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).

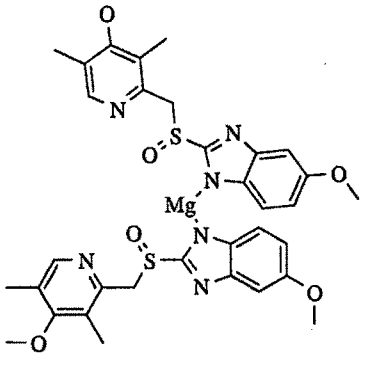

Figure 3.1. Chemical structure of Esomeprazole

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).

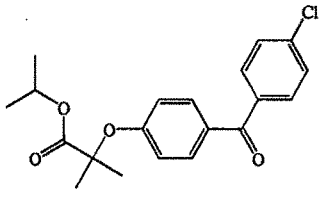

Figure 3.2. Chemical structure of Fenofibrate

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).

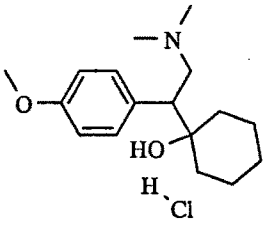

Figure 3.3. Chemical structure of Venlafaxine HCl

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.

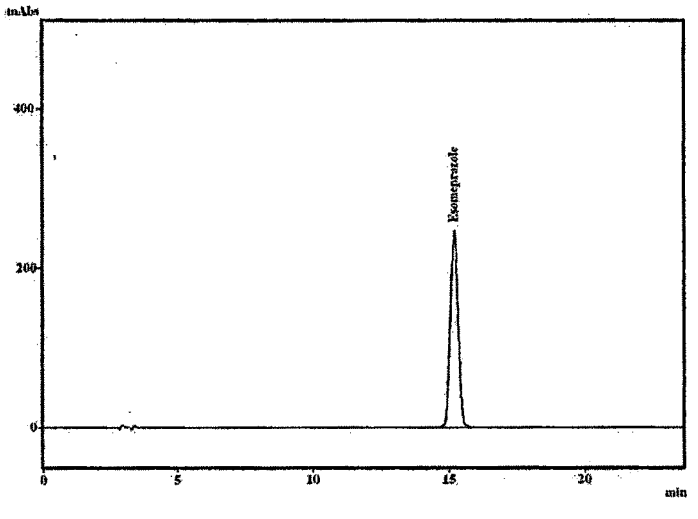

Figure 3.4. Chromatogram showing peak of esomeprazole for 100mg L^{-1} solution

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.

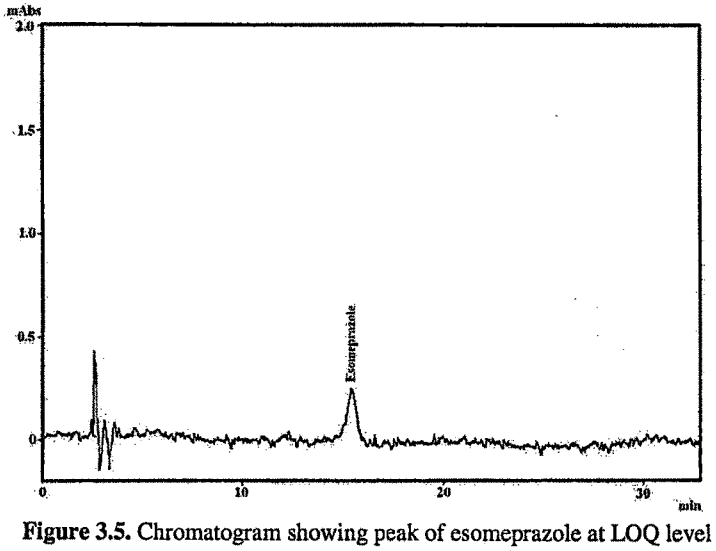

Figure 3.5. Chromatogram showing peak of esomeprazole at LOQ level

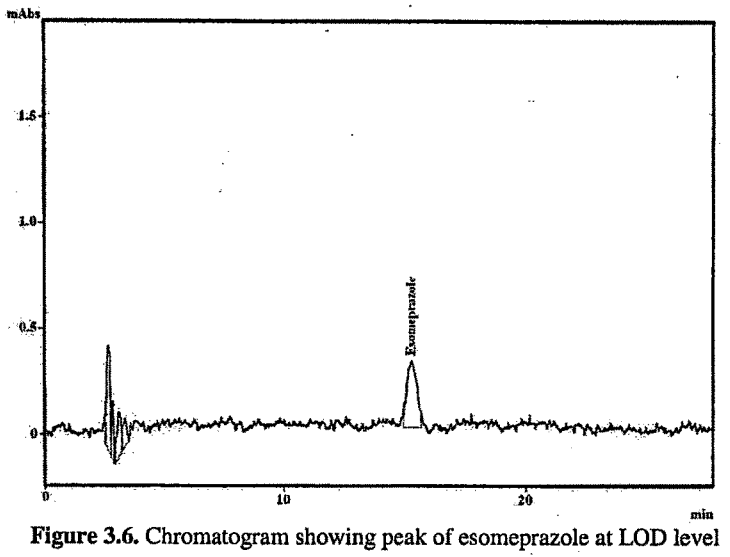

Figure 3.6. Chromatogram showing peak of esomeprazole at LOD level

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.

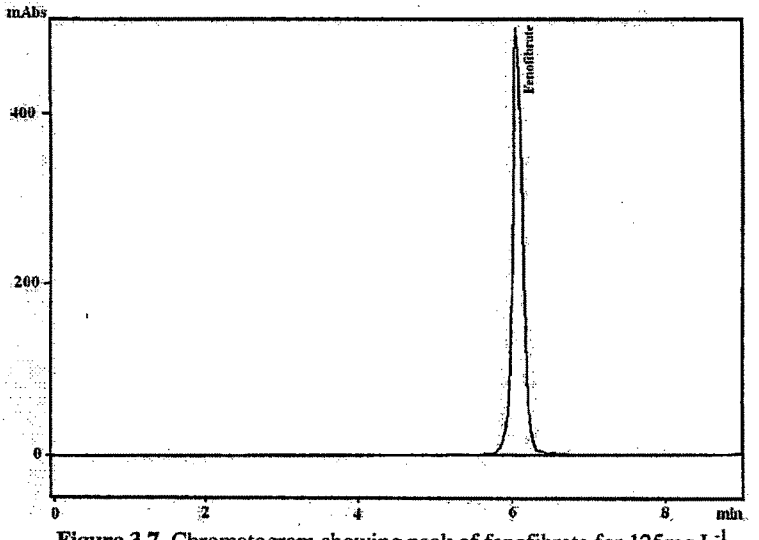

Figure 3.7. Chromatogram showing peak of fenofibrate for 125mg L^{-1}

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.

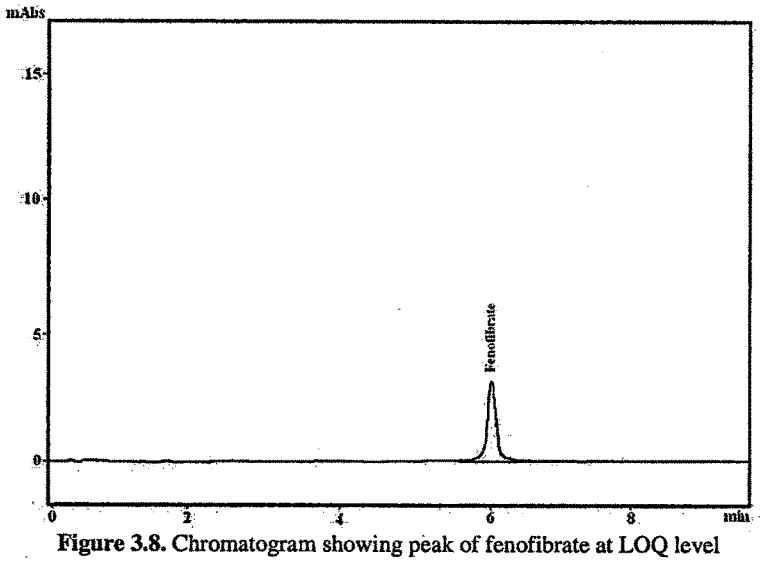

Figure 3.8. Chromatogram showing peak of fenofibrate at LOQ level

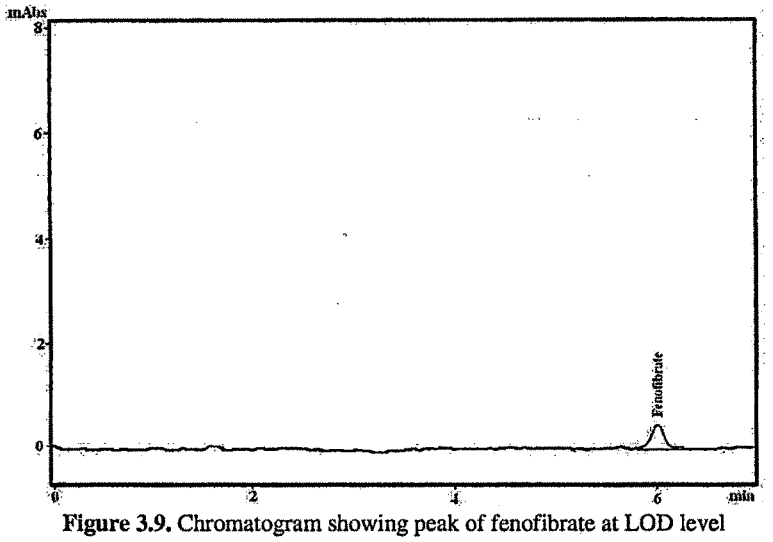

Figure 3.9. Chromatogram showing peak of fenofibrate at LOD level

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.

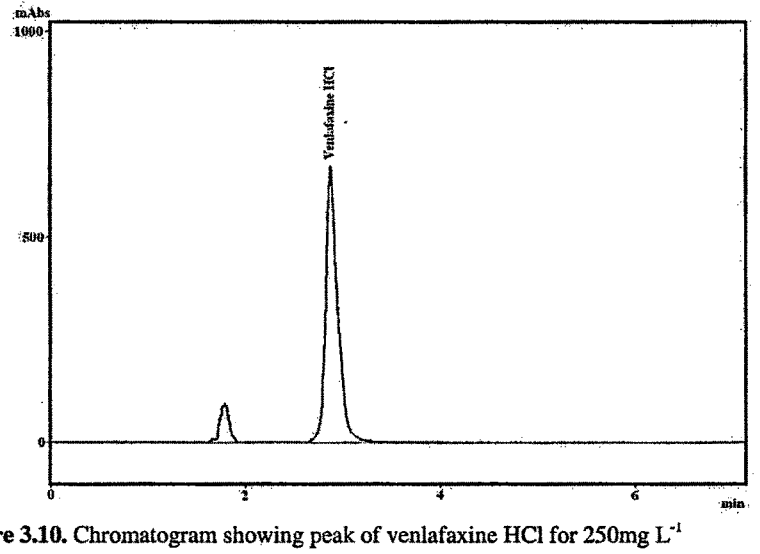

Figure 3.10. Chromatogram showing peak of venlafaxine HCl for 250mg L^{-1}

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.

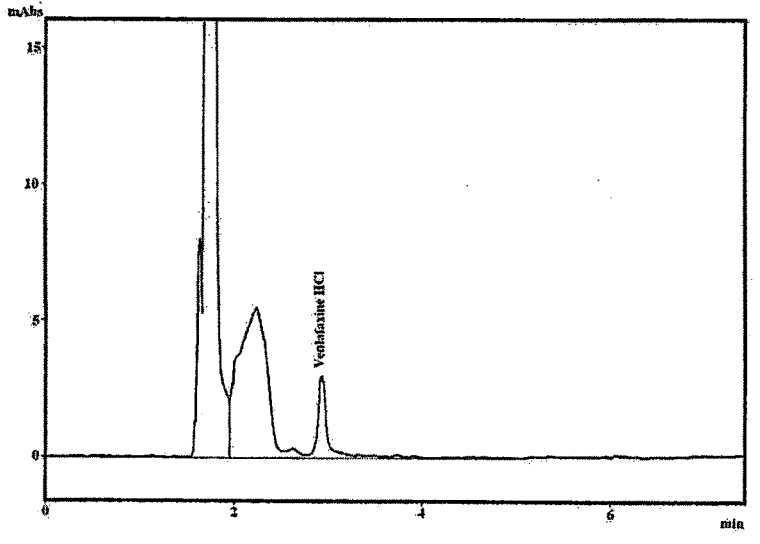

Figure 3.11. Chromatogram showing peak of venlafaxine HCl at LOQ level

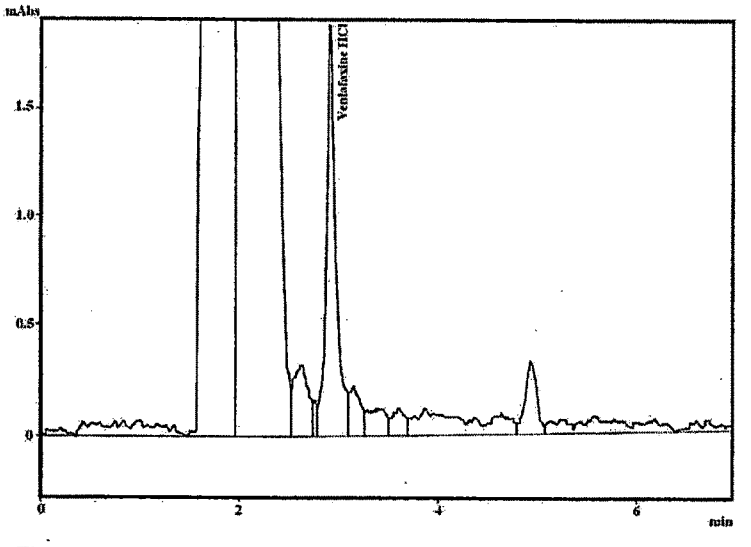

Figure 3.12. Chromatogram showing peak of venlafaxine HCl at LOD level

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.

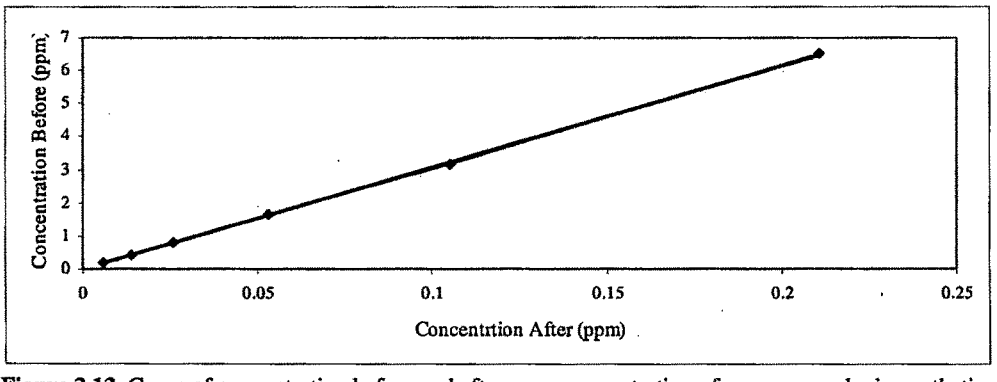

Figure 3.13. Curve of concentration before and after pre – concentration of esomeprazole in synthetic aqueous solution

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.

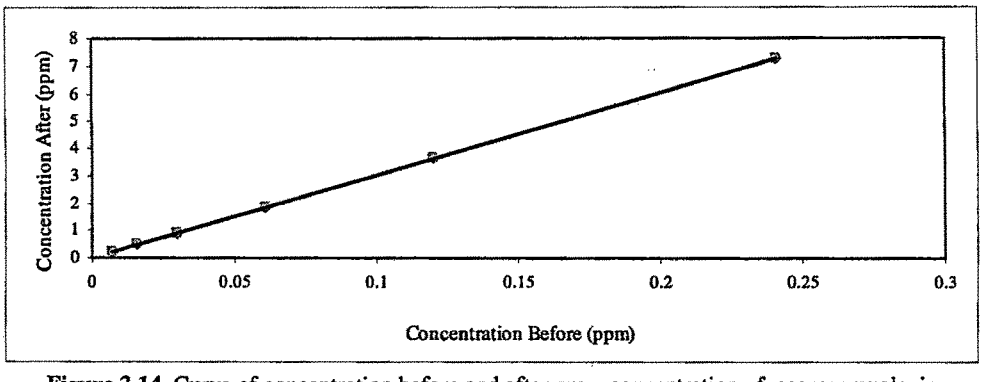

Figure 3.14. Curve of concentration before and after pre – concentration of esomeprazole in environmental water samples

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.

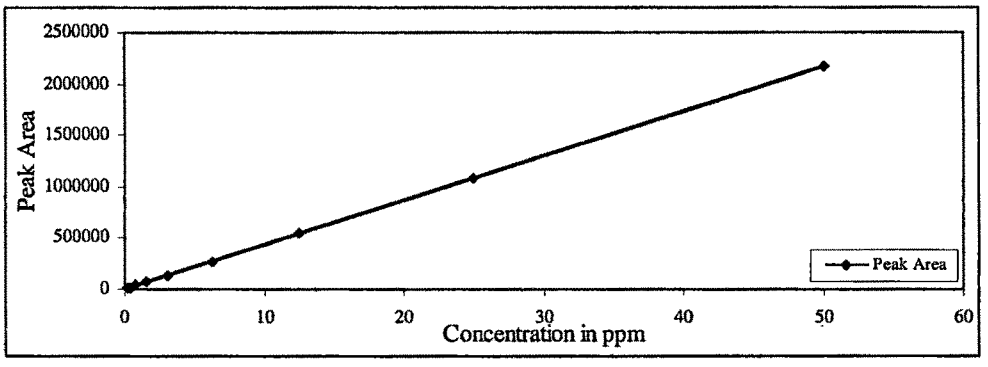

Figure 3.15. Linear working range of esomeprazole in water

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.

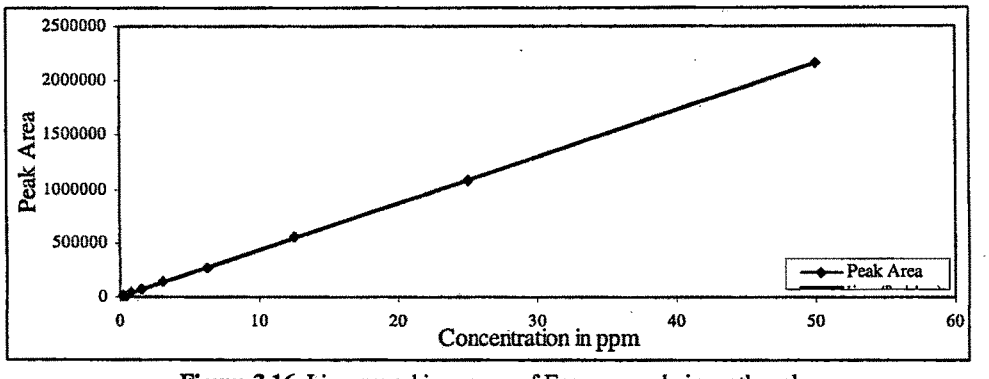

Figure 3.16. Linear working range of Esomeprazole in methanol

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.

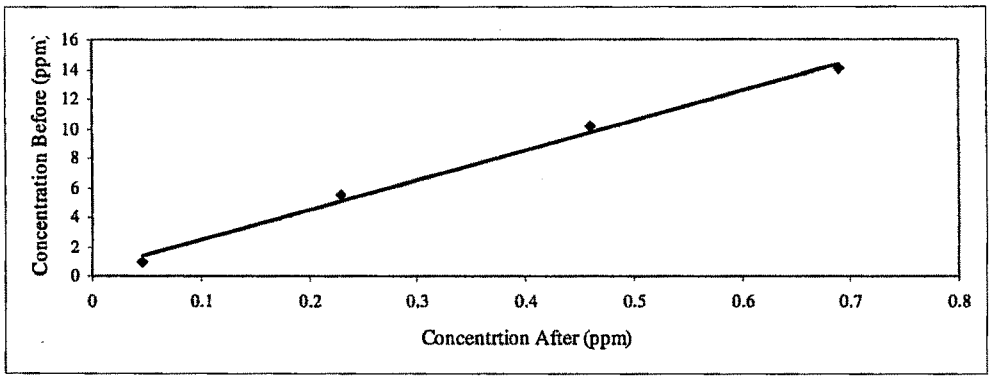

Figure 3.17. Curve of concentration before and after pre – concentration of fenofibrate in synthetic aqueous solution

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.

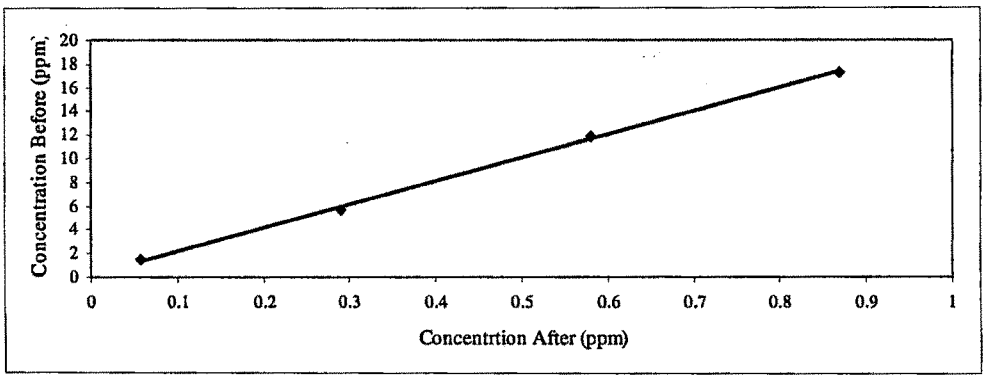

Figure 3.18. Curve of concentration before and after pre – concentration of fenofibrate in environmental water samples

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.

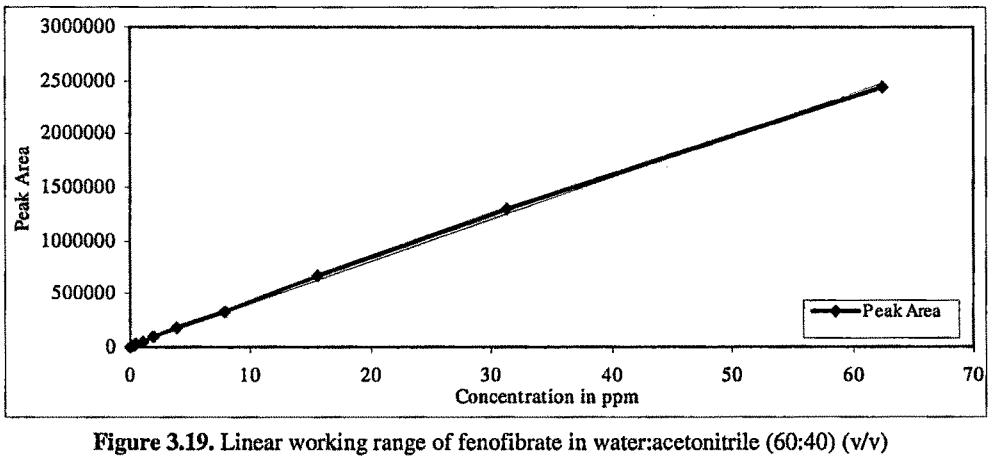

Figure 3.19. Linear working range of fenofibrate in water:acetonitrile (60:40) (v/v)

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.

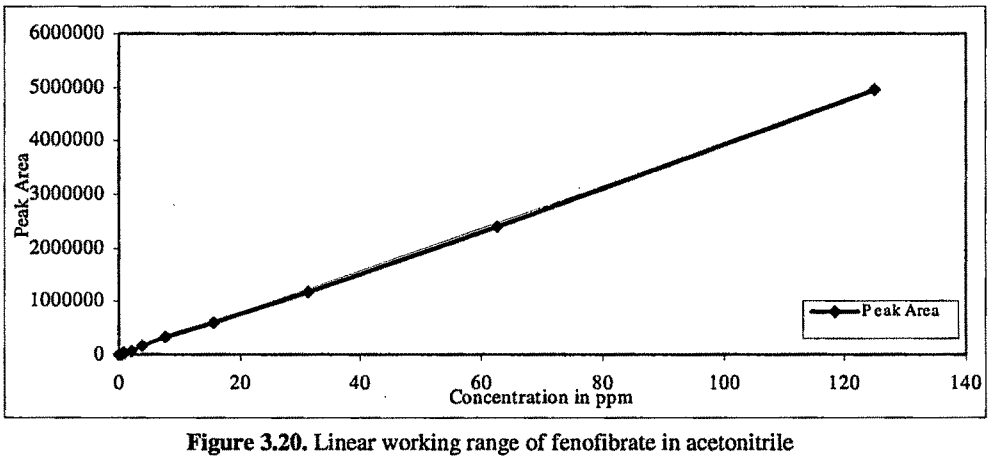

Figure 3.20. Linear working range of fenofibrate in acetonitrile

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.

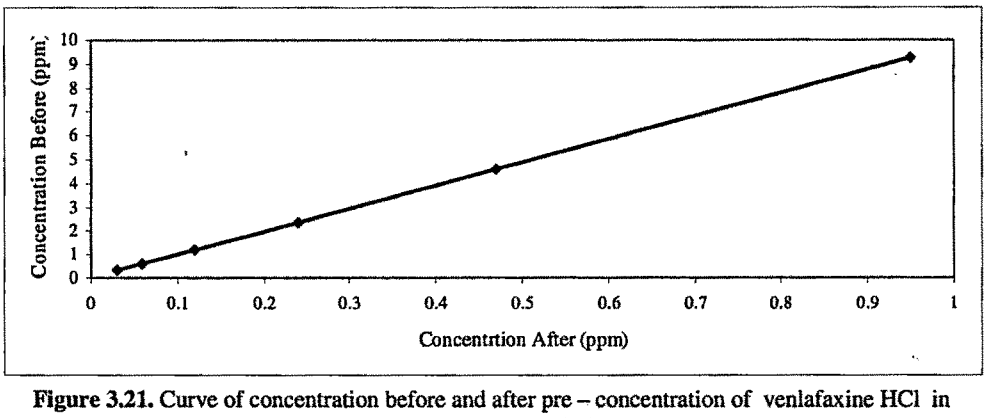

Figure 3.21. Curve of concentration before and after pre – concentration of venlafaxine HCl in synthetic aqueous solution

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.

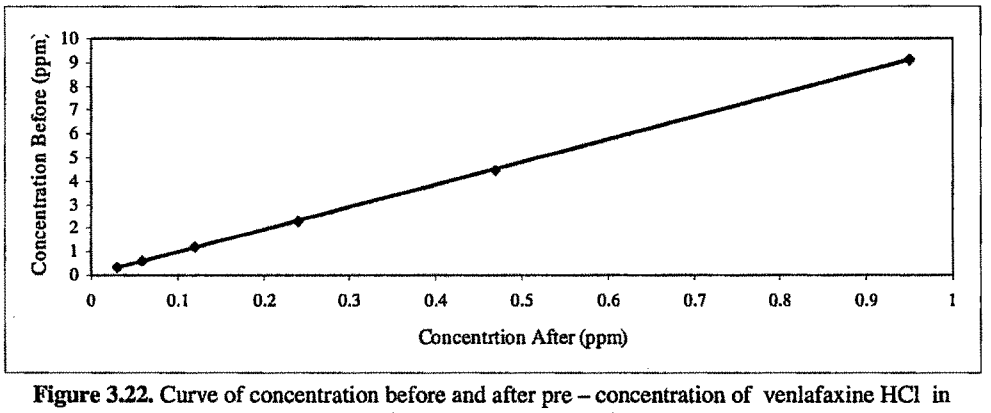

Figure 3.22. Curve of concentration before and after pre – concentration of venlafaxine HCl in environmental water sample

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.

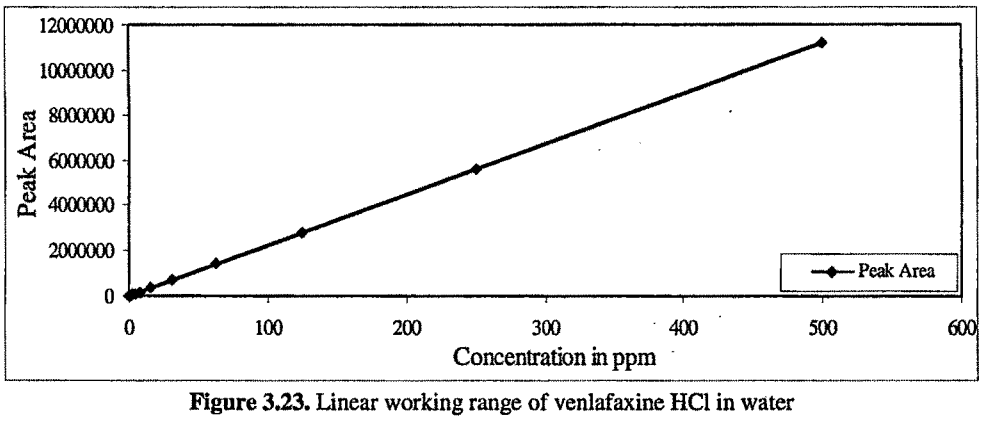

Figure 3.23. Linear working range of venlafaxine HCl in water

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.

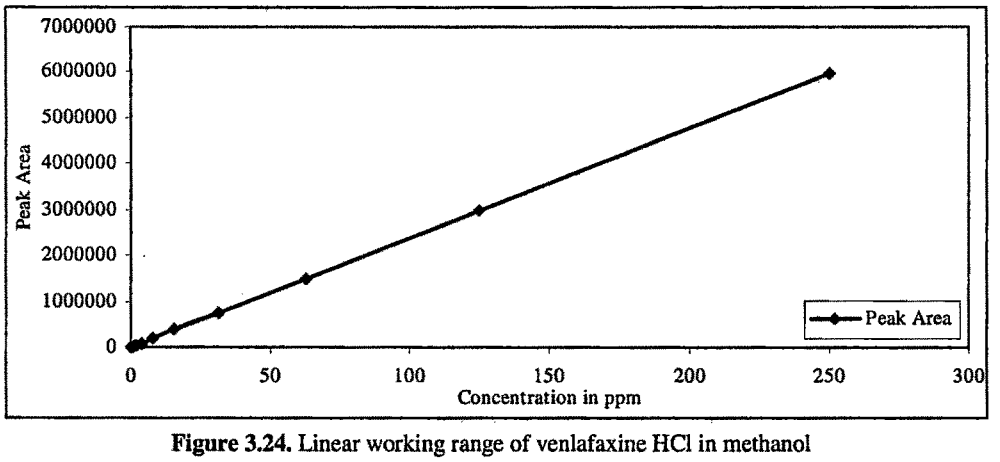

Figure 3.24. Linear working range of venlafaxine HCl in methanol

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.