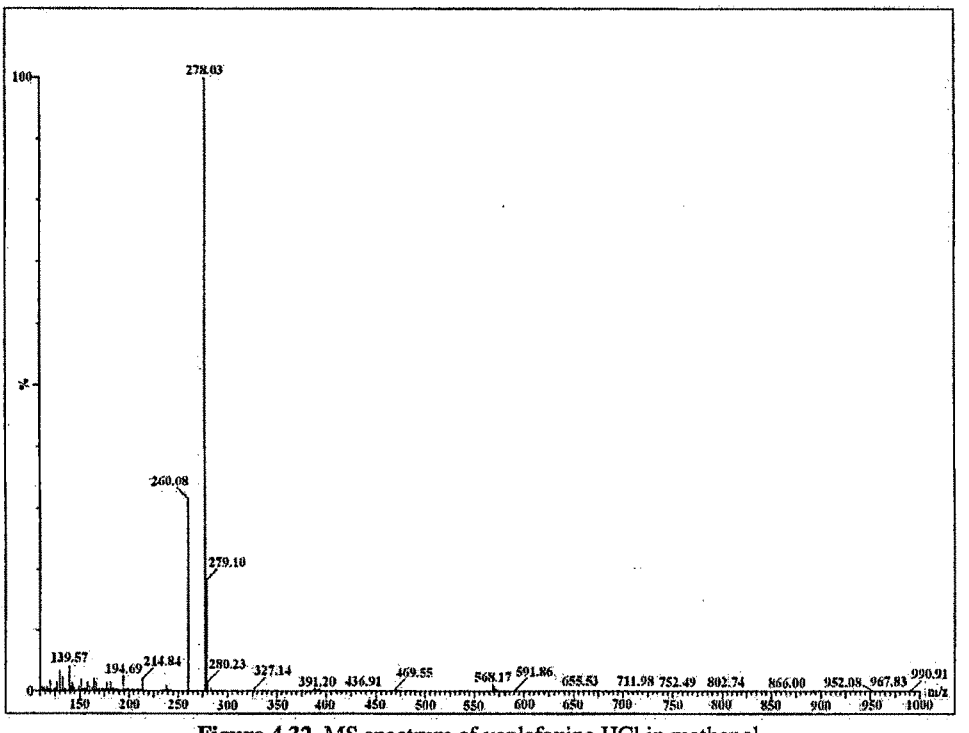

Figure 4.32. MS spectrum of venlafaxine HCl in methanol

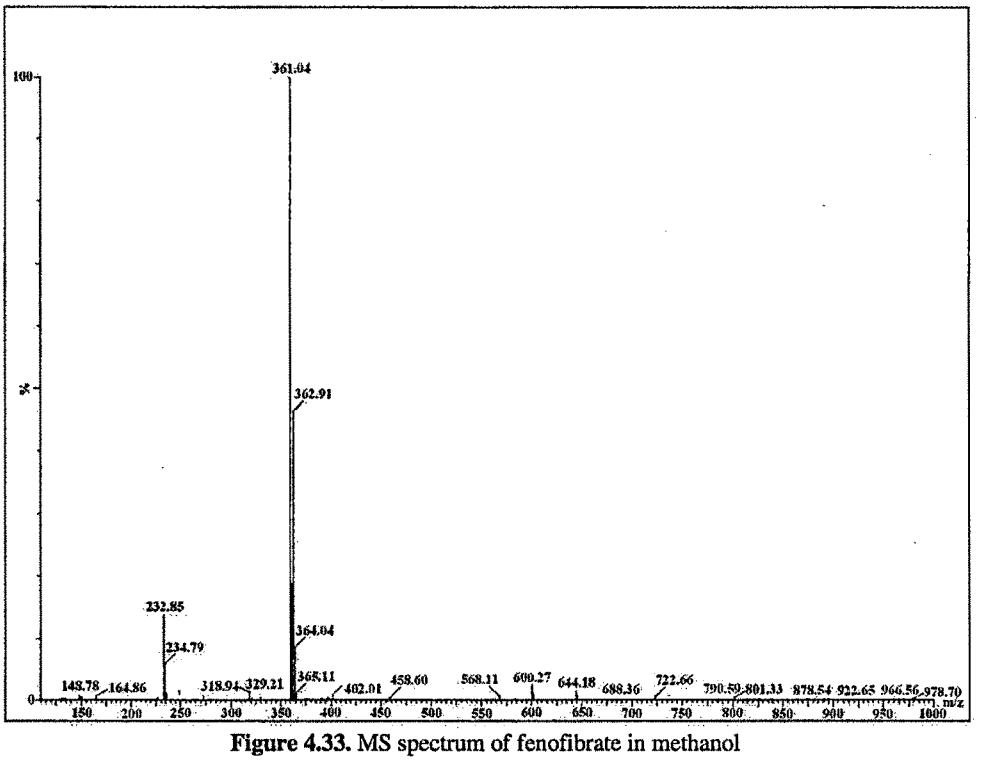

Figure 4.33. MS spectrum of fenofibrate in methanol

Separation and Identification of Esomeprazole, Venlafaxine HCl and Fenofibrate by LC – MS.

HPLC of environmental water sample with PDA detector did not show any peaks correspond to the three drugs when the environmental water sample was analysed without pre – concentration. LC – MS for the Environmental water sample without pre – concentration did not show presence of the three drugs. The representative chromatograms of 100mg L^{-1} esomeprazole, venlafaxine HCl and fenofibrate in methanol obtained by HPLC, PDA detector are shown in Figure 4.34, 4.35 and 4.36 respectively.

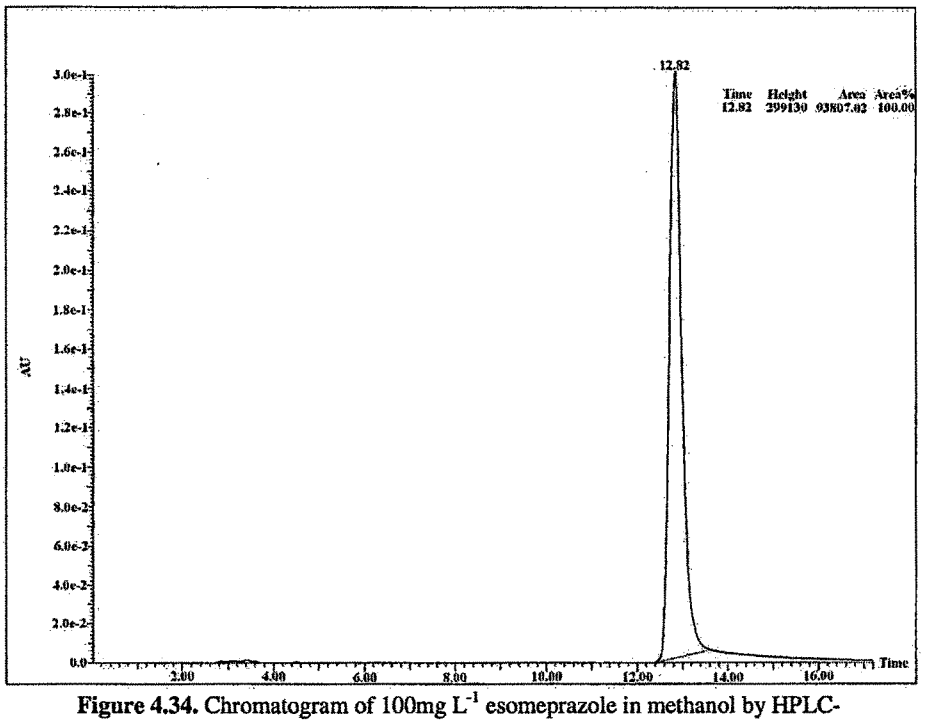

Figure 4.34. Chromatogram of 100mg L^{-1} esomeprazole in methanol by HPLC-

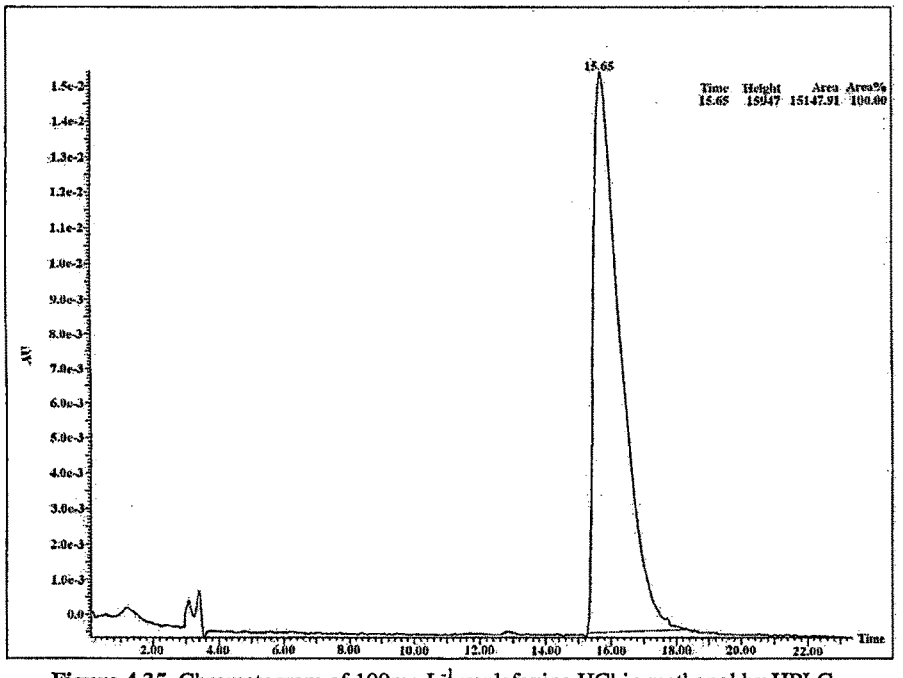

Figure 4.35. Chromatogram of 100mg L^{-1} venlafaxine HCl in methanol by HPLC-

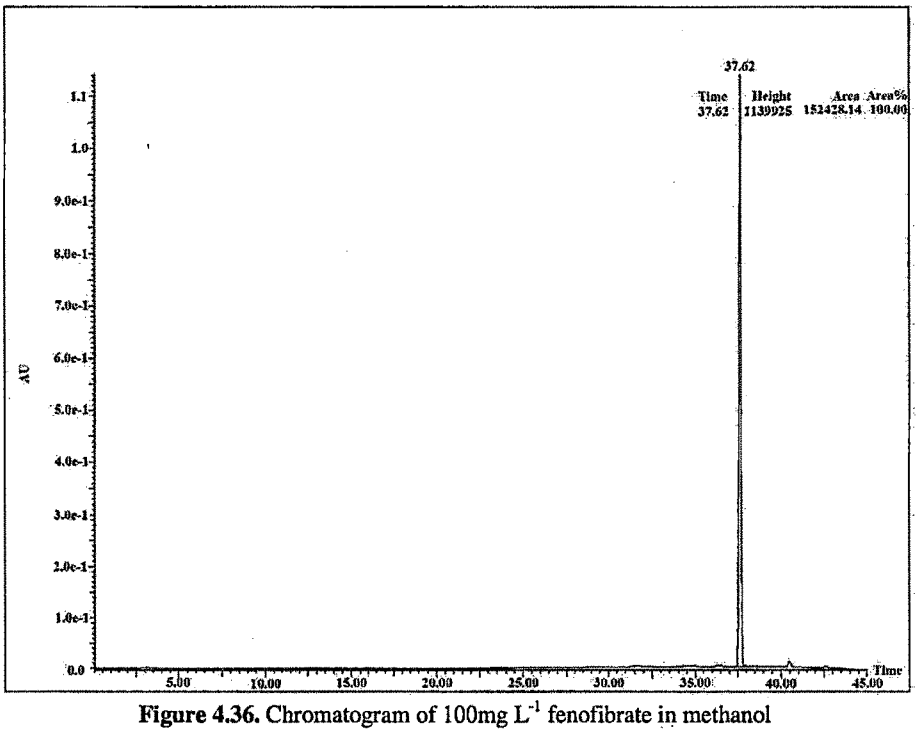

Figure 4.36. Chromatogram of 100mg L^{-1} fenofibrate in methanol

The representative chromatograms of 5, 10 and 15µL of environmental water sample analyzed by HPLC – PDA detector are shown in Figure 4.37, 4.38 and 4.39.

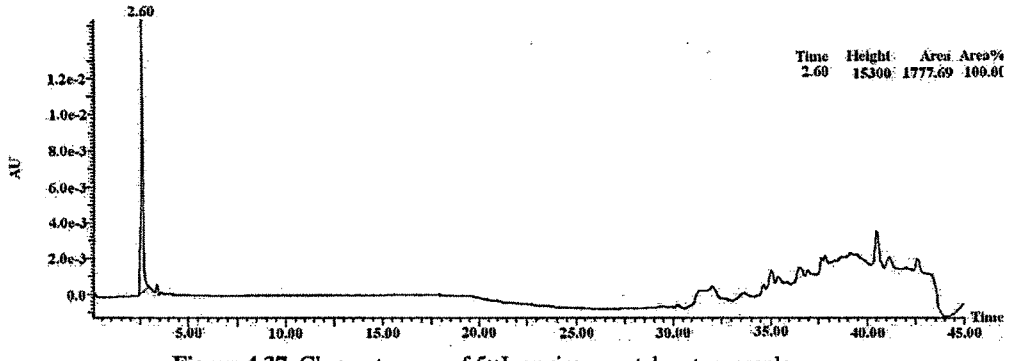

Figure 4.37. Chromatogram of 5µL environmental water sample

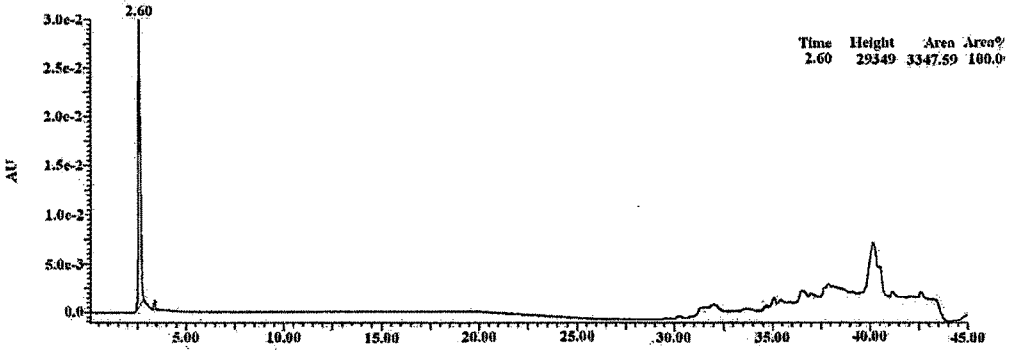

Figure 4.38. Chromatogram of 10µL environmental water sample

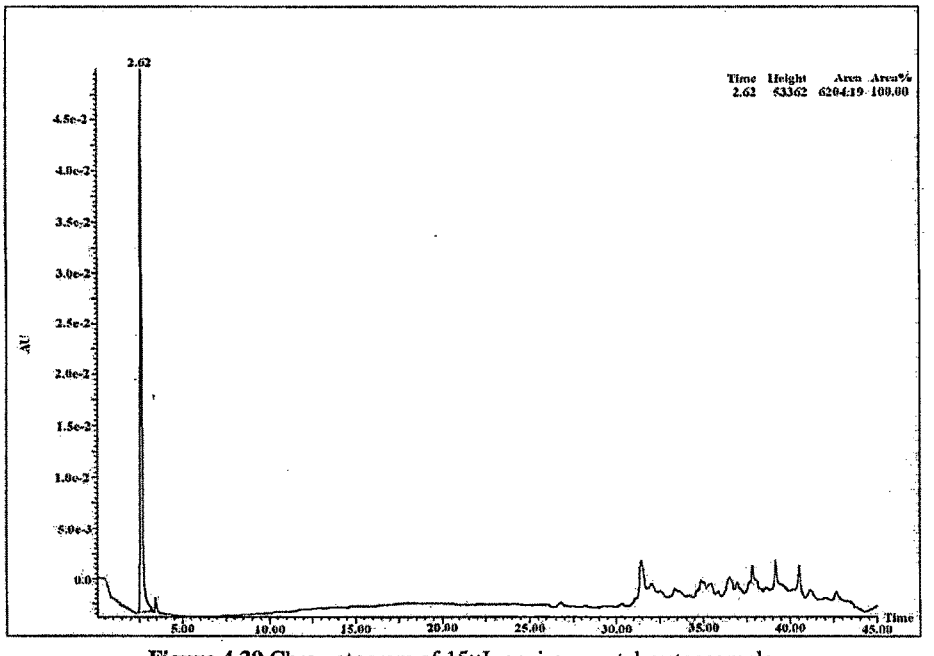

Figure 4.39 Chromatogram of 15µL environmental water sample

The MS spectrums of environmental water sample is shown in Figure 4.40.

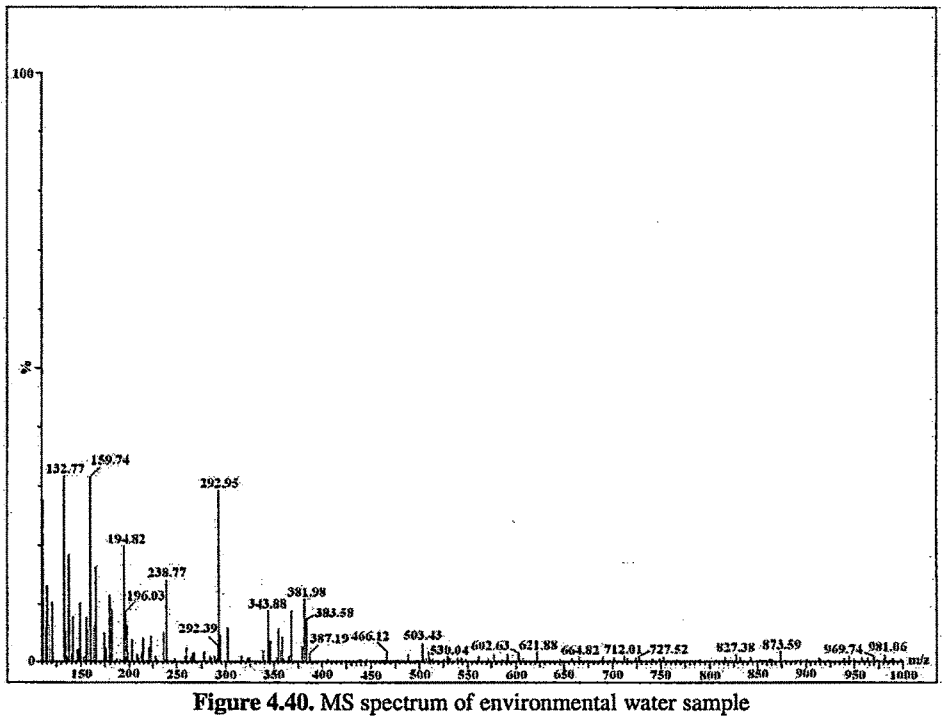

Figure 4.40. MS spectrum of environmental water sample

CONCLUSION

The gradient RP – LC method developed for determination of esomeprazole, venlafaxine HCl and fenofibrate is precise, accurate and specific. The developed, validated method could separate esomeprazole, venlafaxine HCl and fenofibrate with good resolution. The method can be used for routine analysis.

From LC – MS, results does not show presence of esomeprazole, venlafaxine HCl or fenofibrate in environmental water sample. This indicates water samples collected from STP (Vadodara – India) after treatment does not show presence of esomeprazole, venlafaxine HCl and fenofibrate. This may be due to extensive dilution occurring during the treatment process or the STP is efficient in removing the drug effectively.

CHAPTER 5

REMOVAL OF DRUGS FROM AQUEOUS SOLUTION USING CARBON BASED MATERIALS

Drugs have been shown to pass intact through conventional STPs, into water ways, lakes and aquifers and discharged pharmaceuticals may end up at landfill sites posing a threat to underlying ground water (Jones *et al.* 2003). Presently, all STPs are not designed to completely remove most pharmaceuticals and these compounds are consequently released into surface water (Zuccato *et al.* 2008; Carballa *et al.* 2004; Stackelberg *et al.* 2007), making it important to develop new methods to treat water containing such pollutants.

Whether or not trace pollutants can be eliminated in a WWTP essentially depends on the biological treatment stage. In Europe, biological wastewater treatment has been adapted step – by – step during the past 40 years in response to the tightening of discharge quality regulations.

Sorption: One of the most important elimination processes in WWTPs is sorption to suspended solids in the wastewater and subsequent removal by sedimentation as primary and secondary sludge. Sorption mainly occurs by absorption, involving hydrophobic interactions of the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms and the fat fractions of the sludge, and by adsorption, where electrostatic interactions of positively charged groups (e.g., amino groups) with the negatively charged surfaces of the microorganisms are of importance. The quantity of a substance sorbed per liter of wastewater (C_{sorbed}) is expressed as a simplified linear equation:

$$C_{sorbed} = K_d \cdot SS \cdot C_{dissolved} \qquad (1)$$

where K_d is the sorption constant, defined as the partition of a compound between the sludge and the water phase; SS is the concentration of suspended solids in the raw wastewater; and $C_{dissolved}$ is the dissolved concentration of the substance. An example is the antibiotic ciprofloxacin, which was administered in the United States after anthrax attacks as a reserve antibiotic and is excreted as a metabolite of enrofloxacin.

Despite being an extremely polar compound, ciprofloxacin sorbs onto the suspended solids of the sewage sludge to a high degree (Golet *et al.* 2003). At

neutral pH, the sorption is likely to be based mainly on electrostatic interactions between the positively charged amino group and the negatively charged surfaces of the microorganisms. Microorganisms in the secondary sludge make up the greatest proportion of the suspended solids; therefore, a relatively high sorption constant of K_d 20 liters per gram of suspended solids (Lg_{SS}^{-1}) and a relatively high sorbed fraction was observed. However, primary sludge contains few microorganisms and has a large fat fraction, so the K_d of ciprofloxacin in the primary sludge is only - 2 L/g_{SS}. This means that ~20% of the ciprofloxacin is sorbed onto the primary sludge, whereas more than double this load partitions onto the secondary sludge.

Thus, when municipal sludge is applied to the land, substantial loading of ciprofloxacin may take place. On the other hand, the contraceptive 17 – ethinylestradiol exhibited similar K_d values (0.28 and 0.35 L g_{SS}^{-1}) for both primary and secondary sludge, which means that the removal via sorption is <10% (Ternes *et al.* 2004b). Musk fragrances like tonalide (AHTN) have much higher sorption portions. Because they lack functional moieties (such as –OH, –COOH, or $–NH_2$), these compounds are not charged at neutral pH; hence, the sorption is probably caused by nonspecific sorption interactions. Many acidic pharmaceuticals, such as the anti – inflammatory ibuprofen and acetylsalicylic acid and the lipid regulators clofibric acid and bezafibrate, are negatively charged at neutral pH, because their carboxylic moieties are deprotonated. For all these polar pharmaceuticals, sorption onto sludge was found to be negligible. Because of the high polarity, significant sorption by nonspecific interactions can be ruled out for many pharmaceuticals. Until now, specific interactions of pharmaceuticals have only been reported for fluorochinolones and tetracyclines; the latter tend to precipitate with Mg^{2+}, Ca^{2+}, or Fe^{3+}.

Biological degradation. In wastewater, PPCPs occur primarily at concentrations of $<10^{-4}$g L^{-1} (Ternes 1998; Ternes 2000; Haberer 2002b). At these levels, biological transformation or degradation of the trace pollutants occurs only if a primary substrate is available for the corresponding bacteria to grow on. Hence, co-metabolism probably occurs, in which case the bacteria break down or partially converts the trace pollutant and do not use it as a carbon source. In another likely

scenario, mixed-substrate growth takes place and the bacteria use the trace pollutant as a carbon and energy source and may mineralize it totally. A trace pollutant's affinity for the bacterial enzymes in the activated sludge influences the pollutant's transformation or decomposition. Two possible mechanisms could explain this trend. The bacterial population may become more diversified with increasing sludge age (i.e., longer residence time of microorganisms), possibly because slow growing bacteria eventually reach relevant numbers.

Alternatively, the microorganisms may diversify their metabolic activity in response to the lower sludge loading with bulk organics (i.e., lower substrate availability); in this case, an increased PPCP removal might be due only to the broadened enzyme spectrum and not necessarily to the microbial community. The anti-inflammatory diclofenac and the contraceptive 17 – ethinylestradiol are good examples. For both compounds, significant decomposition was observed only when the aerobic sludge age was at least eight days (Kreuzinger 2004; Tilton 2002; Buser *et al.* 1998b).

The redox conditions also affect bacteria's degradation ability. Degradation can occur under aerobic (molecular oxygen available), denitrifying (no molecular oxygen available, nitrate available), or anaerobic (neither molecular oxygen nor nitrate available) conditions. For example, the natural estrogens 17 estradiol and estrone degrade in the aerobic and anoxic tanks of the activated sludge system, whereas the synthetic contraceptive 17 – ethinylestradiol decomposes only under aerobic conditions (Lai 2000; Holbrook 2002; Matsui 2000; Johnson and Sumpter 2001; Andersen *et al.* 2003).

Because of the low concentrations of trace organic pollutants, the decomposition occurs primarily as a first – order reaction:

$$r_{decomposition} = k_{decomposition} \cdot SS \cdot C_{dissolved} \quad (2)$$

where $k_{decomposition}$ is the rate constant and $C_{dissolved}$ is the dissolved concentration of the pollutant. Hence, a cascade of denitrifying and aerated tanks operating at conditions similar to those of a plug – flow reactor is advantageous because it

results in lower discharge concentrations than is the case with a single, fully mixed reactor.

With respect to pharmaceuticals, the metabolites excreted by humans should be accounted for when the mass flux of a compound during wastewater treatment is described. For instance, aspirin and its metabolites can occur in raw wastewater at the $\mu g\ L^{-1}$ level with a removal rate generally >80%. Many pharmaceuticals are conjugated with glucuronic acid or sulfate to enhance their polarity prior to excretion. The conjugates of the natural hormones estrone and estradiol, for example, are generally present in the same concentration range as the free compounds in the raw wastewater (Adler *et al.* 2001).

However, the conjugates can be cleaved in WWTPs, which releases active pharmaceuticals (Ternes *et al.* 1999b). The anti – inflammatory ibuprofen, for instance, is directly conjugated or first hydroxylated and then conjugated. Approximately 15% of ibuprofen is excreted unchanged or as its glucuronide; the remaining percentage is allocated to further metabolites, such as hydroxyl – ibuprofen, carboxy – ibuprofen, and their respective conjugates.

Hence, the fate of metabolites is of major relevance for the mass balance. In the case of an ecotoxicological risk, such as when the receiving water is used for irrigation in agriculture or the WWTP outflow undergoes low dilution in surface water, ozonation of the biologically purified wastewater should be considered. If 5 – 10g m^{-3} of ozone are used, concentrations of many pharmaceuticals are reduced below detection limits (Ternes *et al.* 2004a). The effectiveness of the ozone treatment depends on the chemical properties of the compound and the back ground level of dissolved organic carbon in the waste water (Huber *et al.* 2003). Although ozonation costs only a few cents per cubic meter of wastewater, the energy expenditure is 0.1– 0.2 kilowatt – hours per cubic meter, which is significant in comparison with the total energy consumption of a WWTP. In addition, although initial results indicate significantly reduced toxicity, oxidation products formed during ozonation should be further investigated prior to large – scale application (Huber *et al.* 2004).

Adsorption by activated charcoal is frequently the most efficient and economical method for removing pollutants from water, particularly when these are present in low concentrations, whether it is a batch process or continuous flow treatment method. Literature reports several studies on use of activated charcoal for removal of a variety of pollutants from water (Garcia – Araya *et al.* 2003; Safarik *et al.* 1997). Charcoal, the forerunner of modern activated charcoal has been recognized as the oldest adsorbent known in wastewater treatment. Its ability to purify water dates back to 2000 B.C. Lowitz established the first used of charcoal for the removal of bad tastes and odours from water on an experimental basis in 1789 – 1790.

The credit of developing commercial activated carbon however goes to Raphael von Ostrejko whose inventions were patented in 1900 and 1901. Early applications of carbon in water treatment plant to remove chlorophenolics were reported by Balyis in U.S. and Sierp in Germany in 1929 (Bhatnagar and Minocha 2006). Activated charcoal is also recommended for removing poisonings caused by drugs in human body (Mohd *et al.* 2006; Eddleston *et al.* 2008).

The removal efficiency of activated charcoal for organic compounds may be increased by presence of metal ions or complexes on the surface of activated charcoal. Concept was to use the activated charcoal which has previously been used for removal of metals in effluents. Since disposal of such carbon is a problem (Bhatnagar and Minocha 2006). So before disposing, whether we can use the metal loaded carbon once more for removing pharmaceuticals or organic compounds. Aspirin and paracetamol were considered as target drugs for the present study of removal efficiency of commercially available activated charcoal (granular) and effect of metal complexes on it. Effect of oxygen on the efficiency of activated charcoal loaded with metal complex to remove these compounds was also studied.

It was expected that in presence of metal complexes and oxygen the non – polar part of organic pollutants would be oxidized and become more polar, increasing its affinity for carbon. Hence its removal should be more complete from water. This is because several transition metal ions and metal complexes are known to act as catalyst for oxidation of organic compounds in presence of oxygen (Jana *et al.* 2007; Silva *et al.* 2004; Silva *et al.* 2002).

EXPERIMENTS

Chemicals and Reagents

Copper chloride dihydrate ($CuCl_2.2H_2O$), manganese (II) chloride tetra hydrate ($MnCl_2.4H_2O$), copper sulphate pentahydrate ($CuSO_4.5H_2O$), nickel sulphate heptahydrate ($NiSO_4.7H_2O$), cobalt chloride hexahydrate ($CoCl_2.6H_2O$), nickel chloride hexahydrate ($NiCl_2.6H_2O$), acetylacetone, salicylaldehyde, ethylenediammine, alcohol, liquor ammonia, methanol (CH_3OH), chloroform ($CHCl_3$), acetonitrile (CH_3CN) A.R. grade obtained from Qualigens whereas, activated charcoal (granular) were obtained from National Chemicals.

Instrumentation

A UV/VIS spectrometer (Perkins Elmer Lambert 35) equipped with 1cm quartz cells (4ml each) was used for all absorbance measurements.

Treatment of activated charcoal

100g of charcoal (granular) was washed with conductivity water to remove fine carbon particles. After this, it was dried at temperature 110°C in hot air oven for 3hr. This was then used for further studies in small portions.

Synthesis of metal complexes

Copper Bisacetylacetonate: Bis(acetylacetonate) copper was prepared using procedures adapted from those described in the literature (Lipatova and Nizelskii 1968), by mixing redistilled acetylacetone with an aqueous suspension of copper hydroxide, freshly precipitated with ammonia. The precipitate obtained was filtered, washed with water and finally with alcohol and air dried at room temperature. *Anal. Calcd. ($C_{10}H_{14}O_4Cu$): C, 45.88; H, 5.35.Found C, 45.84; H, 5.37.*

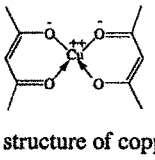

Figure 5.1. Chemical structure of copper bisacetylacetonate

Manganese Salen: The manganese (III) Schiff base complex was prepared using procedures adapted from those described in the literature (Silva *et al.* 2004), by refluxing equimolar quantities of an ethanolic solution of ligand and a methanolic solution of $MnCl_2.4H_2O$. *Anal. Calcd. ($C_{16}H_{14}N_2O_2MnCl$): C, 53.86; H, 3.92; N, 7.84. Found C, 53.2; H, 4.05; N, 7.81.*

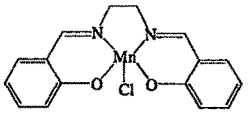

Figure 5.2. Chemical structure of manganese salen

Copper Salen: The copper Schiff base complex was prepared using procedure adapted from that described in the literature (Holm *et al.* 1966), by stirring equimolar quantities of an ethanolic solution of ligand and aqueous solution of $CuCl_2.2H_2O$. *Anal. Calcd. ($C_{16}H_{14}N_2O_2CuI$): C, 58.26; H, 4.24; N, 8.49. Found C, 58.28; H, 4.26; N, 8.50.*

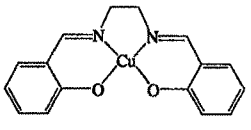

Figure 5.3. Chemical structure of copper salen

Preparation of stock drug solution

Drug Solution

Stock solutions and working standard solution of aspirin and paracetamol were prepared as mentioned in Chapter 2.

Metal Complex Solution

Stock solutions of 2000mg L^{-1} copper bisacetylacetonate and copper salen complex was prepared by dissolving 200mg respective metal complex in 100mL $CHCl_3$. Similarly Stock solution of 2000mg L^{-1} manganese salen complex was prepared in Acetonitrile. Working standard solutions were obtained by diluting standard solutions with respective solvents to obtain 100mL, 1000mg L^{-1} metal

complex solution. To obtain standard curve, solution of different concentration were prepared from stock solutions.

Metal Salt solution

0.2M Copper sulphate solution was prepared by dissolving 12.484g $CuSO_4.5H_2O$ in 250mL DDW. Similarly 0.2M Nickel sulphate, Cobalt chloride and Nickel Chloride solutions were prepared by dissolving 13.143g of $NiSO_4.7H_2O$, 11.896g of $CoCl_2.6H_2O$ and 11.884g of $NiCl_2.6H_2O$ in 250mL DDW respectively. Working standard solutions were obtained by diluting standard solutions with DDW to obtain 100mL, 0.1M metal salt solution. To obtain standard curve, solution of different concentration were prepared from stock solutions.

Loading of metal complexes (MAC)

The copper bisacetylacetonate complex was dissolved in $CHCl_3$ (500mg L^{-1}). 5g of Activated charcoal (AC) was put into the copper bisacetylacetonate $CHCl_3$ solution for 3 hours at room temperature with occasional stirring. The resulting material (MAC_1) was filtered off, washed, dried and stored in bottle.

Similarly manganese salen was laoded on activated charcoal by dissolving in ACN to get manganese salen laoded activated charcoal (MAC_2) and copper salen by dissolving in $CHCl_3$ to get copper salen laoded activated charcoal (MAC_3) respectively.

The amount of the metal complex remaining in the filtrate was determined by recording absorbance of the solution at the λ_{max} of the respective metal solution and computing the concentration from corresponding calibration curve of respective metal solution. The amount of metal complex adsorbed on charcoal was computed using absorbance value for solution before passing it through charcoal.

Leach out test for Metal Complex loaded Activated Charcoal

1.0g of activated charcoal loaded with a particular metal complex was treated with 10.0mL respective solvent with occasional shaking for 30 minute. It was then

filtered and absorbance was recorded in the filtrate using UV – Visible spectrophotometer at respective wavelength of metal complex solution.

Loading of metal ions (MC)

5.0g of activated charcoal was put into 50mL of 0.1M $CuSO_4$ solution for 3hr. at room temperature with occasional stirring. The resultant material (MC_1) was filtered off, washed, dried and stored in bottles.

Similarly $NiSO_4$ was loaded on activated charcoal to get $NiSO_4$ loaded activated charcoal (MC_2), $CoCl_2$ was loaded on activated charcoal to get $CoCl_2$ loaded activated charcoal (MC_3) and $NiCl_2$ was loaded on activated charcoal to get $NiCl_2$ loaded activated charcoal (MC_4)

The amount of the metal salt remaining in the filtrate was determined by recording absorbance of the solution at the λ_{max} of the respective metal solution and computing the concentration from corresponding calibration curve of respective metal solution. The amount of metal salt adsorbed on charcoal was computed using absorbance value for solution before passing it through charcoal.

Leach out test for Metal ion loaded Activated Charcoal

1.0g of activated charcoal loaded with a particular metal salt was treated with 10.0mL conductivity water with occasional shaking for 30 minute. It was then filtered and absorbance was recorded in the filtrate using UV – Visible spectrophotometer at respective wavelength of metal complex solution.

Drug Removal Procedures

The following two sets of experiments were applied to the three aqueous solutions of aspirin, paracetamol and mixture of aspirin and paracetamol.

Set 1: 10mL (50mg L^{-1}) aspirin solution was added into two different stoppered tubes containing AC and MAC respectively for 30 minutes with constant stirring. Then the

resultant solution was filtered and absorbance was measured through UV – Visible Spectrometer.

Set 2: 10mL (50mg L^{-1}) aspirin solution was added into two different stopper tubes containing AC and MAC respectively for 30 min with constant supply of oxygen. Then the resultant solution was filtered and absorbance was measured through UV – Visible Spectrometer.

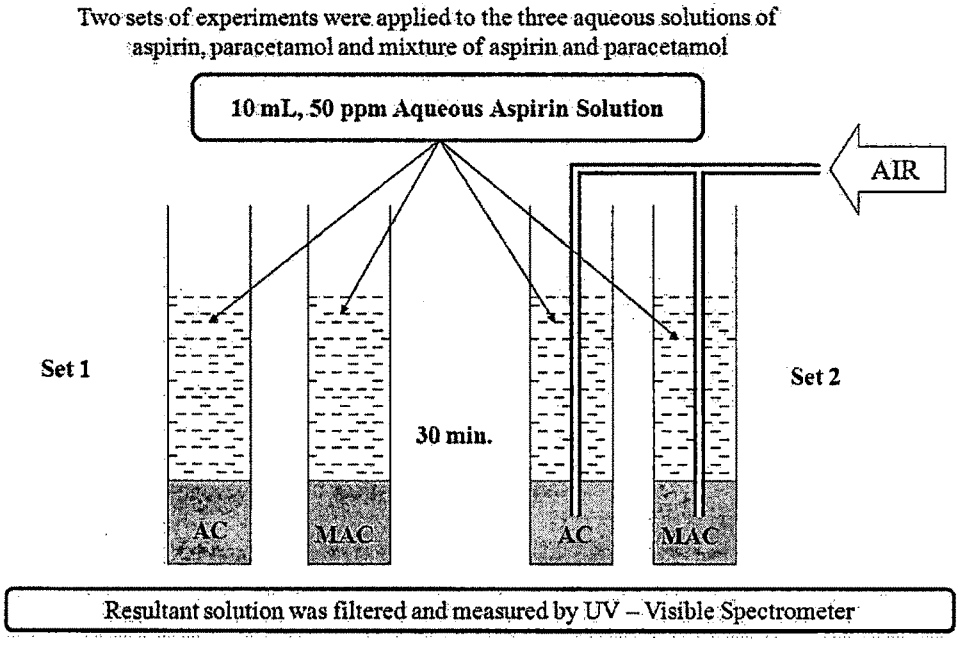

Figure 5.4. Experimental set up for drug removal procedure

The same above mentioned set of experiments were applied individually to all charcoal samples loaded with three metal complexes and metal solutions respectively. The experimental set up used is shown in figure 5.4.

RESULTS AND DISCUSSION

The removal efficiency of AC may be increased by presence of some metal complexes on the surface of AC. In this study the effect of air (oxygen) on three different metal complexes for removal of aspirin and paracetamol from water is reported. For the study two different drugs aspirin and paracetamol are considered. These are the most reported drugs as water pollutant.

The amount of metal complex and metal salts loaded on activated charcoal is given in Table5.1.

Table 5.1. Percentage loading of metal complexes and metal ions on activated charcoal

Adsorbent	Concentration of metal complex (mg L^{-1})					Percentage Loaded
	Before	After	Adsorbed	Leached out	Loaded	
MAC_1	1000	110	890	N.D.	890	89.0
MAC_2	1000	95	905	N.D.	905	90.5
MAC_3	1000	150	850	N.D.	850	85.0
Adsorbent	Concentration of metal ions (M)					Percentage Loaded
	Before	After	Adsorbed	Leached out	Loaded	
MC_1	0.1	0.094	0.006	N.D.	0.006	6.0
MC_2	0.1	0.096	0.004	N.D.	0.004	4.0
MC_3	0.1	0.089	0.011	N.D.	0.011	11.0
MC_4	0.1	0.087	0.013	N.D.	0.013	13.0

N.D. = Not detected. Percentage loaded = (Adsorbed concentration / Initial concentration) x 100.

The MACs were prepared and the drug solutions containing a known concentration of aspirin and paracetamol were treated. The amount of unabsorbed drug in filtrate was measured by UV – Visible spectrometer and the adsorption percent was calculated.

The amount of the individual drugs present in mixture was determined by solving the equations obtained adding Beer – Lambert's law (A = abc), for two components mixture as mentioned in chapter 2. Amount of aspirin and paracetamol in water was determined by the calibration curves given in Figure 2.4 and Figure 2.15 in chapter 2.

AC removes 14.29% aspirin and 47% paracetamol from water when the individual aqueous drug solutions were treated with it, and in presence of oxygen the percentage of removal increases for both drugs. Results shown in Table 5.2. Sr. No. 1 and 2.

Table 5.2. Percentage removal of drugs by activated charcoal

Sr. No.	Drug in aqueous solution		Percentage of drug removed from aqueous solution by AC	
			without O_2	with O_2
1.	Individual	Aspirin	14.29	20.93
2.		Pracetamol	47.00	94.00
3.	Mixture	Aspirin	18.72	58.23
4.		Pracetamol	30.84	97.89

When the mixture of aqueous drug solution was treated with AC, the percentage removal of individual drug increases compared with the individual treatment with AC except paracetamol whose adsorption decreases in absence of oxygen as shown in Table 5.2. Sr. No. 4.

MAC_1 removes 27% aspirin and 39.25% paracetamol from water when the individual aqueous drug solutions were treated with it, and in presence of oxygen the percentage of removal increases for both drugs. Results shown in Table 5.3. Sr. No. 1 and 2.

Table 5.3. Percentage removal of drugs by MAC_1

Sr. No.	Drug in aqueous solution		Percentage of drug removed from aqueous solution by MAC_1	
			without O_2	with O_2
1.	Individual	Aspirin	27.00	70.00
2.		Pracetamol	39.25	90.19
3.	Mixture	Aspirin	17.49	78.50
4.		Pracetamol	19.57	78.54

When the mixture of aqueous drug solution was treated with MAC_1, the percentage removal of individual drug increases in presence of oxygen compared with the treatment in absence of oxygen. The percentage removal of aspirin by MAC_1 with oxygen increases in present of paracetamol. Results shown in Table 5.3. Sr. No. 3 and 4.

MAC_2 removes 12.96% aspirin and 41.98% paracetamol from water when the individual aqueous drug solutions were treated with it, and in presence of oxygen the percentage of removal increases for both drugs. Results shown in Table 5.4. Sr. No. 1 and 2.

Table 5.4. Percentage removal of drugs by MAC_2

Sr. No.	Drug in aqueous solution		Percentage of drug removed from aqueous solution by MAC_2	
			without O_2	with O_2
1.	Individual	Aspirin	12.96	20.37
2.		Pracetamol	41.98	92.59
3.	Mixture	Aspirin	27.23	66.39
4.		Pracetamol	42.31	99.74

When the mixture of aqueous drug solution was treated with MAC_2 in presence of oxygen, the removal percentage of drugs form aqueous solution increases. Results shown in Table 5.4. Sr. No. 3 and 4.

Similar trends were observed with MAC_3. In presence of oxygen, drug removal efficiency of MAC_3 increases. Results shown in Table 5.5.

Table 5.5. Percentage removal of drugs by MAC_3

Sr. No.	Drug in aqueous solution		Percentage of drug removed from aqueous solution by MAC_3	
			without O_2	with O_2
1.	Individual	Aspirin	25.58	34.88
2.		Pracetamol	50.00	89.65
3.	Mixture	Aspirin	19.22	62.95
4.		Pracetamol	52.64	99.97

In case of individual drug solutions, MAC_1 removes maximum amount of aspirin from water when compared with other three adsorbents in absence of oxygen. In presence of oxygen the trend remains same but removal efficiency of MAC_1 for aspirin increases to 38% compared to that with absence of oxygen. Whereas MAC_3 separates maximum amount of paracetamol from water when compared with other three adsorbent in absence of oxygen. In presence of oxygen the removal efficiency of MAC_1, MAC_2 and MAC_3 increases with maximum. Comparison of results is shown in Table 5.6.

Table 5.6. Percentage removal of drugs individually by MAC_s

Sr. No.	Adsorbent	Aspirin		Paracetamol	
		without O_2	with O_2	without O_2	with O_2
1.	AC	14.29	20.93	47.00	94.00
2.	MAC1	27.00	70.00	39.25	90.19
3.	MAC2	12.96	20.37	41.98	92.59
4.	MAC3	25.58	34.88	50	89.65

In case of treatment of drugs in presence of each other, MAC_2 separates maximum amount of aspirin from water when compared with other three adsorbent in absence of oxygen, whereas in presence of oxygen MAC_1 separates maximum amount of aspirin. When paracetamol is considered, MAC_3 adsorbs maximum in absence of oxygen and almost 100% in presence of oxygen. Comparison of results is shown in Table 5.7.

Table 5.7. Percentage removal of drugs in presence of each other by MAC_s

Sr. No.	Adsorbent	Aspirin		Paracetamol	
		without O_2	with O_2	without O_2	with O_2
1.	AC	18.72	58.23	30.84	97.89
2.	MAC1	17.49	78.50	19.57	78.54
3.	MAC2	27.23	66.39	42.31	99.74
4.	MAC3	19.22	62.95	52.64	99.97

MC_1 removes 62.5% aspirin and 48.84% paracetamol from water when the individual aqueous drug solutions were treated with it, and in presence of oxygen the percentage of removal remains same for both drugs. Results shown in Table 5.8. Sr. No. 1 and 2.

Table 5.8. Percentage removal of drugs by MC_1

Sr. No.	Drug in aqueous solution		Percentage of drug removed from aqueous solution by MC_1	
			without O_2	with O_2
1.	Individual	Aspirin	62.5	62.5
2.		Pracetamol	48.84	48.84
3.	Mixture	Aspirin	54.51	54.47
4.		Pracetamol	55.27	55.48

When the mixture of aqueous drug solution was treated with MC_1, the percentage removal of aspirin drug decreases compared with its individual treatment with MC_1 and the percentage of removal of paracetamol increases compared with its individual treatment with MC_1 as shown in Table 5.8. Sr. No. 3 and 4. Presence of oxygen does not change the drug removal efficiency of MC_1 as shown in Table 5.8.

MC_2 removes 50% aspirin and 55.82% paracetamol from water when the individual aqueous drug solutions were treated with it, and in presence of oxygen the percentage of removal remains same for both drugs. Results shown in Table 5.9. Sr. No. 1 and 2.

Table 5.9. Percentage removal of drugs by MC_2

Sr. No.	Drug in aqueous solution		Percentage of drug removed from aqueous solution by MC2	
			without O_2	with O_2
1.	Individual	Aspirin	50.00	50.00
2.		Pracetamol	55.82	54.65
3.	Mixture	Aspirin	62.71	62.74
4.		Pracetamol	62.02	62.00

When the mixture of aqueous drug solution was treated with MC_2, the percentage removal of individual drug increases compared with the individual treatment with MC_2 and in presence of oxygen the percentage of removal remains same for both drugs. Results shown in Table 5.9.

MC_3 removes 60.42% aspirin and 48.84% paracetamol from water when the individual aqueous drug solutions were treated with it, and in presence of oxygen the percentage of removal remains same for both drugs. Results shown in Table 5.10. Sr. No. 1 and 2.

Table 5.10. Percentage removal of drugs by MC_3

Sr. No.	Drug in aqueous solution		Percentage of drug removed from aqueous solution by MC_3	
			without O_2	with O_2
1.	Individual	Aspirin	60.42	60.42
2.		Pracetamol	48.84	48.84
3.	Mixture	Aspirin	42.41	42.40
4.		Pracetamol	54.30	54.31

When the mixture of aqueous drug solution was treated with MC_3, the percentage removal of aspirin drug decreases compared with its individual treatment with MC_3 and the percentage of removal of paracetamol increases compared with its individual treatment with MC_3 as shown in Table 5.10. Sr. No. 3 and 4. Presence of oxygen does not change the drug removal efficiency of MC_3 as shown in Table 5.10.

MC_4 removes 87.5% aspirin and 58.14% paracetamol from water when the individual aqueous drug solutions were treated with it and in presence of oxygen the percentage of removal for aspirin decreases where as for paracetamol it increases. Results shown in Table 5.11. Sr. No. 1 and 2.

Table 5.11. Percentage removal of drugs by MC_4

Sr. No.	Drug in aqueous solution		Percentage of drug removed from aqueous solution by MC_4	
			without O_2	with O_2
1.	Individual	Aspirin	87.5	77.03
2.		Pracetamol	58.14	60.46
3.	Mixture	Aspirin	32.25	32.27
4.		Pracetamol	43.20	43.16

When the mixture of aqueous drug solution was treated with MC_4, the percentage removal of aspirin and paracetamol decreases compared with its individual

treatment with MC_3 as shown in Table 5.11. Sr. No. 3 and 4. Presence of oxygen does not change the drug removal efficiency of MC_4 as shown in Table 5.11.

In case of individual drug solutions, MC_4 separates maximum amount of aspirin from water when compared with other four adsorbents in absence of oxygen. In presence of oxygen the trend remains same but removal efficiency of MC_4 for Aspirin decreases to 77.03% compared to that with absence of oxygen i.e. 87.5%. Similarly MC_4 separates maximum amount of paracetamol from water when compared with other four adsorbent in absence of oxygen. Presence of oxygen does not affect the drug removal efficiency of all adsorbents except AC and MC_4. Comparison of results is shown in Table 5.12.

Table 5.12. Percentage removal of drugs individually by MC_s

Sr. No.	Adsorbent	Aspirin		Paracetamol	
		without O_2	with O_2	without O_2	with O_2
1.	AC	14.29	20.93	47	94
2.	MC_1	62.5	62.5	48.84	48.84
3.	MC_2	50.0	50.0	55.82	54.65
4.	MC_3	60.42	60.42	48.84	48.84
5.	MC_4	87.5	77.03	58.14	60.46

In case of treatment of drugs in presence of each other, MC_2 separates maximum amount of aspirin and paracetamol from water when compared with other four adsorbent in absence of oxygen. Presence of oxygen does not affect the drug removal efficiency of all four adsorbents except AC. Comparison of results is shown in Table 5.13.

Table 5.13. Percentage removal of drugs in presence of each other by MC_s

Sr. No.	Adsorbent	Aspirin		Paracetamol	
		without O_2	with O_2	without O_2	with O_2
1.	AC	18.72	58.23	30.84	97.89
2.	MC_1	54.51	54.47	55.27	55.48
3.	MC_2	62.71	62.74	62.02	62.0
4.	MC_3	42.41	42.40	54.30	54.31
5.	MC_4	32.25	32.27	43.20	43.16

The relation of percentage removal of drugs by activated charcoal loaded with copper complex and copper ion was also studied. The relation shows, the removal efficiency of activated charcoal loaded by copper complex increases in presence of

oxygen whereas no change is observed in case of activated charcoal loaded by copper ion in presence of oxygen. The relation between percentage removal of activated charcoal loaded with copper ion and copper complex is shown in Table 5.14.

Table 5.14. Percentage removal of drugs by copper complexes and copper ion

Adsorbent	Drug solution		Without O_2	With O_2
Activated Charcoal	Individual	Aspirin	14.29	20.93
		Paracetamol	47.00	94.00
	Mixture	Aspirin	18.72	58.23
		Paracetamol	30.84	97.89
89.0% Copper Biacetylacetonnate Loaded Activated Charcoal	Individual	Aspirin	27.00	70.00
		Paracetamol	39.25	90.19
	Mixture	Aspirin	17.49	78.50
		Paracetamol	19.57	78.54
85.0% Copper Salen Loaded Activated Charcoal	Individual	Aspirin	25.58	34.88
		Paracetamol	50.00	89.65
	Mixture	Aspirin	19.22	62.95
		Paracetamol	52.64	99.97
6.0% Copper Sulphate Loaded Activated Charcoal	Individual	Aspirin	62.50	60.42
		Paracetamol	48.84	48.84
	Mixture	Aspirin	54.51	55.27
		Paracetamol	54.47	55.48

Similarly percentage removal of drugs by activated charcoal loaded with nickel ion was studied. Removal efficiency of activated charcoal increases in presence of nickel but in presence of oxygen no improvement was observed. The relation between percentage removal of activated charcoal loaded with nickel ion is shown in Table 5.15.

Table 5.15. Percentage removal of drugs by nickel ions

Adsorbent	Drug solution		Without O_2	With O_2
Activated Charcoal	Individual	Aspirin	14.29	20.93
		Paracetamol	47.00	94.00
	Mixture	Aspirin	18.72	58.23
		Paracetamol	30.84	97.89
4.0% Nickel Sulphate Loaded Activated Charcoal	Individual	Aspirin	50.00	50.00
		Paracetamol	55.82	54.65
	Mixture	Aspirin	62.17	62.74
		Paracetamol	62.02	62.00
13.0% Nickel Chloride Loaded Activated Charcoal	Individual	Aspirin	87.50	77.03
		Paracetamol	58.14	60.46
	Mixture	Aspirin	32.25	32.27
		Paracetamol	43.20	43.16

The result also shows, in case of metal ions, nickel removes maximum amount of drugs from water although much difference is not seen in presence of oxygen. Whereas in case of metal complexes more than 98% of drugs are removed from water particularly paracetamol when treated in presence of oxygen. The percentage removal of drugs by metal loaded activated charcoal increase in presence of oxygen.

Surface studies (BET) were done to study the surface properties of activated charcoal and metal loaded activated charcoal. Surface studies shows that surface area of metal loaded activated charcoal decreases after removing drugs from water. This shows the adsorption of drugs on metal loaded activated charcoal.

Analytical performance characteristics

Linearity was established with a series of working standard solutions prepared by diluting the stock solution with respective solvents individually to the final concentrations for each metal complex. Calibration curves were obtained by measuring the UV absorbance of the standard solutions of Metal complex in a range of 200 – 1000mg L^{-1} and Metal salt solution in a range of 0.002 – 0.1M at their respective wavelengths. Absorbance of each concentration was measured in triplicate and the mean value of peak area was taken for the calibration curve.

Copper Bisacetylacetonate

Maximum absorbance was measured at 547nm for copper bisacetylacetonate in chloroform. Linearity experiment in the range of 200 – 1000mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 5.16.

Table 5.16. Linearity experiment for copper biacetylacetonate in chloroform: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	200	0.1774
2.	400	0.3492
3.	600	0.5205
4.	800	0.7054
5.	1000	0.8693

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 5.17. The plot of absorbance Vs concentration for copper biacetylacetonate in $CHCl_3$ was shown in Figure 5.5.

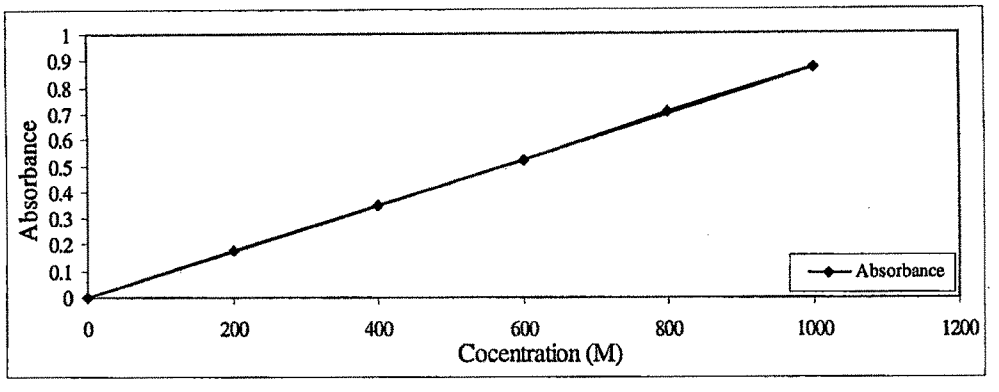

Figure 5.5. Linear working range of copper bisacetylacetonate in chloroform

Table 5.17. Results of regression analysis: Copper bisacetylacetonate in chloroform

Parameters	Copper Bisacetylacetonate in $CHCl_3$
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9997
Slope, a	0.0009
Intercept,	0.0022
No. of observations	5

The calibration graph is described by the following equation: $y = 0.0009x + 0.0022$ ($r^2 = 0.9997$).

Manganese salen

Maximum absorbance was measured at 480nm for manganese salen in acetonitrile. Linearity experiment in the range of 200 – 1000mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 5.18.

Table 5.18. Linearity experiment for manganese salen in acetonitrile: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	200	0.4766
2.	400	0.9366
3.	600	1.3835
4.	800	1.8566
5.	1000	2.3166

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 5.19. The plot of absorbance Vs concentration for manganese salen in CH_3CN was shown in Figure 5.6.

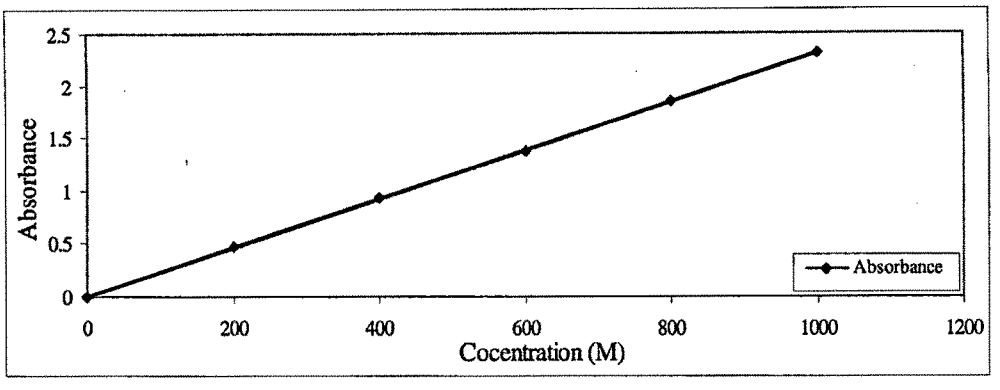

Figure 5.6. Linear working range of manganese salen in acetonitrile

Table 5.19. Results of regression analysis : Manganese salen in acetonitrile

Parameters	Manganese Salen in Acetonitrile
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9999
Slope, a	0.0023
Intercept,	0.0066
No. of observations	5

The calibration graph is described by the following equation: $y = 0.0023x + 0.0066$ ($r^2 = 0.9999$).

Copper Salen

Maximum absorbance was measured at 565nm for copper salen in chloroform. Linearity experiment in the range of 200 – 1000mg L^{-1} was carried out. The absorbance values with respective concentrations are tabulated in Table 5.20

Table 5.20. Linearity experiment for copper salen in chloroform: Concentration Vs absorbance

Observation No.	Concentration (mg L^{-1})	Absorbance
1.	200	0.0354
2.	400	0.0757
3.	600	0.1114
4.	800	0.1462
5.	1000	0.1853

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 5.21. The plot of absorbance Vs concentration for copper salen in $CHCl_3$ is shown in Figure 5.7.

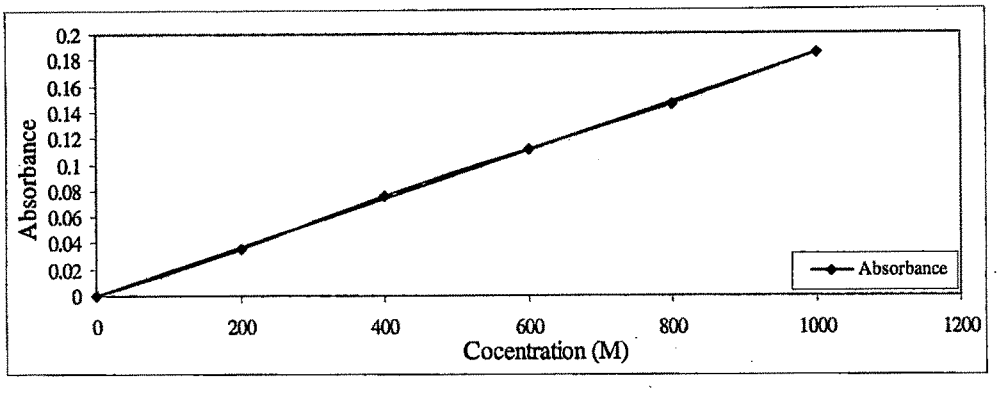

Figure 5.7. Linear working range of copper salen in chloroform

Table 5.21. Results of regression analysis: Copper salen in chloroform

Parameters	Copper Salen in $CHCl_3$
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9996
Slope, a	0.0002
Intercept,	0.0002
No. of observations	5

The calibration graph is described by the following equation: $y = 0.0002x + 0.0002$ ($r^2 = 0.9996$).

Copper Sulphate

Maximum absorbance was measured at 800nm for copper sulphate in DDW. Linearity experiment in the range of 0.002 – 0.1M was carried out. The absorbance values with respective concentrations are tabulated in Table 5.22.

Table 5.22 Linearity experiment for copper sulphate in water: Concentration Vs absorbance

Observation No.	Concentration (M)	Absorbance
1.	0.002	0.0253
2.	0.004	0.0522
3.	0.006	0.0794
4.	0.008	0.1044
5.	0.01	0.1276
6.	0.02	0.2612
7.	0.04	0.5066
8.	0.06	0.7433
9.	0.08	0.9862
10.	0.1	1.2083

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 5.23. Figure 5.8. shows the plot of absorbance Vs concentration for copper sulphate in DDW.

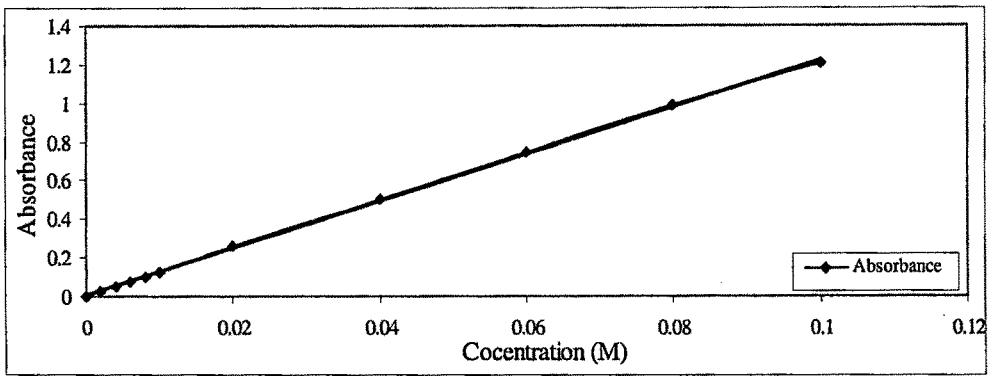

Figure 5.8. Linear working range of copper sulphate in water

Table 5.23 Results of regression analysis: Copper sulphate in water

Parameters	Copper Sulphate in water
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9996
Slope, a	12.161
Intercept,	0.0073
No. of observations	10

The calibration graph is described by the following equation: $y = 12.161x + 0.0073$ ($r^2 = 0.9996$).

Nickel Sulphate

Maximum absorbance was measured at 393nm for nickel sulphate in water. Linearity experiment in the range of 0.002 – 0.1M was carried out. The absorbance values with respective concentrations are tabulated in Table 5.24.

Table 5.24 Linearity experiment for nickel sulphate in water: Concentration Vs absorbance

Observation No.	Concentration (M)	Absorbance
1.	0.002	0.008
2.	0.004	0.017
3.	0.006	0.0246
4.	0.008	0.0335
5.	0.01	0.0425
6.	0.02	0.0852
7.	0.04	0.1768
8.	0.06	0.2664
9.	0.08	0.356
10.	0.1	0.447

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 5.25. The plot of absorbance Vs concentration for nickel sulphate in DDW is shown in Figure5.9.

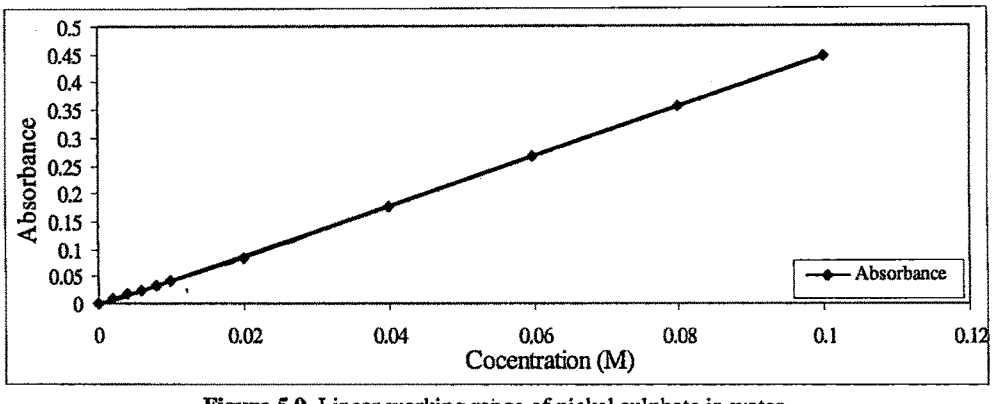

Figure 5.9. Linear working range of nickel sulphate in water

Table 5.25 Results of regression analysis: Nickel sulphate in water

Parameters	Nickel Sulphate in water
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9999
Slope, a	4.4763
Intercept,	- 0.002
No. of observations	10

The calibration graph is described by the following equation: $y = 4.4763x - 0.002$ ($r^2 = 0.9999$).

Cobalt Chloride

Maximum absorbance was measured at 510nm for cobalt chloride in DDW. Linearity experiment in the range of 0.002 – 0.1M was carried out. The absorbance values with respective concentrations are tabulated in Table 5.26.

Table 5.26 Linearity experiment for cobalt chloride in water: Concentration Vs absorbance

Observation No.	Concentration (M)	Absorbance
1.	0.002	0.004
2.	0.004	0.0128
3.	0.006	0.0217
4.	0.008	0.0306
5.	0.01	0.0386
6.	0.02	0.0856
7.	0.04	0.1724
8.	0.06	0.2642
9.	0.08	0.3486
10.	0.1	0.4376

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 5.27. The plot of absorbance Vs concentration for cobalt chloride in DDW was shown in Figure 5.10.

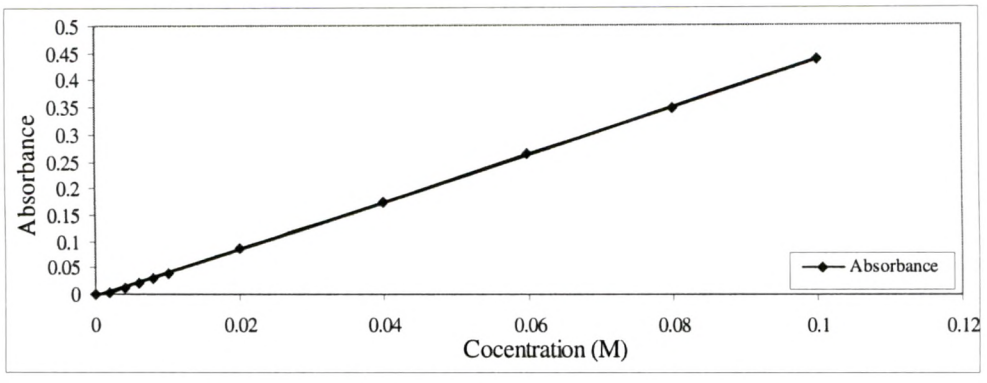

Figure 5.10. Linear working range of cobalt chloride in water

Table 5.27 Results of regression analysis: Cobalt chloride in water

Parameters	Cobalt Chloride in water
Regression Equation (y)	
Correlation Coefficient (r^2)	0.9999
Slope, a	4.419
Intercept,	- 0.0039
No. of observations	10

The calibration graph is described by the following equation: $y = 4.419x - 0.0039$ ($r^2 = 0.9999$).

Nickel Chloride

Maximum absorbance was measured at 394nm for nickel chloride in DDW. Linearity experiment in the range of 0.002 – 0.1M was carried out three times. The absorbance values with respective concentrations are tabulated in Table 5.28.

Table 5.28 Linearity experiment for cobalt chloride in water: Concentration Vs absorbance

Observation No.	Concentration (M)	Absorbance
1.	0.002	0.0092
2.	0.004	0.0201
3.	0.006	0.0301
4.	0.008	0.0352
5.	0.01	0.0454
6.	0.02	0.0854
7.	0.04	0.1756
8.	0.06	0.2581
9.	0.08	0.3441
10.	0.1	0.4352

The calibration data was subjected to regression analysis. The result of the regression analysis is given in Table 5.29. The plot of absorbance Vs concentration for nickel chloride in DDW was shown in Figure 5.11.

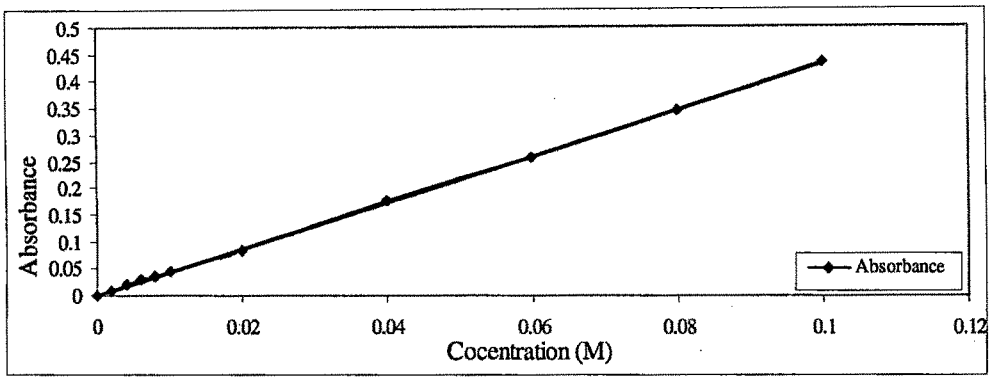

Figure 5.11. Linear working range of nickel chloride in water

Table 5.29 Results of regression analysis: Nickel chloride in water

Parameters	Nickel Chloride in water
Regression Equation (y)	
Correlation Coefficient (r^2)	0.999
Slope, a	4.312
Intercept,	0.001
No. of observations	10

The calibration graph is described by the following equation: $y = 4.312x + 0.001$ ($r^2 = 0.999$).

Acceptability of linearity data is judged by examining the coefficient of determination and the y – intercept as follows.

a. The plot of concentration Vs absorbance (mean of three observations) for the linear working range is depicted in Table 5.16 for copper bisacetylacetonate in chloroform, in Table 5.18 for manganese salen and in Table 5.20 for copper salen in chloroform. The plot shows that a linear relationship exists between concentration and absorbance in the range of concentration 200 – 1000mg L^{-1} obeying Beer's – Lambert's law for determination of all three metal complexes in their respective solution. The plot of concentration Vs absorbance (mean of three observations) for the linear working range is depicted in Table 5.22 for copper sulphate, in Table 5.24 for

nickel sulphate, in Table 5.26 for cobalt chloride and in Table 5.28 for nickel chloride. The plot shows that a linear relationship exists between concentration and absorbance in the range of concentration 0.002 – 0.1M obeying Beer's – Lambert's law for determination of all three metal salts in DDW.

b. The coefficient of determination i.e. 0.9997 for copper bisacetylacetonate in chloroform, 0.9999 for manganese salen in acetonitrile, 0.9996 for copper salen in chloroform, 0.9996 for copper sulphate in DDW and 0.9999 for nickel sulphate in DDW, 0.9999 for cobalt chloride in DDW and 0.999 nickel chloride in DDW means that almost 99.9% of variation in y i.e. the change in the response of the analyte can be explained by the change in x i.e. concentration of the analyte in the respective solutions. The correlation coefficient is a measure of goodness of the fit of the calculated line to the sample data.

c. The slope of the regression line is 0.0009 for copper bisacetylacetonate in chloroform, 0.0023 for manganese salen in acetonitrile, 0.0022 for copper salen in chlorofrom,12.161 for copper sulphate in DDW and 4.4771 for nickel sulphate in DDW, 4.419 for cobalt chloride in DDW and 4.312 nickel chloride in DDW this indicates that one unit increase in the concentration of copper bisacetylacetonate in chloroform, manganese salen in acetonitrile, copper salen in chloroform, copper sulphate, nickel sulphate, cobalt chloride and nickel chloride in DDW will result in an increase in the absorbance value by 0.0022, 0.0009, 0.0023, 12.161, 4.4771, 4.419 and 4.312 units respectively.

CONCLUSION

Almost in all cases of metal complex loaded activated charcoal, the adsorption of drugs increases in presence of oxygen with respect to their corresponding adsorbent. In case of MAC_1 a distinct difference on the adsorption behavior of aspirin and paracetamol is observed: aspirin and paracetamol in presence of each other adsorbed less compared to individual treatment in absence of oxygen, but in presence of oxygen adsorption of aspirin increases and paracetamol decreases in presence of each other. Similarly in case of MAC3 amount of aspirin adsorbs in less amount when treated with paracetamol in absence of oxygen compared to its individual treatment

but in presence of oxygen adsorption efficiency of MAC_3 increases both for aspirin and paracetamol in presence of each other as compared to individual treatment. At this juncture we are unable to provide suitable explanation for the observed trend.

All most in all cases of metal salts loaded activated charcoal, the adsorption of drugs increases in absence of oxygen with respect to activated charcoal which is not loaded with metal salts. The effect of oxygen was not observed on the absorption of adsorbents except AC. MC_2 removes maximum about of drugs in absence of oxygen and presence of oxygen increases removal efficiency of MC_2. In case of MC_1 and MC_3 a distinct difference on the adsorption behavior of aspirin and paracetamol is observed: When treated individual aspirin adsorbed more compared to paracetamol and where as paracetamol adsorbed more compared to aspirin when treated in presence of each other. In case of MC_4, the percentage removal of both aspirin and paracetamol in presence of each other decreases compared to its individual treatment.

From the removal study it can be concluded that metal complex and metal ion loaded activated charcoal can remove drugs present in water. Its removal efficiency increases in presence of oxygen. Metal loaded charcoal can be used for removal of drugs present in water as tertiary treatment in water treatment plants. However, no specific trend could be observed in terms of use of metal salt or metal complex.

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APPENDIX 1

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A Simple Method For Simultaneous Determination Of Aspirin And Paracetamol In Treated Municipal Sewage Water In Vadodara

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ABSTRACT

A clean method for simultaneous determination of pharmaceutical compounds in treated sewage water has been developed based on UV-Vis spectrometry. This study is a part of larger work for determination of pharma products in water bodies in Vadodara, India. We report in this article data for determination of two drugs: aspirin and paracetamol. These drugs, used as model compounds, were separated from dilute aqueous solution by solid phase extraction(SPE). Macroporous beads of polystyrene divinyl benzene polymer or an anion exchanger were used for pre-concentration followed by spectrophotometric determination. Experimental parameters were optimized. The developed method was used for determination of the drugs in water sample collected from a sewage treatment facility. Presence of these drugs was not detected at the detection limits of the methods, viz. 0.1ppm. The drugs were also not detected in an HPLC method up to concentration of 0.039ppm.

KEYWORDS

Solid phase extraction;
Pre-concentration;
Aspirin;
Paracetamol;
UV-spectrometer;
HPLC.

INTRODUCTION

There is growing concern about the presence of pharmaceuticals or drugs in the aquatic environment. Following use, pharmaceuticals/drugs are excreted as the parent compound, water soluble conjugate or as metabolites and thus enter sewage treatment works (STWs). Disposal of unused pharmaceuticals can also be a route to the aquatic environment either through dumping to sewer via the toilet or drain, or to landfills in domestic refuse or as special water by licensed waste contractors[1] Despite their likely continuous discharge, little is known about the ultimate fate and transport of many drug substances after their intended application. This has led to pharmaceuticals attracting increasing attention as water pollutants due to their possible environmental effects[2]. The type/group of pharmaceuticals detected are fairly broad e.g. contraceptive hormones, lipid regulators, pain-killers, antibiotics, anti-cancer drugs, anti-epileptic drugs and those regulating blood pressure. Where

pharmaceuticals have been detected in sewage effluents or surface water, the levels are generally at the ng L^{-1} or at most, low mg L^{-1} level[1].

Such environmental trace analyses of organic pollutants usually is preferred by Gas Chromatography-Mass Spectrometry(GC-MS)or Liquid Chromatography-Mass Spectrometry(LC-MS)[2,3]. However, these techniques are relatively expensive and not easily available at several places. Need of the hour; therefore, is to develop simpler techniques which however, require higher concentration of drug molecules. Therefore, prior to the instrumental analysis, attention has to be paid to the sample preparation, and enrichment procedure.

Solid Phase Extraction(SPE) is the preferred method of sample enrichment/pre-concentration in environmental analytical chemistry. There are several solid phases on which the sample can be concentrated[3,4].

In the present work, drugs were pre-concentrated using Polystyrene Divinyl Benzene or anion-exchanger as solid phase, recovered by different solvents and later analyzed by UV-Spectrometer. After optimizing the conditions the drug samples of Aspirin and Paracetamol individually; the optimized conditions were used for mixture aqueous solution of aspirin and paracetamol together. Later the optimized method was applied for environmental sample.

EXPERIMENTS

A UV/Vis spectrometer(Perkin Elmer Lambert 35) equipped with 1cm quartz cells(4ml each) was used for all absorbance measurements. A glass column with stop-cock and packed with adsorbent material was used for pre-concentration. Chemicals and solvents used were: Double Distilled Water(DDW), methanol(qualigens), acetonitrile(qualigens), concentrated HCl(qualigens), aspirin{synthesized in laboratory[5] and characterized}, Paracetamol (API, collected from a drug industry), Polymer-Styrene Divinyl Benzene(PSDVB) beads macro-porous(8% and 12%) cross-linking and gel type(Kind gift from doshi ion-exchange, Ahmedabad, India) and anion-exchanger(Amberlite IRA-93).

For HPLC chromatographic determination, HPLC(Waters-Model number 2965 Seperation Module with empower pro software) using UV(2487 Channel 1) with measurements at 280nm and using a chromatographic column{Hyperssil BDS C_{18} (250×4.6)mm5μ} and the following solutions were also used: H_3PO_4/H_2O buffer 1ml L^{-1} and pH=3.0 with Triethyl amine(TEA)} and Acetonitrile(all of HPLC grade).

Stock solutions of drugs

Stock solutions of Aspirin and Paracetamol (1000ppm) were prepared by separately dissolving 100mg of each compound in 100ml DDW. Working standard solutions were obtained by diluting standard solution with DDW to obtain the concentration of 100ppm. The standard mixture solutions were prepared by mixing the working standard solutions to obtain 100ml, 25ppm of Aspirin and 25ppm of Paracetamol mixture respectively. To obtain standard curve, solutions of different concentrations were prepared from stock standard solutions.

Pre-concentration using PSDVB beads

Preliminary studies were conducted to work out the experimental conditions for the optimum adsorption and recovery. A typical experiment was performed by taking synthetic samples of Aspirin and Paracetamol. 100ml, 100ppm drug solution was passed through a column packed with 1gm of the adsorbent material. Adsorbed drug was recovered in 10 ml solvent. Amount of drug adsorbed was determined in eluate and the amount of drug recovered in solvent, by recording absorbance at 225nm for Aspirin. The adsorbent for SPE was activated using 5ml Acetonitrile followed by acetonitrile : DDW (80:20)(v/v).To optimize the experimental conditions nine different experimental parameters where changed (keeping all other parameters and conditions same for each experiment) and analysed for aspirin. With the optimized experimental conditions of Aspirin, paracetamol was pre-concentrated and its amount was determined by recording absorbance at 244nm. Different experimental parameters and its conditions are summarized in TABLE 1, including the results obtained from the each experiment.

Pre-concentration using anion exchanger

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TABLE 1 : Experimental parameters, conditions and their results for pre-concentration by PSDVB beads

Ex. No.	Adsorbent		Flow rate (ml min^{-1})	Aqueous solution Before Adsorption			Drug present in aqueous solution adsorbed on adsorbent				Solution used for recovery		Recovery of drug adsorbed			
	Type	Weight (gms)		Volume (ml)	Drug Present in solution (mg)		As (Weight)		Pr (Weight)		Type	Volume (ml)	As (Weight)		Pr (Weight)	
					As[A]	Pr[B]	mgs	%	mgs	%			mgs	%	mgs	%
i.	Macro12%	1	1	100	10	-[C]	0.55	5.5	-	-	H_2O	10	0.086	15.6	-	-
ii.	Gel	1	1	100	10	-	0.4	4	-	-	H_2O	10	-	-	-	-
iii.	Macro 12%	1	1	100	10	-	0.55	5.5	-	-	H_2O	10	0.086	15.6	-	-
iv.	Macro 8%	1	1	100	10	-	1.2	12	-	-	H_2O	10	0.083	6.92	-	-
v.	Macro 8%	0.5	1	100	10	-	0.4	4	-	-	H_2O	10	0.066	16.5	-	-
vi.	Macro 8%	1.5	1	100	10	-	1.4	14	-	-	H_2O	10	0.082	5.86	-	-
vii.	Macro 8%	1	0.1	100	10	-	2.1	21	-	-	H_2O	10	0.083	3.95	-	-
viii.	Macro 8%	1	1	100	10	-	1.2	12	-	-	5% CH_3OH	10	0.083	6.92	-	-
ix.	Macro 8%	1	1	100	10	-	1.2	12	-	-	20% CH_3OH	10	0.084	6.92	-	-
x.	Macro 8%	1	1	100	-	10	-	-	0.3	3	H_2O	10	-	-	0.016	5.33

In every experiment mentioned in the table, 5ml Acetonitrile was used for activation of solid phase and later Acetonitrile: Water (80:20) was used for washing

[A]As represents Aspirin ; [B]Pr represents Paracetamol ; [C]Dash represents that drug is not present in that experiment.

TABLE 2 : Experimental parameters, conditions and their results for pre-concentration by anion-exchangers

Ex. No.	Drug Present in solution (mg)		Drug present in aqueous solution adsorbed on adsorbent				Solution used for recovery		Recovery of drug absorbed			
	As[A]	Pr[B]	As(Weight)		Pr(Weight)		Type	Volume (ml)	As(Weight)		Pr(Weight)	
			mgs	%	mgs	%			mgs	%	mgs	%
i.	10	-[C]	9.9	99	-	-	1% HCl	10	0.59	5.96	-	-
ii.	10	-	9.9	99	-	-	1% HCl	10+10	0.81	8.18	-	-
iii.	-	10	-	-	1.4	14	1% HCl	10	-	-	0.33	23.57
iv.	2.5	-	2.5	100	-	-	1% HCl	10	0.42	16.8	-	-
v.	-	3.4	-	-	1.0	29.41	1% HCl	10	-	-	0.28	28.0
vi.	3.7944	2.1804	2.718	71.63	0.5647	25.90	1% HCl	10	0.8028	29.54	0.1765	31.26
vii.	3.8538	2.1804	2.961	76.83	0.5647	25.90	1% HCl	10+10	0.9997	33.76	0.3171	56.15
viii.	3.7674	2.1442	2.7824	73.85	0.3982	18.57	2% HCl	10	0.2828	10.16	0.1522	38.22
ix.	3.7674	2.1442	2.8476	75.59	0.459	21.41	2% HCl	10+10	0.9799	34.41	0.328	71.46

In every experiment mentioned in the table, 100 ml drug containing aqueous solution is passed through 1gm anion-exchanger (Amberlite IRA-93) with a flow rate 1 ml min^{-1}.

[A]As represents Aspirin ; [B]Pr represents Paracetamol ; [C]Dash represents that drug is not present in that experiment

1gm of anion exchanger was kept overnight in 20ml DDW. It was washed with 20ml DDW and activated by passing 50ml 2M HCl solution. Then the column(containing anion exchanger) was washed with 50ml DDW. Through this washed activated column, 100ppm, 100ml aspirin aqueous solution was allowed to pass at 1ml min^{-1}. The adsorbed drug was recovered with 10ml 1%HC. The same conditions were used to pre-concentrate paracetamol from water using anion-exchanger. Using these optimized conditions, the aqueous solution containing both Aspirin and Paracetamol was pre-concentrated. The samples were analyzed by UV-Spectrometer. Different experimental parameters and its conditions are summarized in TABLE 2, including the results obtained from the each experiment. For each experiment, 100ml of drug solution was passed through 1gm of anion-exchanger with a 1ml min^{-1} flow rate.

Chromatography procedure

Synthetic samples of known concentration of aspirin and paracetamol were analysed by HPLC us-

ing Buffer 80%(v/v)(1ml H_3PO_4/Liter H_2O) plus Acetonitrile 20%(v/v) as mobile phase, volume of sample injected was 20µL. The mobile phase flow rate was kept at 1.5ml /min. Under these conditions the retention time were 2.4 min for Paracetamol and 7.4min for Aspirin. Synthetic aqueous samples of mixture Aspirin and Paracetamol and environmental samples of, before and after optimized pre-concentration method using anion-exchanger were also analysed under these HPLC conditions.

Analysis of environmental samples

To test the applicability of the optimized ion-exchanger-UV-Spectrometer method, treated water samples were collected from Sewage Treatment Plant(STP) whose working principle is Up-flow Anaerobic Sludge Blanket(UASB) (capacity 43 MLD) located in Vadodara, Gujarat, India. 2.5L(volume) sample was collected from the outlet of secondary clarifier of the treatment plant in a glass container. Samples were immediately used for experiment after collection.

All samples were analyzed by UV-spectrometer and were compared with results of same samples analyzed by HPLC(Chromatographic procedure).

RESULTS AND DISCUSSION

The preliminary experimental data, TABLE 1{Sr.No.(i)and(ii)} indicate that the adsorption capacity of macroporous polymer of PSDVB is better campared to gel type polymer. Macroporous polymers used are of two different cross-linking: 12 % and 8%. Data from TABLE 1{Sr.No.(iii) and(iv)} show that macroporous polymer with 8% cross-linking is better adsorbent. For further experiments macroporous polymer having 8% cross-linking was used. The adsorption of drug from aqueous solution increases when the amount of adsorbent increases. However, the amount of drug recovered remains almost same for 10ml eluent as shown in TABLE 1 {Sr.No.(v), (vi)and(vii)}. Data of TABLE 1 {Sr.No.(viii)} show that by decreasing the flow rate the adsorption is better but the recovery after adsorption is same as that of 1 ml min^{-1} flow. Keeping the flow 1 ml min^{-1}, the polarity of recovering solvent was increased by taking 5% and 20% methanol/water respectively. From TABLE 1{(ix) and (x)}, it is seen that the recovery remains same even if solvent polarity is increased. So considering above all reasons the condition for 100 ml aqueous solution of Aspirin for pre-concnetration were optimized to 1gm of adsorbent and 10ml volume of water (Solvent for recovery) with flow of 1ml min^{-1}. Same optimized conditions were used for aqueous solution of paracetamol TABLE 1 {Sr.No.(xii)}.

To get better adsorption anion-exchanger was used as adsorbent keeping all above optimized experimental conditions same. For better recovery of drug adsorbed on anion-exchanger, 1% HCl aqueous solution was used. The efficiency of recovery was observed better when the volume of solvent for recovery was doubled, TABLE 2. From the results it is also observed that the percentage of adsorption of Paracetamol decreases in presence of Aspirin in water and with the double volume of 2% HCl solution the percentage of recovery increases for Paracetamol.

After optimizing the conditions for anion-exchanger for aqueous aspirin samples, aqueous solution of paracetamol was pre-concentrated with the same optimized conditions. These optimized conditions were used for mixture of aqueous solution of Aspirin and Paracetamol together. The amount of the individual drugs present in mixture can be derived by solving the equations(1)and(2), which are obtained adding Beer-Lambert's law.(A=abc), for two components mixture of X and Y, the mixture's absorbance, am is

$$(Am)_{\lambda 1} = (\epsilon_X)_{\lambda 1} bC_x + (\epsilon_Y)_{\lambda 1} bC_Y \quad (1)$$

Where λ1 is the wavelength at which the absorbance of component X is measured.

$$(Am)_{\lambda 2} = (\epsilon_X)_{\lambda 2} bC_x + (\epsilon_Y)_{\lambda 2} bC_Y \quad (2)$$

Where λ2 is the wavelength at which the absorbance of component Y is measured. ϵ is absorptivity (L $g^{-1}cm^{-1}$); its value is determined for each component at both wavelengths. B is path length(cm); and, C the concentration(M)[6]. With this developed pre-concentration method, environmental sample was pre-concentrated and analyzed for the two

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TABLE 3 : Optical characteristics, linearity and sensitivity of the compounds

Parameters	Aspirin					Paracetamol				
	Aqueous solution	1% HCl	2% HCl	5% methanol	20% methanol	Aqueous solution	1% HCl	2% HCl	5% methanol	20% methanol
	Absorptive(1 $mol^{-1}cm^{-1}$)									
at 225 nm	7747.143	-A	-	-	-	4660.606	-	-	-	-
at 244 nm	2540	-	-	-	-	7278.788	-	-	-	-
Correlation Coefficient (r^2)	0.9626	0.9899	0.9985	0.9998	0.9996	0.9989	1	0.9996	0.9964	0.9958
	Regression Equation (y)									
Slope, a	0.0366	0.0401	0.0367	0.0371	0.0451	0.0675	0.0455	0.0670	0.0717	0.0745
Intercept, b	-0.0052	0.0171	0.0222	0.0085	0.0122	0.0087	0.0017	0.0323	0.0856	0.0894

λ_{max} for Aspirin is 225nm and for Paracetamol is 244nm, Limit of Detection of Aspirin and Paracetamol is 0.1ppm.
[A]Dash represents the value is taken same as that of respective drug in aqueous solution.

TABLE 4 : Analytical parameters and method validation results of HPLC method

	Aspirin	Paracetamol
Correlation Coefficient (r^2)	0.9998	0.9999
Limit of detection (LOD)	0.0390ppm	0.0390ppm
Limitof quantification (LOQ)	0.3125ppm	0.0390ppm

drugs(Aspirin and Paracetamol).

Analytical performance characteristics

For UV-spectrometer

Analytical performance characteristics including linearity, precision, and accuracy, limit of detection and limit of quantitation for the analysis of Aspirin and Pracetamol by spectrometry are recorded in TABLE 3. Calibration curves were obtained by measuring the UV absorbance of the standard solutions of Aspirin and Pracetamol in a range of 25-100mg L^{-1} at wavelength 225nm for Aspirin and 244nm for Paracetamol. Linear regression and correlation coefficient(r^2) were calculated using Microsoft Excel®program. Precision of the method was determined by repetitive measurements(n=6) of the UV absorbance of the pure Aspirin and Pracetamol solutions and percent relative standard deviations(% RSD) were calculated.

The validity of the methods for the analysis of Aspirin and Pracetamol was examined using the proposed procedures. Summary of analytical performance characteristics is shown in TABLE 3. The calibration graphs involved at least four experimental points for each compound and they are described by the following equations: for Aspirin at wavelength 225nm were in DDW, $y=0.0366x-0.0052$($r^2=0.9997$), in 1%HCl $y=0.0401x+0.0171$ ($r^2=0.9899$), in 2%HCl $y=0.0367x+0.0222$($r^2=0.9985$), in 5% Methanol $y=0.0371x+0.0085$($r^2=0.9998$), in 20% Methanol $y=0.0451x+0.0122$($r^2=0.9996$) and for Paracetamol at 244nm were in DDW $y=0.0675x+0.0087$($r^2=0.9989$), in 1%HCl $y=0.0455x+0.0017$($r^2=1$), in 2% HCl $y=0.067x+0.0323$ ($r^2=0.9991$), in 5% Methanol $y=0.0717x+0.0856$($r^2=0.9964$), in 20% Methanol $y=0.0745x+0.0898$($r^2=0.9958$) Precision of the method was substantiated by calculating %RSDs of the absorbance of Aspirin and Pracetamol at wavelengths of 225 and 244nm, which were less than 0.008 for all cases(n=6).

For HPLC

The validity of chromatographic procedure was established through a study of linearity, sensitivity, repeatability. Linearity was established with a series of working solutions prepared by diluting the stock solution with mobile phase to the final concentrations. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve. The calibration graphs involved at least five experimental points for each compound and they are described by the following equations: for Aspirin $y=10899x-1056.7$($r^2=0.9998$); for Paracetamol $y=25015x+1248.4$($r^2=0.9999$). Limit of detection (LOD) and quantitation(LOQ) were calculated form visual determination method of %RSD of area. The validity of the methods for the analysis of Aspirin and Pracetamol was examined. Summary of analytical performance characteristics

is shown in TABLE 4.

The results of quantitation of drugs by HPLC and UV-visible spectrometer were obtained similar for mixture of 25 ppm Aspirin and 25 ppm Paracetamol in aqueous solution.

Application of pre-concentration method for environmental samples

The developed method was used for environmental sample of treated water from sewage treatment plant. The collected sample was immediately and directly used for pre-concentration using the developed method for pre-concentration. Later the samples were analysed by the HPLC method (Chromatography procedure). The results shows that Aspirin and Paracetamol in the environmental samples where very low beyond the detection limit of the HPLC method(0.039mg L^{-1}).

CONCLUSIONS

The method developed for pre-concentration of aqueous samples containing Aspirin and Paracetamol selected using UV-Spectrometer for quantification, is accurate, sensitive and reliable and enables the determination of the target pharmaceuticals in water samples at 0.025mg ml^{-1} for Aspirin and Paracetamol., By using, easily available and less in cost macroporous polymer of PSDVB with 8% cross-linking and anion-exchanger(Amberlite IRA-93), in simple laboratory conditions an increase in concentration by a factor of 1.30 for Aspirin and 0.76 for Paracetamol can be obtained. Quantitative analysis of Aspirin and Para cetamol individual and together can be done by UV-spectrometer even at low levels.

The water sample collected from STP(Vadodara-India) after treatment does not show presence of Aspirin and Paracetamol up to the detection level of 0.03ppm. This means either the concentration of these drugs is below the levels or STP removes these drugs effectively.

Further studies are underway for determination of other pharmaceutical compounds and also use of other commercial available adsorbents for pre-concentration.

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APPENDIX 2

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Liquid Chromatography

PRECONCENTRATION AND QUANTITATIVE DETERMINATION OF ESOMEPRAZOLE MAGNESIUM PRESENT IN WATER

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A simple, accurate, and sensitive preconcentration method for determination of esomeprazole magnesium in treated sewage water was developed based on HPLC. A preconcentration method was developed for aqueous solution containing pure drug using solid phase extraction. Macroporous beads of polystyrene divinyl benzene (PSDVB) polymer were used for preconcentration followed by chromatographic determination. Experimental parameters were optimized. This optimized method can detect esomeprazole magnesium concentration up to 0.003 mg L^{-1} after preconcentration. This method was used for determination of esomeprazole magnesium in water collected from a sewage treatment facility. Esomeprazole magnesium could not be detected in the treated sewage water sample collected for the study.

Keywords: Esomeprazole magnesium; HPLC; Preconcentration; Solid phase extraction

INTRODUCTION

Esomeprazole magnesium trihydrated, bis (5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrated is a proton pump inhibitor (PPI) developed as an optical isomer (S-Esomeprazole) for the treatment of acid related diseases (Lind et al. 2000). Esomeprazole is a potent inhibitor of gastric acid secretion and accumulates in the acidic compartment of the parietal cells where the molecule is transformed to its active sulfonamide form. Esomeprazole does not undergo chiral inversion in vivo (Andersson et al. 2001) and, therefore, esomeprazole can be determined using the same methodology

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as for its racemate, omeprazole. Esomeprazole is also excreted in an unaltered form in the same low proportion and its presence in aquatic environment has been reported (Hernando et al. 2007). Understanding the fate of such drugs in water systems could lead to better waste water treatment options that would lead to more complete removal of such compounds. To aid in this understanding, an analytical method that accurately measures low concentrations of esomeprazole in water is an essential tool. The literature survey reveals that esomeprazole was analyzed in environmental water samples using solid phase extraction followed by LC-MS (Hernando et al. 2007; Castiglioni et al. 2005). Esomeprazole has been determined in blood plasma by liquid chromatography with UV detection (Lagerstrom and Persson 1984; Yeung et al. 1998; Yuen et al. 2001) and this technique has also been employed for a simultaneous assay of the two major metabolites (Grundevik et al. 1986). In recent years the combination of liquid chromatography and mass spectrometry (LC–MS) has been used for omeprazole and metabolites (Woolf and Matuszewski 1998; Kanazawa et al. 2002; Hoffman et al. 2006) and for esomeprazole and other PPIs (Shimizu et al. 2006; Oliveira et al. 2003). Enantioselective methods for detection of esomeprazole by liquid chromatography have also been presented that employ mass spectrometric (Wang et al. 2005) or UV-detection (Stenhoff, Blomqvist, and Lagerstrom 1999). To our knowledge, none of these methods have been applied for analysis of treated waste water for the detection of esomeprazole using HPLC.

The purpose of this work was to develop an analytical method for quantitation of esomeprazole in water samples obtained from a waste water treatment plant using a relatively simple and yet sensitive SPE method in combination with a HPLC detection method. Preconcentration of esomeprazole from aqueous solution was carried out using PSDVB beads. After adsorption the drug was recovered from the solid phase using methanol. The resultant solution was subjected to quantitation using HPLC method optimized for esomeprazole. After optimizing the preconcentration method, it was applied to a treated water sample collected from a local Sewage Treatment Plant (STP).

EXPERIMENTS

Chemicals and Reagents

Esomeprazole was obtained from local drug industry in India, where as macroporous polystyrene divinyl benzene beads (8% cross linking) was a kind gift from Doshi Ion-Exchange, Ahmedabad, India. All other solvents and reagents were purchased from Qualigens and were of analytical or HPLC grade. These were used as obtained and Milli Q water was prepared with Millipore Elix®-3.

Instrumentation

The chromatograph system comprised of Shimadzu LC-10 AS equipped with Rheodyne injector (20 μL capacity) and UV-Vis detector (SPD-10A). Data integration was done using a software package (LC-10). The column used was BDS Hypersil C8 (4.6 × 250 mm, 5 μ).

Preconcentration using SPE was carried out using a glass column with stop-cock packed with adsorbent material.

Stock Solutions

Stock solution of esomeprazole was prepared by dissolving 100 mg of drug in 100 mL Milli-Q water. Working standard solution was prepared by diluting stock solution with Milli-Q water to obtain the concentration of 500 mg L^{-1}. To obtain standard curve, solutions of different concentrations were prepared by diluting appropriate volumes of working standard solution. Similarly, stock solution of esomeprazole in methanol solvent was also prepared. The stock solutions were refrigerated and were consumed within three days.

Chromatography Procedure

Synthetic samples of known concentration of esomeprazole were analyzed by HPLC using mobile phase, which was prepared by mixing buffer and acetonitrile in a ratio of 70:30 v/v. Buffer was prepared by mixing 5 mL of triethyl amine and 995 mL Milli-Q water containing 1.2 g ammonium dihydrogen orthophosphate. The flow rate was 1.0 mL min^{-1}. Detection was carried out at wavelength 302 nm. All determinations were performed at room temperature. The injection volume was 20 µL. Under these conditions the retention time of Esomeprazole prepared in methanol and water was in the range of 15.0 to 15.3 min.

Pre-Concentration Studies

For pre-concentration using PSDVB beads, preliminary studies were conducted to work out the experimental conditions for the optimum adsorption and recovery. A typical experiment was performed using a synthetic sample of esomeprazole. A sample of 100 mg L^{-1} esomeprazole aqueous solution of 100 mL volume was prepared by diluting an appropriate aliquot of stock solution. The column packed with 1.0 gm of the adsorbent material (PSDVB polymer beads) was activated by passing 5 mL acetonitrile through it followed by 5 mL of acetonitrile:water (80:20) (v/v), and then by 5 mL of water. The aqueous drug sample was passed through the activated column at the rate of 0.66 mL/min. The adsorbed drug was eluted with 10 mL of methanol. Amounts of drug adsorbed and recovered in methanol were determined by HPLC analysis. To optimize the experimental conditions for preconcentration of esomeprazole, different experimental parameters where changed, one-by-one, while keeping other factors constant.

Analysis of Environmental Sample

Treated waste water sample was collected from STP operating with Up-Flow Anaerobic Sludge Blanket (UASB) principle. The plant has working capacity of 43 MLD and is located in Vadodara, Gujarat, India. A 2.5 L (volume) sample was collected from the outlet of the secondary clarifier of the treatment plant in a glass container. For sample preparation, the collected water sample was filtered through

Whatman filter paper (No. 41) and into the filtrate for extraction, 75 μL of 40% H_2SO_4 and a scoop of disodium ethylene diamine tetra acetate (Na_2EDTA) were added (Lindsey, Meyer, and Thurman 2001). An aliquot of the sample was then subjected to HPLC analysis as such; whereas, the other was subjected to an optimized preconcentration step, the adsorbed drug recovered by acetonitrile, and this was also analyzed using HPLC.

RESULTS AND DISCUSSION

Preconcentration Studies

We have earlier reported preconcentration studies for aspirin and paracetamol on different adsorbents from aqueous solution (Samnani et al. 2007). Optimized conditions obtained in the study viz., 1.0 gm adsorbent, 100 mL aqueous solution of drug and 5 mL solvent for recovery were used as starting set of conditions for the present work. Initially, the effect of flow rate of aqueous esomeprazole solution on adsorption was studied. With an increase in the flow rate, the adsorption of the drug on adsorbent decreased. For subsequent experiments the 0.66 mL min^{-1} flow rate was maintained, which resulted in a maximum drug adsorption up to 70%. The effect of changing the volume of aqueous solution containing the drug and the effect of changing the amount of adsorbent while keeping volume and concentration of drug solution constant were studied and optimized in the present work for esomeprazole. Studies show that more than 70% esomeprazole adsorbs on 1 gm of polymer beads when 100 mL of its aqueous solution is passed through the column. Whereas, with 50 mL of the initial drug solution, the percentage of drug adsorption increased to 87.95. With this percentage of adsorption, methanol was used for recovery. The percentage of recovery was studied with four different volumes of methanol (3, 5, 7, and 10 mL). A maximum drug recovery up to 102.91% was observed with 10 mL of methanol, which resulted in a preconcentration factor of 6.05. With the decrease in the volume of methanol for recovery, the percentage of drug recovered decreased but the preconcentration factor increased. Considering this trend, the condition for recovery of drug adsorbed on 1 gm of adsorbent was optimized to 5 mL of methanol. With these optimized conditions for recovery, preconcentration experiments were performed taking higher volumes of aqueous drug solutions while keeping the amount of drug the same. These experiments show that with an increase in volume of the initial aqueous drug solution, the percentage of amount of drug adsorbed remains almost the same; however, after their recovery with a 5 mL methanol, the preconcentration factor for the respective experiments increased. However, this trend changed with higher volumes of the initial drug solution. To study the effect of the initial volume, different sets of experiments were done with 50, 100, 150, 250, and 500 mL of initial aqueous drug solution. With 250 mL initial drug solution, a maximum of 96.51% drug was adsorbed. Results also showed that the volume of 5 mL methanol can recover 100% of the drug at lower amounts up to 1.47 mg.

The optimized conditions for maximum adsorption of drug and its recovery with a better preconcentration factor for Esomeprazole are: 250 mL of initial aqueous drug solution passed through 1 gm PSDVB beads with a flow rate of 0.66 mL per minutes, followed by 5 mL methanol used for recovery of drug adsorbed solution.

A developed optimized method for preconcentration of esomeprazole was applied to an environmental water sample collected from STP. Before preconcentration, the sample was also analyzed by HPLC. In this case, no peaks were observed in the chromatogram for esomeprazole. The samples were spiked with a known amount of drug (1 mg L^{-1}) and analyzed, but the signal enhancement for the 1 mg L^{-1} added drug was not seen. Results indicated no presence of esomeprazole in the sample collected from the STP, which was confirmed by a LC-MS method. Details of results for the LC-MS experiment for esomeprazole, along with other drugs, are being processed as a separate paper.

Accuracy of the Preconcentration Method

The optimized conditions were used to determine accuracy of the preconcentration method by fortifying known amounts of Esomeprazole to the synthesized aqueous solution at a concentration range of 30 times less than LOQ level. Thus, 0.006 mg L^{-1}, 0.014 mg L^{-1}, 0.026 mg L^{-1}, 0.053 mg L^{-1}, 0.105 mg L^{-1}, and 0.211 mg L^{-1} aqueous solutions of esomeprazole could be preconcentrated to 0.191 mg L^{-1}, 0.422 mg L^{-1}, 0.793 mg L^{-1}, 1.664 mg L^{-1}, 3.186 mg L^{-1}, and 6.512 mg L^{-1}, respectively, with a preconcentration factor of more than 30, confirming that the designed level of preconcentration was achieved in the target concentration range in synthetic aqueous sample.

Similarly, the optimized conditions were used to determine the matrix effect by fortifying a known amount of esomeprazole to the live water sample at a concentration range of 30 times less than the LOQ level. Thus, 0.007 mg L^{-1}, 0.016 mg L^{-1}, 0.03 mg L^{-1}, 0.061 mg L^{-1}, 0.12 mg L^{-1}, and 0.241 mg L^{-1} could be preconcentrated to 0.216 mg L^{-1}, 0.491 mg L^{-1}, 0.921 mg L^{-1}, 1.857 mg L^{-1}, 3.654 mg L^{-1}, and 7.283 mg L^{-1}, respectively, with a preconcentration factor of more than 30, confirming that the designed level of preconcentration was achieved in the target concentration range in live water samples.

Analytical Performance Characteristics

The validity of the chromatographic procedure was established through a study of linearity, sensitivity, and repeatability. Linearity was established with a series of working standard solutions prepared by diluting the stock solution with both

Table 1. Analytical Performance Characteristics

	Esomeprazole mangnesium	
Parameters	In water	In methanol
Regression Equation (y)		
Correlation Coefficient (r^2)	1	1
Slope, a	43243	43257
Intercept,	3294.5	2539.5
Limit of Quantification (mg L^{-1})	0.19	0.19
Limit of Detection (mg L^{-1})	0.09	0.09

water and methanol, individually to the final concentrations. Each concentration was injected in triplicate and the mean value of the peak area was taken for the calibration curve. The calibration graphs involved at least five experiment points for the compound, and they are described by the following equations: for esomeprazole in water: $y = 43243x + 3294.5$ ($r^2 = 1$); for esomeprazole in methanol: $y = 43257\ x + 2539.5$ ($r^2 = 1$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area. The validity of the methods for the analysis of esomeprazole was examined. Summary of analytical performance characteristics is shown in Table (1).

CONCLUSION

The method developed for preconcentration of aqueous solutions containing esomeprazole using the HPLC method for quantification is accurate, sensitive, and reliable and enables the determination of the target drug in water sample at $0.006\,mg\,L^{-1}$. In simple laboratory conditions, aqueous solutions of esomeprazole can be preconcentrated by a factor of 30 by using commercially an available macro porous polymer of PSDVB with 8% cross-linking.

The water sample collected from STP (Vadodara-India) after treatment does not show presence of esomeprazole up to the detection level of $0.003\,mg\,L^{-1}$ considering the preconcentration factor in optimized conditions. This means concentration of this drug is below this level or the STP is efficient in removing the drug effectively.

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