CHAPTER 2

PRE – CONCENTRATION AND QUANTITATIVE DETERMINATION OF ASPIRIN AND PARACETAMOL

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

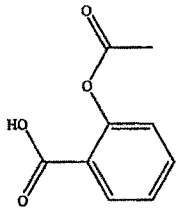

Figure 2.1. Chemical structure of aspirin

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

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

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

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

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

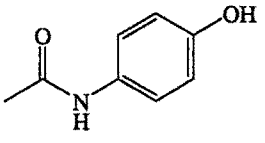

Figure 2.2. Chemical structure of paracetamol

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

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

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

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

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

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

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

EXPERIMENTS

Chemicals and Reagents

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

Instrumentation

For Pre – concentration

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

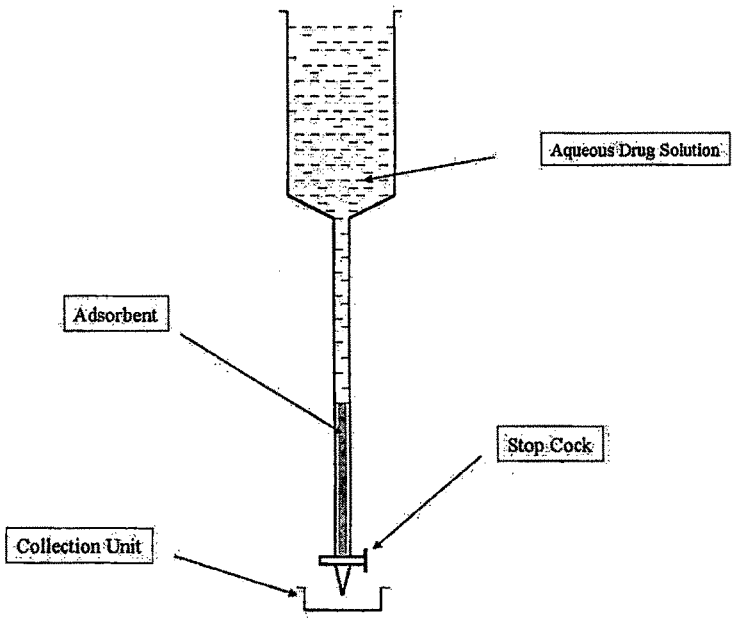

Figure 2.3. Pre – concentration method

For UV – Visible spectrophotometer

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

For Chromatographic method

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

Stock Solutions

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

Treatment to PSDVB beads

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

Pre – concentration Studies

Pre – concentration using PSDVB beads

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

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

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

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

Pre – concentration using anion – exchanger

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

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

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

Environmental Sample

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

Chromatographic Procedure

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

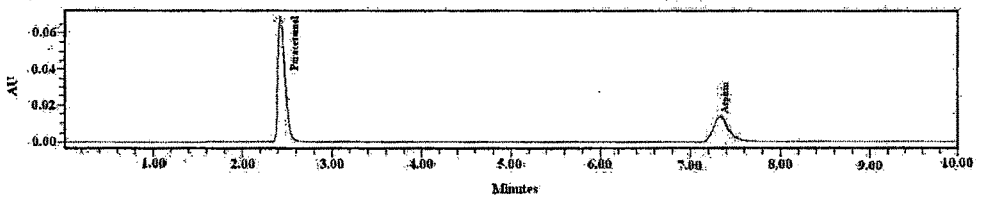

Figure 2.3. Chromatogram showing peaks of aspirin and paracetamol for 100mg L^{-1}

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

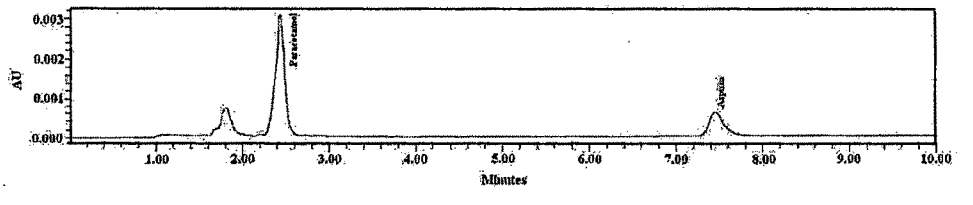

Figure 2.3. Chromatogram showing peak of aspirin and paracetamol at LOQ level

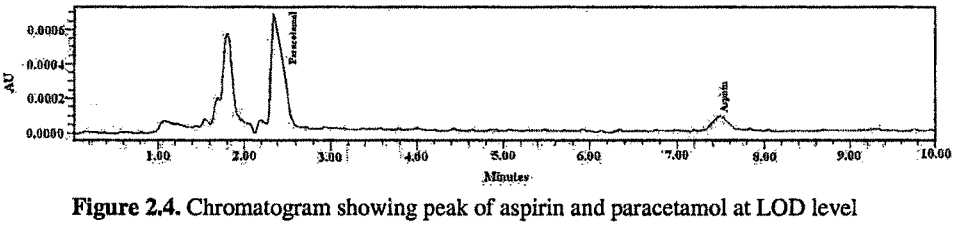

Figure 2.4. Chromatogram showing peak of aspirin and paracetamol at LOD level

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Analytical Performance Characteristics

For UV – Visible Spectrometer

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

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

Aspirin in water

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

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

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

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

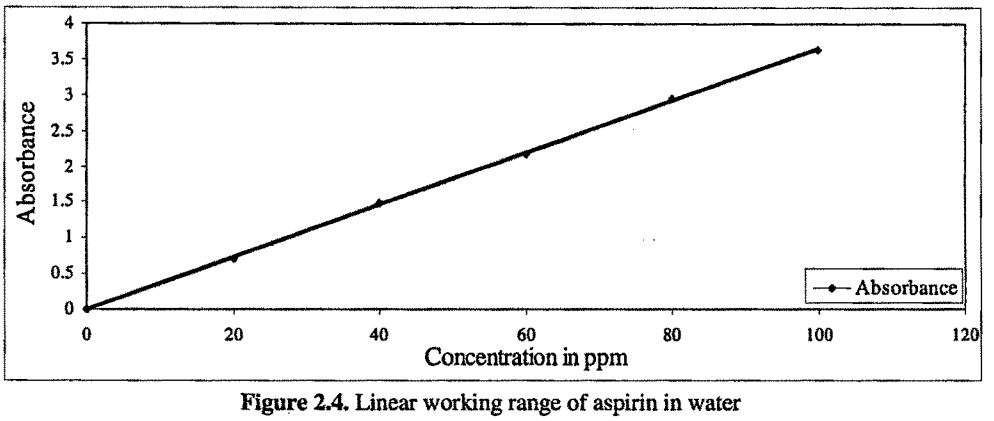

Figure 2.4. Linear working range of aspirin in water

Table 2.13. Results of regression analysis: Aspirin in water

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

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

Aspirin in 1% HCl

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

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

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

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

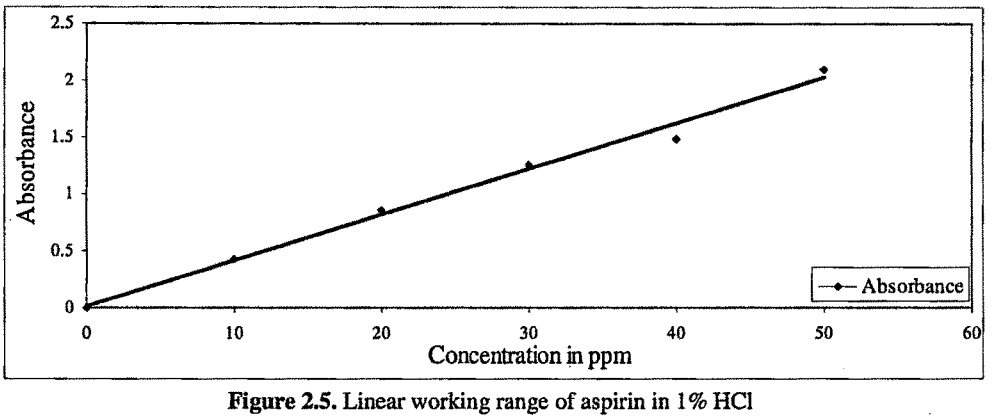

Figure 2.5. Linear working range of aspirin in 1% HCl

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

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

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

Aspirin in 2% HCl

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

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

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

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

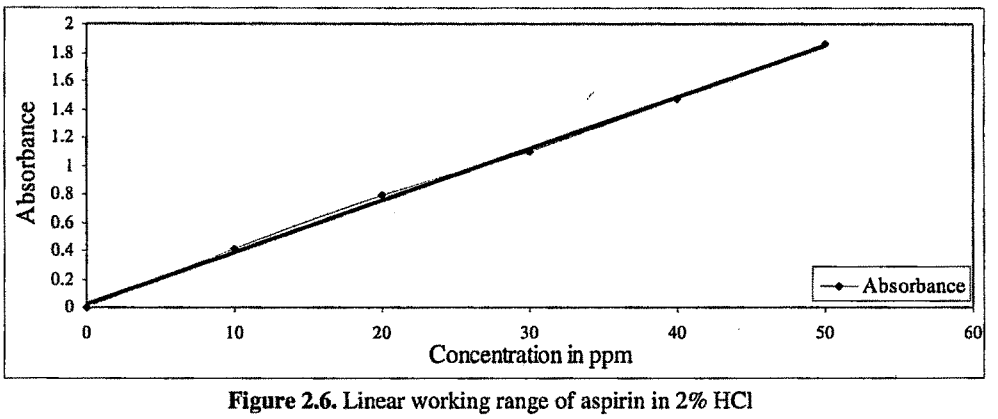

Figure 2.6. Linear working range of aspirin in 2% HCl

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

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

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

Aspirin in 5% methanol

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

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

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

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

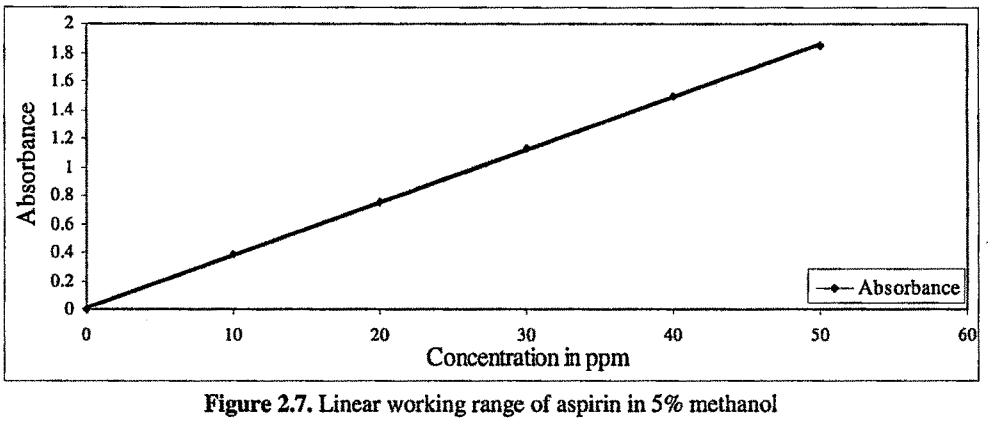

Figure 2.7. Linear working range of aspirin in 5% methanol

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

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

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

Aspirin in 20% methanol

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

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

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

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

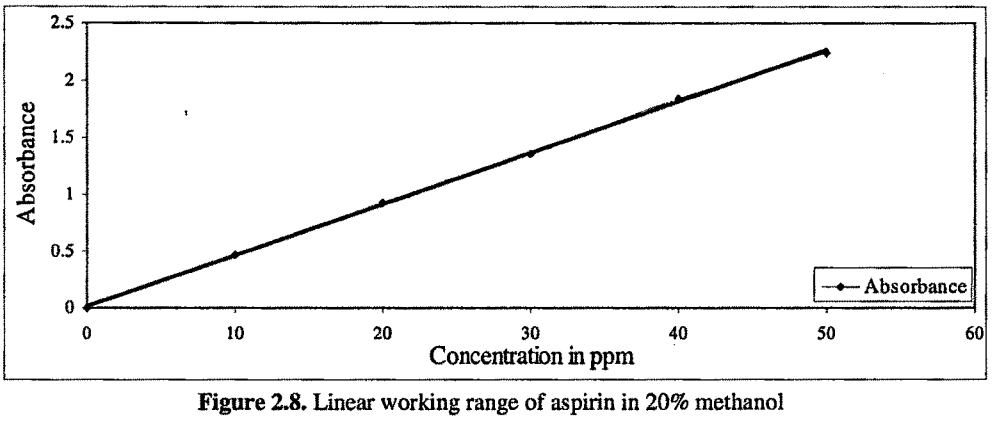

Figure 2.8. Linear working range of aspirin in 20% methanol

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

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

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

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

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

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

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

Paracetamol in water

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

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

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

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

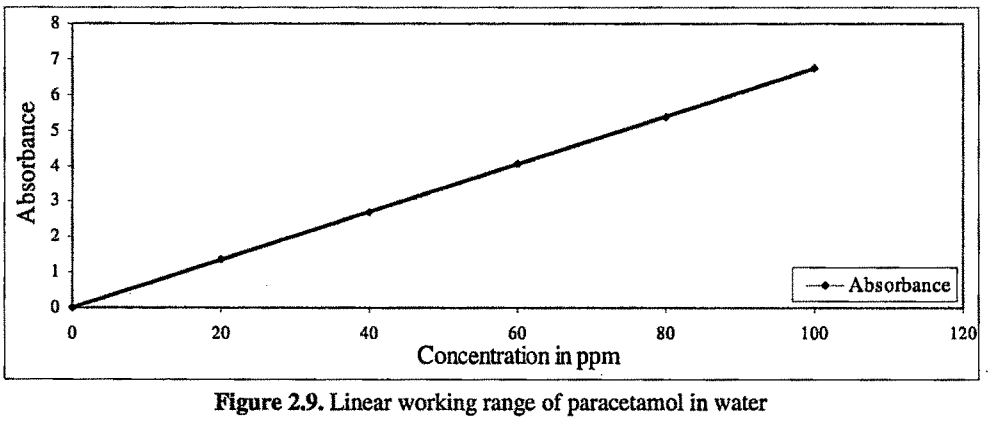

Figure 2.9. Linear working range of paracetamol in water

Table 2.23. Results of regression analysis: Paracetamol in water

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

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

Paracetamol in 1% HCl

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

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

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

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

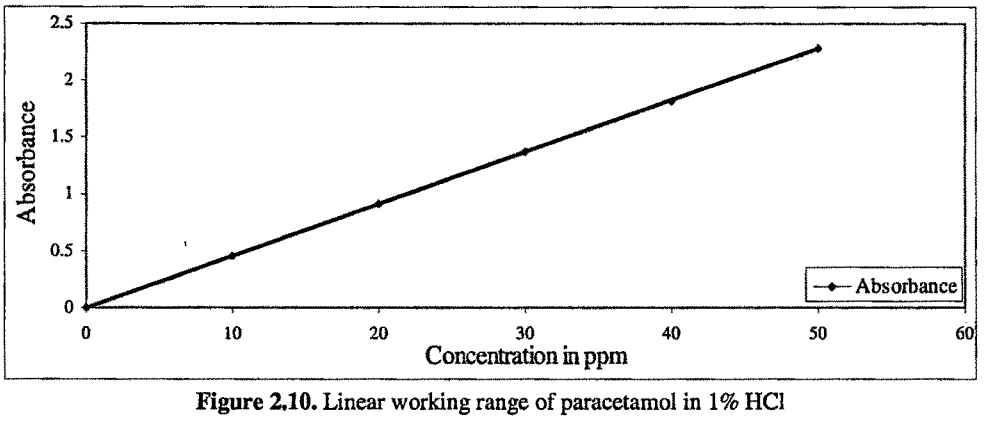

Figure 2.10. Linear working range of paracetamol in 1% HCl

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

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

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

Paracetamol in 2% HCl

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

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

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

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

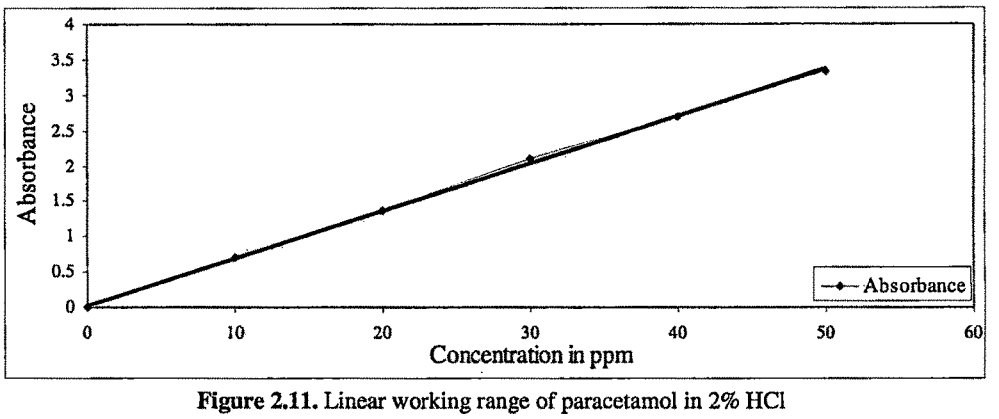

Figure 2.11. Linear working range of paracetamol in 2% HCl

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

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

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

Paracetamol in 5% methanol

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

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

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

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

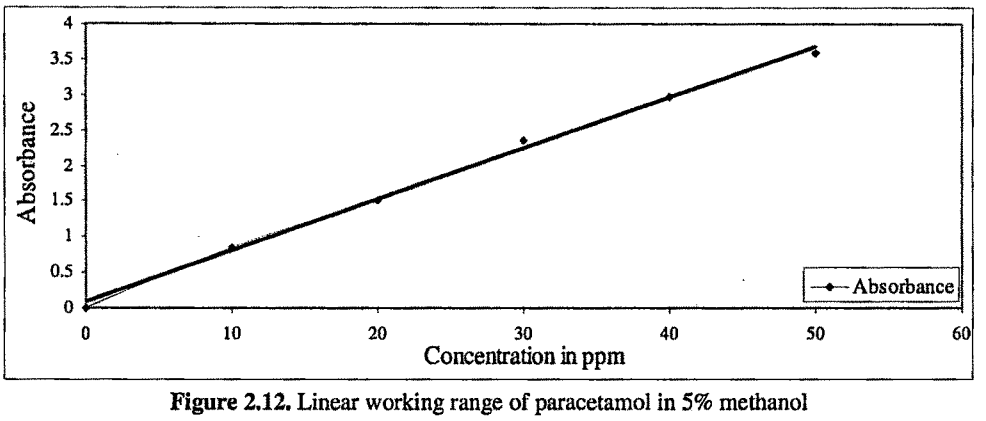

Figure 2.12. Linear working range of paracetamol in 5% methanol

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

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

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

Paracetamol in 20% methanol

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

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

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

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

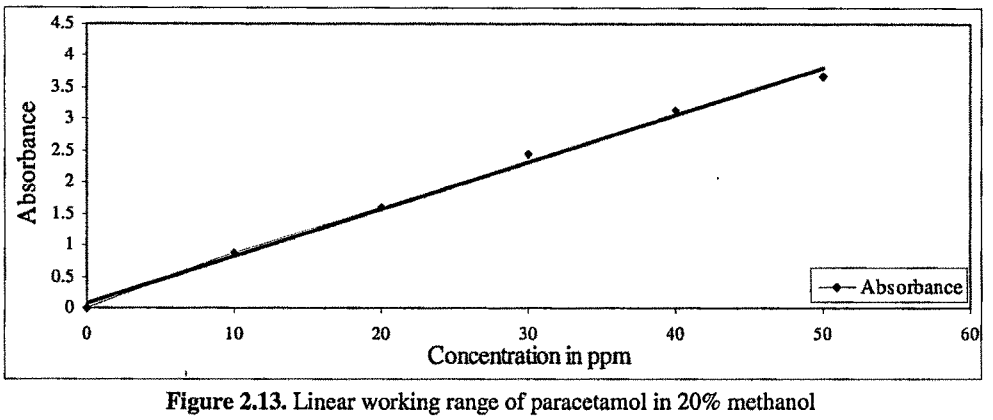

Figure 2.13. Linear working range of paracetamol in 20% methanol

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

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

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

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

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

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

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

For HPLC

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

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

Aspirin

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

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

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

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

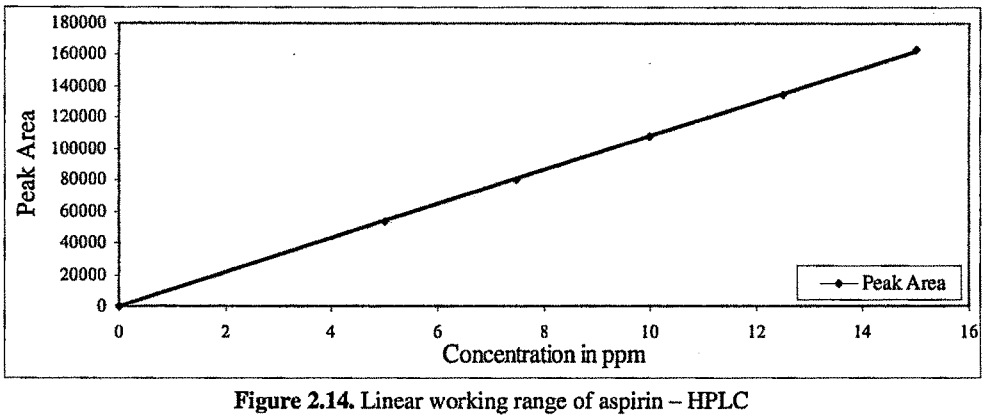

Figure 2.14. Linear working range of aspirin – HPLC

Table 2.33. Results of regression analysis: Aspirin – HPLC

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

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

Paracetamol

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

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

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

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

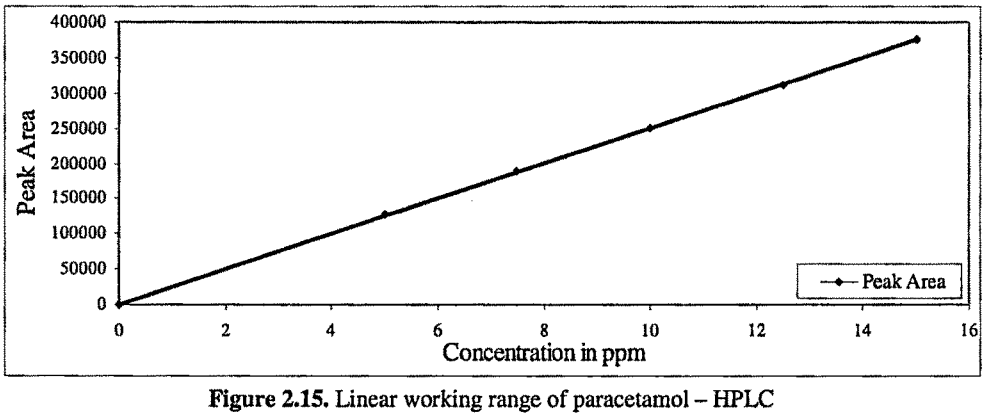

Figure 2.15. Linear working range of paracetamol – HPLC

Table 2.35. Results of regression analysis: Paracetamol – HPLC

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

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

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

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

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

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

CONCLUSION

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

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