International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.6, No.1, pp 838-844, Jan-March 2014

Development and Validation of a New HPLC Method for Simultaneous determination of Esomeprazole, Venlafaxine HCl and Fenofibrate

K. S. Kumar[1] and P. B. Samnani[2]*

[1]School of Engineering and Technology, Navrachana University, Vadodara – 391 410, India.

[2] Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara – 390 002, India.

Corres. Author: ksk.india@gmail.com[1],pbsamnani2009@gmail.com[2]
Phone No: +912652795552

Abstract: A new gradient reversed-phase liquid chromatographic method was developed for the simultaneous determination of Esomeprazole, Venlafaxine HCl and Fenofibrate in water. The chromatographic separation of these analytes was done by C18 column using a 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 a wavelength of 230 nm. The Linearity of the method was found to be in the concentration range of 10.37 – 518.40 mg / L for three drugs with correlation coefficient greater than 0.999.
Keywords: Esomeprazole, Venlafaxine HCl and Fenofibrate, HPLC and Gradient.

INTRODUCTION

Esomeprazole magnesium structure shown in Figure No. 1, is a proton pump inhibitor (PPI) developed as an optical isomer (S – Esomeprazole) for the treatment of acid – related diseases.[1] Esomeprazole does not undergo chiral inversion in vivo[2] and therefore can be determined using the same methodology as for its racemate, Omeprazole. The literature survey revels that Omeprazole has been analyzed by various methods.[3,4] Venlafaxine HCl structure shown in Figure No. 2, is a non-tricyclic antidepressant. There are various HPLC methods[5,6] reported in literature for quantitation of Venlafaxine HCl for different purposes. Fenofibrate structure shown in Figure No. 3 is fibric acid derivative, used for regulating plasma lipids and treatment of hyperlipoproteinaemias.[7] The literature survey reveals that fenofibrate has been analyzed by various methods.[8-15]

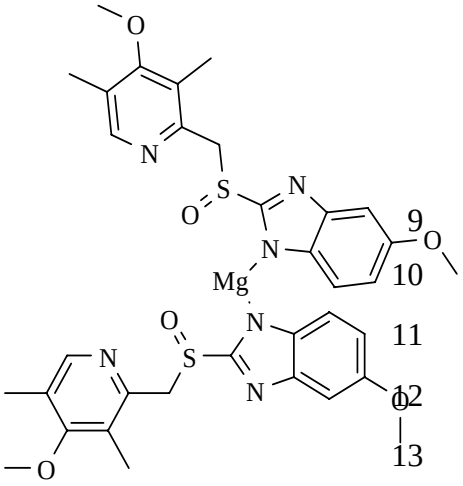

Figure No. 1: Structure of Esomeprazole Magnesium.

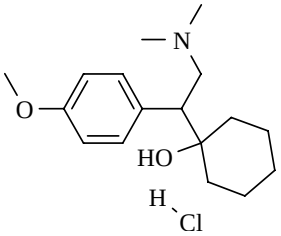

Figure No. 2: Structure of Venlafaxine HCl.

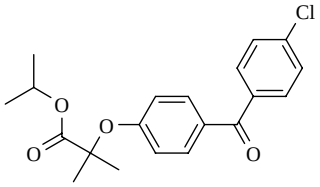

Figure No. 3: Structure of Fenofibrate.

Several studies have reported presence of personal care and pharma products in aquatic system.[16-20] These pharma compounds find different ways to enter into surface waters through sewage treatment plants. Omeprazole is excreted in an unaltered form in the same low proportion and its presence in aquatic environment has been reported.[4] 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 drain. Number of reports on the occurance of a wide variety of antidepressants in the aquatic environment have been increasing steadily in recent times.[16] Presence of fenofibrate in aquatic environment has been reported recently.[8,20]

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 this 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.

EXPERIMENTAL

Materials and Reagents

Samples of Esomeprazole, Venlafaxine HCl and Fenofibrate were received from local drug industry in India. HPLC grade acetonitrile, methanol, formic acid, ammonium acetate were purchased from Qualigens and used as such, and milli-Q water was prepared with Millipore Water Purification system type -1.

Instrumentation

The LC system, used was a Schmadzu 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.

Chromatographic Conditions

Separation was carried out on a C18 column (150 cm x 4.6 mm, 3.5 µm 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 0.45 µm nylon filter and degassed. The flow rate was kept at 1.1 ml 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 230 nm. The injection volume was 10 µL.

Preparation of Standard Solutions

Standard stock solutions

A stock solutions of Esomeprazole, Venlafaxine HCl and Fenofibrate each was prepared at about 4000 mg L^{-1} by dissolving an appropriate amount in methanol and diluted to the desired concentration. Working standard solutions of 1000 mg L^{-1} and 100 mg L^{-1} were prepared from the stock solution by diluting with acetonitrile for each drug. Standard mixture solution was prepared by diluting 2.5 mL of each of the stock solutions of Esomeprazole, Venlafaxine HCl and Fenofibrate to 10 mL with acetonitrile to achieve 1000 mg L^{-1}. System suitability solution was prepared by diluting 1.0 mL standard mixture to 10 mL with acetonitrile to achieve 100 mg L^{-1}.

Environmental Sample Preparation

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. 2.5 L (volume) sample was collected from the outlet of secondary clarifier of the treatment plant in a glass container. For sample preparation, procedure was followed as mentioned in the literature.[21] An aliquate of the sample was then subjected to recovery studies for the three drugs.

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.[22]

Specificity

The specificity of the method was studied by injecting acetonitrile, mobile phase, methanol, standard solutions of the three drugs.

Precision

Precision of the developed method was determined at two levels, 10 mg L^{-1} and 200 mg 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.

Limit of Detection and Limit of Quantification

For calculating the LOD and LOQ values, solutions with known decreased 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.

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

System Suitability

System suitability solution was injected on to HPLC in six replications and %RSD calculated for retention time and peak area of Esomeprazole, Venlafaxine HCl and Fenofibrate respectively.

RESULTS AND DISCUSSION

Method Development and Optimization of Chromatographic Conditions

In the present work, a new HPLC method developed for separation and quantification of Esomeprazole, Venlafaxine HCl and Fenofibrate is reported. The method has been validated. Different stationary phases (C18, C8), mobile phases containing buffers like formic acid, ammonium acetate and using 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 4.6 mm, 5µ particle size) using a mixture of acetonitrile: buffer (0.13 % formic acid, 15.50 % 0.1 mol / L 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 302 nm with flow rate 1.0 mL / min. with run time 45 min. The gradiant LC conditions were mentioned in Table No. 1. To reduce the run time chromatographic conditions were changed. This was achieved on a C18 (150 cm x 4.6 mm, 3.5 µm 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 230 nm 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 No. 4. The tailing factor for Esomeprazole, Venlafaxine HCl and Fenofibrate was 1.288, 1.478 and 1.290 respectively.

Table No.1: Gradiant LC conditions for C8.

Time	Percentage of Mobile Phase A	Percentage of Mobile Phase B
0.00	100.0	0.0
15.00	100.0	0.0
35.00	30.0	70.0
40.00	30.0	70.0
41.00	100.0	0.0
45.00	100.0	0.0

Method Validation

Precision

The % RSD for the area of Esomeprazole in the method was within 0.39, for Venlafaxine HC was within 0.91 and Fenofibrate was within 0.35 confirming the good precision of the method.

Limit of Detection and Limit of Quantification

The limit of detection of Esomeprazole, Venlafaxine HCl and Fenofibrate was 1.02 mg L^{-1}, 1.02 mg L^{-1} and 1.05 mg L^{-1} (for test concentration) respectively; the limit of quantification was 5.18 mg L^{-1}, 5.09 mg L^{-1} and 5.22 mg L^{-1} (of test concentration) respectively.

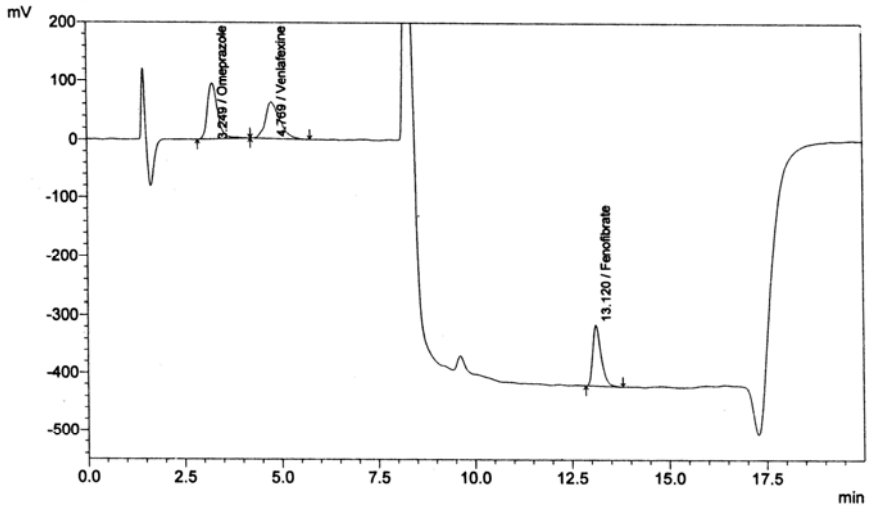

Figure No. 4: Typical LC chromatogram of 100 mg L^{-1} Esomeprazole, Venlafaxine HCl and Fenofibrate solution.

Linearity

A linear calibration plot for the method for Esomeprazole, Venlafaxine HCl and Fenofibrate was obtained over the calibration ranges 10.37 mg L^{-1} – 518.40 mg L^{-1} with a correlation coefficient greater that 0.999. The computed equations of the calibration curve for the three drugs are: Esomeprazole: y = 16375.54 x – 3513.49, for Venlafaxine HCl: y = 15400.66 x + 30904.46, and for Fenofibrate: y = 15356.84 x + 15485.60. The results show that an excellent correlation existed between the peak area and concentration of Esomeprazole, Venlafaxine HCl and Fenofibrate.

Accuracry

The percentage recovery of Esomeprazole was 99.61%, Venlafaxine HCl was 74.66% and Fenofirate was 74.14 % with 4.46, 9.56 and 7.9 %RSD respectively for 5.16 mg L^{-1}, 5.09 mg L^{-1} and 5.22 mg L^{-1} of respective drugs. The percentage recovery of Esomeprazole was 73.1 %, Venlafaxine HCl was 75.36 % and Fenofibrate was 73.72 % with 2.73, 2.23 and 2.03 %RSD respectively for 51.84 mg L^{-1}, 50.89 mg L^{-1} and 52.24 mg L^{-1} of respective drugs.

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.

CONCLUSIONS

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, Vanlafaxine HCl and Fenofibrate with good resolution. The method can be used for routine analysis.

ACKNOWLEDGMENT

Sincere thanks are due to University Grants Commission, Government of India for financial support and fellowship to K.S.Kumar, The Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, for the support and providing facilities, Mr. S.P.Sahoo, Sun Pharma Advance Research Centre (SPARC), Tandalja, Vadodara for technical discussion.

REFERENCES

1. Lind T., Rydberg L., Kyleback A., Jonsson A., Andersson, T., Hasselgren, G., Holmberg, J. and Rohss K., Esomeprazole provides improved acid control vs. omeprazole in patients with symptoms of gastro-oesophageal reflux disease. Aliment Pharmacol Ther., 2000, 14, 861 – 867.
2. Andersson T., Hassan-Alin M., Hasselgren G., Röhss K. and Weidolf L., Pharmacokinetic studies with esomeprazole, the (S)-isomer of omeprazole. Clin. Pharmacokinet., 2001, 40, 411 – 426.
3. Bosch M.E., Sánchez A.J.R., Rojas F.S. and Ojeda C.B., Analytical methodologies for the determination of omeprazole: An overview. J. Pharm. Biomed. Anal., 2007, 44, 831 – 844.
4. Hernando M.D., Gomez M.J., Aguera A. and Fernandez-Alba A.R., LC – MS analysis of basic pharmaceuticals (beta-blockers and anti-ulcer agents) in wastewater and surface water., Trends Anal. Chem., 2007,Vol 26, 581 – 594.
5. Hicks D., Wolaniuk D., Russell A., Cavanaugh N. and Kraml M., A high – performance liquid chromatographic method for the simultaneous determination of venlafaxine and o-desmethylvenlafaxine in biological fluids., Thr. Drug Monitor., 1994,16, 100 – 107.
6. Vu R., Helmeste D., Albers L. and Reist C., Rapid determination of venlafaxine and O-desmethylvenlafaxine in human plasma high-performance liquid chromatography with flourimetric detection., J. Chromatogr B., 1997,703, 195 – 201.
7. Sweetman S.C., *Martindale – The Complete Drug Reference.* 33rd ed., London, Pharmaceutical Press, 2002.
8. Reddersen K. and Heberer T., Multi-compound methods for the detection of pharmaceutical residues in various waters applying solid phase extraction (SPE) and gas chromatography with mass spectrometric (GC-MS) detection., *J. Sep. Sci*., 2003, 26, 1443 – 1450.
9. Sacher F., Lange F.T., Brauch H.J. and Blankenhorn I., Pharmaceuticals in groundwaters: Analytical methods and results of a monitoring program in Baden-Württemberg, Germany. *J. Chromatrogr. A.*, 2001, 938, 199 – 210.
10. British Phramacopeia, 2007.
11. Romanyshyn L.A. and Tiller P.R., Ultra-short columns and ballistic gradients: considerations for ultra-fast chromatographic liquid chromatographic–tandem mass spectrometric analysis, *J. Chromatrogr. A.*, 2001, 928, 41 – 51.
12. Streel B., Hubert P. and Ceccato A., Determination of fenofibric acid in human plasma using automated solid-phase extraction coupled to liquid chromatography., *J. Chromatogr. B. Biomed. Appl.*, 2000, 742, 391 – 400.
13. Masnatta L.D., Cuniberti L.A., Rey R.H. and Werba J.P., Determination of bezafibrate, ciprofibrate and fenofibric acid in human plasma by high-performance liquid chromatography, J. *Chromatrogr. B. Biomed. App.*, 1996, 687, 437 – 442.
14. Rao R.N. and Angora V., An overview of the recent trends in development of HPLC methods for determination of impurities in drugs., *J. Pharm. Biomed. Anal.*, 2003, 33: 335 – 377.
15. Lacroix P.M., Dawson B.A., Sears R.W., Black D.B., Cyr T.D. and Ethier J.C., Fenofibrate raw materials: HPLC methods for assay and purity and an NMR method for purity., *J. Pharm. Biomed. Anal.*, 1998, 18, 383 – 402.
16. Weigel S., Berger U., Jensen E., Kallenborn R., Thoresen H. and Hühnerfuss H., Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromso / Norway with emphasis on ibuprofen and its metabolites., *Chemosphere*, 2004, 56, 6, 583 – 592.
17. Buchberger W.W., Novel analytical procedures for screening of drug residues in water, waste water, sediment and sludge., *Anal. Chem. Acta.* 2007, 593, 2, 129 – 139.
18. Jones O.A.H., Voulvoulis N. and Lester J.N., Analytical method development for the simultaneous determination of five human pharmaceuticals in water and wastewater samples by gas chromatography – mass spectrometry., *Chromatographia*, 2003, 58, 471 – 477.

19. Kanda R., Griffin P., James H.A., Fothergill J., Pharmaceuticals and personal care products in sewage treatment works., *J. Envrion. Monit*., 2003, 5, 823 – 830.
20. Hernando M.D., Mezcua M., Fernandez – Alba A.R. and Barcelo D. LC – MS analysis of basic pharmaceuticals (beta-blockers and anti-ulcer agents) in wastewater and surface water. *Talanta*, 2006, 69, 334 – 342.
21. Lindsey M.E., Meyer M. and Thurman E.M., Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid – phase extraction and liquid chromatography / mass spectrometry. *Anal. Chem.*, 2001, 73, 4640 – 4646.
22. ICH Topic Q2 (R1) "*Validation of Analytical Procedures: Text and Methodology"*, current step 4 version, Parent guideline dated 27 October 1994 (Complementary guidenline on methodogy dated 6 November 1996 incorporated in November 2005).

*****