Pre-concentration and Quantitative Determination of Venlafaxine HCl Present in Treated Sewage Water

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Abstract **- A simple, accurate and sensitive pre-concentration method for determination of Venlafaxine HCl in treated sewage water has been developed based on HPLC. Pre-concentration method was developed for aqueous solution containing pure drug using solid phase extraction. Macroporous beads of polystyrene divinyl benzene (PSDVB) polymer were used for pre-concentration followed by chromatographic determination. Experimental parameters were optimized. The developed method was used for determination of Venlafaxine HCl in water collected from a sewage treatment facility. The developed method can detect Venlafaxine HCl concentration upto 0.003 ppm after pre-concentration.**

Keywords **- Venlafaxine HCl, Pre-concentration, HPLC, STP, Water**

I. INTRODUCTION

Venlafaxine, 1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride 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. [1] – [3] 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. [4] Several studies have reported presence of personal care and pharma products in aquatic system. [4] – [7] and [11] – [13] Understanding the fate of such drug 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 Venlafaxine HCl in water is an essential tool. There are various HPLC [8], and LC-MS methods reported in literature for quantitation of Venlafaxine HCl for different purposes. [13] To our knowledge none of the reported HPLC methods have been applied for analysis of treated sewage water for detection of Venlafaxine HCl only.

The purpose of this work was to develop an analytical method to quantitation of Venlafaxine HCl in water using a relatively simple and yet sensitive SPE method in combination with a HPLC detection method. Pre-concentration of Venlafaxine HCl 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 based upon conditions described in a reported method and optimized for Venlafaxine HCl. [10] After optimizing the pre-concentration method; it was applied to treated water sample collected from a local Sewage Treatment Plant (STP).

II. EXPERIMENTAL

A. Reagents and Chemicals

Venlafaxine hydrochloride was obtained from a local drug industry, India; whereas macro porous polystyrene divinyl benzene beads (8% cross linking) were a kind gift from Doshi Ion-Exchange, Ahmedabad, India. All other reagents and solvents were purchased from Qualigens and were of analytical or HPLC grade. These were used as obtained. Milli – Q water was prepared using with Millipore Elix® -3.

B. Instrumentation

The chromatograph system comprised of Shimadzu LC – 10 AS equipped with rheodyne injector (20µL capacity) and UV detector (SPD – 10A). Data integration was done using a software package (LC – 10). The column used was BDS Hypersil C8 (4.6 x 250 mm, 5 µ).

Pre-concentration using SPE was carried out using a glass column with stop-cock packed with adsorbent material.

C. Stock Solutions

Stock solution of 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 500ppm. To obtain standard curve, solutions of different concentration were prepared by diluting appropriate volumes of working standard solution. Similarly stock solutions of Venlafaxine HCl in Methanol solvent was also prepared. The stock solutions were refrigerated for maximum of three days.

D. Treatment to PSDVB Beads

10 g of PSDVB beads were washed in a soxhlet with 350 mL methanol for 3 h followed by 350 mL of water (10 h) and again with 350 mL of methanol (3 h). Then the beads were

dried in vacuum oven at 50° C for 3 hours. These treated beads were used for pre-concentration experiments.

E. Chromatography procedure

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 1.5 mL/min. 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 2.9 min.

F. Pre-concentration Studies

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 50 ppm Venlafaxine HCl aqueous solution of 50 mL volume was prepared by diluting an appropriate aliquote of stock solution. The column packed with 1.0 gm of the adsorbent material (PSDVB beads) was activated using 5mL acetonitrile followed by 5 mL of acetonitrile: water (80:20) (v/v) and then by 5 mL of water. The aqueous sample was passed through the column at a rate of 0.66 mL min^{-1}. The adsorbed drug was recovered with 10 mL of methanol. Amount of drug adsorbed and the amount of drug recovered in methanol was determined by HPLC analysis. To optimize the experimental conditions for pre-concentration of Venlafaxine HCl different experimental parameters were changed one – by – one, keeping other factors constant. All experimental parameters and various conditions are summarized in following sections including the results obtained from the each experiment.

G. Analysis of Environmental Samples

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, the collected water sample was filtered through Whatman filter paper (No. 41) and into the filtrate 75µL of 40% H_2SO_4 and a scoop of disodium ethylene diamine tetra acetate (Na_2EDTA) were added. [10] 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 HPLC.

III. RESULTS AND DISCUSSION

A. Pre-concentration Studies

We have earlier reported Pre-concentration studies for aspirin and paracetamol [11] and also for esomeprazole magnesium [12] different adsorbents from aqueous solution. 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 I, show that with increase in flow rate adsorption of drug on adsorbent decreases.

TABLE I
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	2.5	49.95	50	10	1.27	126.6	99.96	2.53
2	0.66	2.5	49.95	52	10	1.30	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 52%. 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 II, 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 II
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 III, 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 III
PRE – CONCENTRATION STUDIES OF VENLAFAXINE HCL: EFFECT OF VOLUME OF INITIAL DRUG SOLUTION

Sr. No.	Before			After		Recovered			PF
				Drug Adsorbed		Amount of drug			
	Initial Volume of drug solution	Amount of drug present in solution	Conc. of drug solution	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 III, 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 III, 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.

B. 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, 0.03 mg L^{-1}, 0.06 mg L^{-1}, 0.12 mg L^{-1}, 0.24 mg L^{-1}, 0.47 mg L^{-1} and 0.95 mg L^{-1} aqueous solution of venlafaxine HCl were pre – concentrated using the optimized conditions to achieve LOQ level of HPLC method.

TABLE IV
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 – 0.66 mg L^{-1}, Amount of adsorbent – 1.0g, Type of solvent for recovered – Methanol, Volume of methanol – 5mL.

TABLE IV, shows 0.03 mg L^{-1}, 0.06 mg L^{-1}, 0.12 mg L^{-1}, 0.24 mg L^{-1}, 0.47 mg L^{-1} and 0.95 mg L^{-1} aqueous solution of venlafaxine HCl can be pre – concentrated to 0.31 mg L^{-1}, 0.61 mg L^{-1}, 1.23 mg L^{-1}, 2.37 mg L^{-1}, 4.58 mg L^{-1} and 9.26 mg 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.

The curve of concentration before pre – concentration verses after pre – concentration was linear in the range of 0.03 to 0.95 mg 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, 00.03 mg L^{-1}, 0.06 mg L^{-1}, 0.12 mg L^{-1}, 0.24 mg L^{-1}, 0.47 mg L^{-1} and 0.95 mg L^{-1} environmental water samples of venlafaxine HCl were pre – concentrated using the optimized conditions to achieve LOQ level of HPLC method.

TABLE V
STUDY OF ACCURACY OF THE PRE - CONCENTRATION METHOD FOR VENLAFAXINE HCL: ENVIRONMENTAL WATER SAMPLE

Sr. No.	Before		Recovered		PF
			Amount of drug		
	Amount of drug present in solution	Conc. of drug solution	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 V shows 0.03 mg L^{-1}, 0.06 mg L^{-1}, 0.12 mg L^{-1}, 0.24 mg L^{-1}, 0.47 mg L^{-1} and 0.95 mg L^{-1} environmental water samples of venlafaxine HCl can be pre – concentrated to 0.31 mg L^{-1}, 0.6 mg L^{-1}, 1.21 mg L^{-1}, 2.3 mg L^{-1}, 4.49 mg L^{-1} and 9.1 mg L^{-1} respectively with pre – concentration factor more than 10.

The curve of concentration before pre – concentration verses after pre – concentration was linear in the range of 0.03 to 0.95 mg 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 (1 mg 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.

C. 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 water and methanol individually to the final concentrations. Each concentration was injected triplicate and the mean value of peak area was taken for the calibration curve. The calibration graphs involved at least five experiment points for compound and they are described by the following equations: for Vanlafaxine HCl in water: $y = 22423x + 157.57$ ($r^2 = 1$); for Vanlafaxine HCl in methanol: $y = 23924 x + 167.52$ ($r^2 = 1$). 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 Venlafaxine HCl was examined. Summary of analytical performance characteristics is shown in TABLE VI.

TABLE VI
RESULTS OF REGRESSION ANALYSIS: VENLAFAXINE HCL IN WATER AND METHANOL

Parameters	Venlafaxine HCl	
	In water	In methanol
Regression Equation (y)		
Correlation Coefficient (r^2)	1.0	1.0
Slope, a	22423	23924
Intercept, b	157.57	167.52
Limit of Quantification (mg L^{-1})	0.488	0.488
Limit of Detection (mg L^{-1})	0.06	0.06

IV. CONCLUSION

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 0.024 ppm. In simple laboratory conditions aqueous solution of Velnafaxine 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 0.003 ppm 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.

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