APPENDIX 1

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A Simple Method For Simultaneous Determination Of Aspirin And Paracetamol In Treated Municipal Sewage Water In Vadodara

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ABSTRACT

A clean method for simultaneous determination of pharmaceutical compounds in treated sewage water has been developed based on UV-Vis spectrometry. This study is a part of larger work for determination of pharma products in water bodies in Vadodara, India. We report in this article data for determination of two drugs: aspirin and paracetamol. These drugs, used as model compounds, were separated from dilute aqueous solution by solid phase extraction(SPE). Macroporous beads of polystyrene divinyl benzene polymer or an anion exchanger were used for pre-concentration followed by spectrophotometric determination. Experimental parameters were optimized. The developed method was used for determination of the drugs in water sample collected from a sewage treatment facility. Presence of these drugs was not detected at the detection limits of the methods, viz. 0.1ppm. The drugs were also not detected in an HPLC method up to concentration of 0.039ppm.

KEYWORDS

Solid phase extraction;
Pre-concentration;
Aspirin;
Paracetamol;
UV-spectrometer;
HPLC.

INTRODUCTION

There is growing concern about the presence of pharmaceuticals or drugs in the aquatic environment. Following use, pharmaceuticals/drugs are excreted as the parent compound, water soluble conjugate or as metabolites and thus enter sewage treatment works (STWs). Disposal of unused pharmaceuticals can also be a route to the aquatic environment either through dumping to sewer via the toilet or drain, or to landfills in domestic refuse or as special water by licensed waste contractors[1] Despite their likely continuous discharge, little is known about the ultimate fate and transport of many drug substances after their intended application. This has led to pharmaceuticals attracting increasing attention as water pollutants due to their possible environmental effects[2]. The type/group of pharmaceuticals detected are fairly broad e.g. contraceptive hormones, lipid regulators, pain-killers, antibiotics, anti-cancer drugs, anti-epileptic drugs and those regulating blood pressure. Where

pharmaceuticals have been detected in sewage effluents or surface water, the levels are generally at the ng L^{-1} or at most, low mg L^{-1} level[1].

Such environmental trace analyses of organic pollutants usually is preferred by Gas Chromatography-Mass Spectrometry(GC-MS)or Liquid Chromatography-Mass Spectrometry(LC-MS)[2,3]. However, these techniques are relatively expensive and not easily available at several places. Need of the hour; therefore, is to develop simpler techniques which however, require higher concentration of drug molecules. Therefore, prior to the instrumental analysis, attention has to be paid to the sample preparation, and enrichment procedure.

Solid Phase Extraction(SPE) is the preferred method of sample enrichment/pre-concentration in environmental analytical chemistry. There are several solid phases on which the sample can be concentrated[3,4].

In the present work, drugs were pre-concentrated using Polystyrene Divinyl Benzene or anion-exchanger as solid phase, recovered by different solvents and later analyzed by UV-Spectrometer. After optimizing the conditions the drug samples of Aspirin and Paracetamol individually; the optimized conditions were used for mixture aqueous solution of aspirin and paracetamol together. Later the optimized method was applied for environmental sample.

EXPERIMENTS

A UV/Vis spectrometer(Perkin Elmer Lambert 35) equipped with 1cm quartz cells(4ml each) was used for all absorbance measurements. A glass column with stop-cock and packed with adsorbent material was used for pre-concentration. Chemicals and solvents used were: Double Distilled Water(DDW), methanol(qualigens), acetonitrile(qualigens), concentrated HCl(qualigens), aspirin{synthesized in laboratory[5] and characterized}, Paracetamol (API, collected from a drug industry), Polymer-Styrene Divinyl Benzene(PSDVB) beads macro-porous(8% and 12%) cross-linking and gel type(Kind gift from doshi ion-exchange, Ahmedabad, India) and anion-exchanger(Amberlite IRA-93).

For HPLC chromatographic determination, HPLC(Waters-Model number 2965 Seperation Module with empower pro software) using UV(2487 Channel 1) with measurements at 280nm and using a chromatographic column{Hyperssil BDS C_{18} (250×4.6)mm5μ} and the following solutions were also used: H_3PO_4/H_2O buffer 1ml L^{-1} and pH=3.0 with Triethyl amine(TEA)} and Acetonitrile(all of HPLC grade).

Stock solutions of drugs

Stock solutions of Aspirin and Paracetamol (1000ppm) were prepared by separately dissolving 100mg of each compound in 100ml DDW. Working standard solutions were obtained by diluting standard solution with DDW to obtain the concentration of 100ppm. The standard mixture solutions were prepared by mixing the working standard solutions to obtain 100ml, 25ppm of Aspirin and 25ppm of Paracetamol mixture respectively. To obtain standard curve, solutions of different concentrations were prepared from stock standard solutions.

Pre-concentration using PSDVB beads

Preliminary studies were conducted to work out the experimental conditions for the optimum adsorption and recovery. A typical experiment was performed by taking synthetic samples of Aspirin and Paracetamol. 100ml, 100ppm drug solution was passed through a column packed with 1gm of the adsorbent material. Adsorbed drug was recovered in 10 ml solvent. Amount of drug adsorbed was determined in eluate and the amount of drug recovered in solvent, by recording absorbance at 225nm for Aspirin. The adsorbent for SPE was activated using 5ml Acetonitrile followed by acetonitrile : DDW (80:20)(v/v).To optimize the experimental conditions nine different experimental parameters where changed (keeping all other parameters and conditions same for each experiment) and analysed for aspirin. With the optimized experimental conditions of Aspirin, paracetamol was pre-concentrated and its amount was determined by recording absorbance at 244nm. Different experimental parameters and its conditions are summarized in TABLE 1, including the results obtained from the each experiment.

Pre-concentration using anion exchanger

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TABLE 1 : Experimental parameters, conditions and their results for pre-concentration by PSDVB beads

Ex. No.	Adsorbent		Flow rate (ml min^{-1})	Aqueous solution Before Adsorption			Drug present in aqueous solution adsorbed on adsorbent				Solution used for recovery		Recovery of drug adsorbed			
	Type	Weight (gms)		Volume (ml)	Drug Present in solution (mg)		As (Weight)		Pr (Weight)		Type	Volume (ml)	As (Weight)		Pr (Weight)	
					As[A]	Pr[B]	mgs	%	mgs	%			mgs	%	mgs	%
i.	Macro12%	1	1	100	10	-[C]	0.55	5.5	-	-	H_2O	10	0.086	15.6	-	-
ii.	Gel	1	1	100	10	-	0.4	4	-	-	H_2O	10	-	-	-	-
iii.	Macro 12%	1	1	100	10	-	0.55	5.5	-	-	H_2O	10	0.086	15.6	-	-
iv.	Macro 8%	1	1	100	10	-	1.2	12	-	-	H_2O	10	0.083	6.92	-	-
v.	Macro 8%	0.5	1	100	10	-	0.4	4	-	-	H_2O	10	0.066	16.5	-	-
vi.	Macro 8%	1.5	1	100	10	-	1.4	14	-	-	H_2O	10	0.082	5.86	-	-
vii.	Macro 8%	1	0.1	100	10	-	2.1	21	-	-	H_2O	10	0.083	3.95	-	-
viii.	Macro 8%	1	1	100	10	-	1.2	12	-	-	5% CH_3OH	10	0.083	6.92	-	-
ix.	Macro 8%	1	1	100	10	-	1.2	12	-	-	20% CH_3OH	10	0.084	6.92	-	-
x.	Macro 8%	1	1	100	-	10	-	-	0.3	3	H_2O	10	-	-	0.016	5.33

In every experiment mentioned in the table, 5ml Acetonitrile was used for activation of solid phase and later Acetonitrile: Water (80:20) was used for washing

[A]As represents Aspirin ; [B]Pr represents Paracetamol ; [C]Dash represents that drug is not present in that experiment.

TABLE 2 : Experimental parameters, conditions and their results for pre-concentration by anion-exchangers

Ex. No.	Drug Present in solution (mg)		Drug present in aqueous solution adsorbed on adsorbent				Solution used for recovery		Recovery of drug absorbed			
	As[A]	Pr[B]	As(Weight)		Pr(Weight)		Type	Volume (ml)	As(Weight)		Pr(Weight)	
			mgs	%	mgs	%			mgs	%	mgs	%
i.	10	-[C]	9.9	99	-	-	1% HCl	10	0.59	5.96	-	-
ii.	10	-	9.9	99	-	-	1% HCl	10+10	0.81	8.18	-	-
iii.	-	10	-	-	1.4	14	1% HCl	10	-	-	0.33	23.57
iv.	2.5	-	2.5	100	-	-	1% HCl	10	0.42	16.8	-	-
v.	-	3.4	-	-	1.0	29.41	1% HCl	10	-	-	0.28	28.0
vi.	3.7944	2.1804	2.718	71.63	0.5647	25.90	1% HCl	10	0.8028	29.54	0.1765	31.26
vii.	3.8538	2.1804	2.961	76.83	0.5647	25.90	1% HCl	10+10	0.9997	33.76	0.3171	56.15
viii.	3.7674	2.1442	2.7824	73.85	0.3982	18.57	2% HCl	10	0.2828	10.16	0.1522	38.22
ix.	3.7674	2.1442	2.8476	75.59	0.459	21.41	2% HCl	10+10	0.9799	34.41	0.328	71.46

In every experiment mentioned in the table, 100 ml drug containing aqueous solution is passed through 1gm anion-exchanger (Amberlite IRA-93) with a flow rate 1 ml min^{-1}.

[A]As represents Aspirin ; [B]Pr represents Paracetamol ; [C]Dash represents that drug is not present in that experiment

1gm of anion exchanger was kept overnight in 20ml DDW. It was washed with 20ml DDW and activated by passing 50ml 2M HCl solution. Then the column(containing anion exchanger) was washed with 50ml DDW. Through this washed activated column, 100ppm, 100ml aspirin aqueous solution was allowed to pass at 1ml min^{-1}. The adsorbed drug was recovered with 10ml 1%HC. The same conditions were used to pre-concentrate paracetamol from water using anion-exchanger. Using these optimized conditions, the aqueous solution containing both Aspirin and Paracetamol was pre-concentrated. The samples were analyzed by UV-Spectrometer. Different experimental parameters and its conditions are summarized in TABLE 2, including the results obtained from the each experiment. For each experiment, 100ml of drug solution was passed through 1gm of anion-exchanger with a 1ml min^{-1} flow rate.

Chromatography procedure

Synthetic samples of known concentration of aspirin and paracetamol were analysed by HPLC us-

ing Buffer 80%(v/v)(1ml H_3PO_4/Liter H_2O) plus Acetonitrile 20%(v/v) as mobile phase, volume of sample injected was 20µL. The mobile phase flow rate was kept at 1.5ml /min. Under these conditions the retention time were 2.4 min for Paracetamol and 7.4min for Aspirin. Synthetic aqueous samples of mixture Aspirin and Paracetamol and environmental samples of, before and after optimized pre-concentration method using anion-exchanger were also analysed under these HPLC conditions.

Analysis of environmental samples

To test the applicability of the optimized ion-exchanger-UV-Spectrometer method, treated water samples were collected from Sewage Treatment Plant(STP) whose working principle is Up-flow Anaerobic Sludge Blanket(UASB) (capacity 43 MLD) located in Vadodara, Gujarat, India. 2.5L(volume) sample was collected from the outlet of secondary clarifier of the treatment plant in a glass container. Samples were immediately used for experiment after collection.

All samples were analyzed by UV-spectrometer and were compared with results of same samples analyzed by HPLC(Chromatographic procedure).

RESULTS AND DISCUSSION

The preliminary experimental data, TABLE 1{Sr.No.(i)and(ii)} indicate that the adsorption capacity of macroporous polymer of PSDVB is better campared to gel type polymer. Macroporous polymers used are of two different cross-linking: 12 % and 8%. Data from TABLE 1{Sr.No.(iii) and(iv)} show that macroporous polymer with 8% cross-linking is better adsorbent. For further experiments macroporous polymer having 8% cross-linking was used. The adsorption of drug from aqueous solution increases when the amount of adsorbent increases. However, the amount of drug recovered remains almost same for 10ml eluent as shown in TABLE 1 {Sr.No.(v), (vi)and(vii)}. Data of TABLE 1 {Sr.No.(viii)} show that by decreasing the flow rate the adsorption is better but the recovery after adsorption is same as that of 1 ml min^{-1} flow. Keeping the flow 1 ml min^{-1}, the polarity of recovering solvent was increased by taking 5% and 20% methanol/water respectively. From TABLE 1{(ix) and (x)}, it is seen that the recovery remains same even if solvent polarity is increased. So considering above all reasons the condition for 100 ml aqueous solution of Aspirin for pre-concnetration were optimized to 1gm of adsorbent and 10ml volume of water (Solvent for recovery) with flow of 1ml min^{-1}. Same optimized conditions were used for aqueous solution of paracetamol TABLE 1 {Sr.No.(xii)}.

To get better adsorption anion-exchanger was used as adsorbent keeping all above optimized experimental conditions same. For better recovery of drug adsorbed on anion-exchanger, 1% HCl aqueous solution was used. The efficiency of recovery was observed better when the volume of solvent for recovery was doubled, TABLE 2. From the results it is also observed that the percentage of adsorption of Paracetamol decreases in presence of Aspirin in water and with the double volume of 2% HCl solution the percentage of recovery increases for Paracetamol.

After optimizing the conditions for anion-exchanger for aqueous aspirin samples, aqueous solution of paracetamol was pre-concentrated with the same optimized conditions. These optimized conditions were used for mixture of aqueous solution of Aspirin and Paracetamol together. The amount of the individual drugs present in mixture can be derived by solving the equations(1)and(2), which are obtained adding Beer-Lambert's law.(A=abc), for two components mixture of X and Y, the mixture's absorbance, am is

$$(Am)_{\lambda 1} = (\epsilon_X)_{\lambda 1} bC_x + (\epsilon_Y)_{\lambda 1} bC_Y \quad (1)$$

Where λ1 is the wavelength at which the absorbance of component X is measured.

$$(Am)_{\lambda 2} = (\epsilon_X)_{\lambda 2} bC_x + (\epsilon_Y)_{\lambda 2} bC_Y \quad (2)$$

Where λ2 is the wavelength at which the absorbance of component Y is measured. ϵ is absorptivity (L $g^{-1}cm^{-1}$); its value is determined for each component at both wavelengths. B is path length(cm); and, C the concentration(M)[6]. With this developed pre-concentration method, environmental sample was pre-concentrated and analyzed for the two

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TABLE 3 : Optical characteristics, linearity and sensitivity of the compounds

Parameters	Aspirin					Paracetamol				
	Aqueous solution	1% HCl	2% HCl	5% methanol	20% methanol	Aqueous solution	1% HCl	2% HCl	5% methanol	20% methanol
	Absorptive(1 $mol^{-1}cm^{-1}$)									
at 225 nm	7747.143	-A	-	-	-	4660.606	-	-	-	-
at 244 nm	2540	-	-	-	-	7278.788	-	-	-	-
Correlation Coefficient (r^2)	0.9626	0.9899	0.9985	0.9998	0.9996	0.9989	1	0.9996	0.9964	0.9958
	Regression Equation (y)									
Slope, a	0.0366	0.0401	0.0367	0.0371	0.0451	0.0675	0.0455	0.0670	0.0717	0.0745
Intercept, b	-0.0052	0.0171	0.0222	0.0085	0.0122	0.0087	0.0017	0.0323	0.0856	0.0894

λ_{max} for Aspirin is 225nm and for Paracetamol is 244nm, Limit of Detection of Aspirin and Paracetamol is 0.1ppm.
[A]Dash represents the value is taken same as that of respective drug in aqueous solution.

TABLE 4 : Analytical parameters and method validation results of HPLC method

	Aspirin	Paracetamol
Correlation Coefficient (r^2)	0.9998	0.9999
Limit of detection (LOD)	0.0390ppm	0.0390ppm
Limitof quantification (LOQ)	0.3125ppm	0.0390ppm

drugs(Aspirin and Paracetamol).

Analytical performance characteristics

For UV-spectrometer

Analytical performance characteristics including linearity, precision, and accuracy, limit of detection and limit of quantitation for the analysis of Aspirin and Pracetamol by spectrometry are recorded in TABLE 3. Calibration curves were obtained by measuring the UV absorbance of the standard solutions of Aspirin and Pracetamol in a range of 25-100mg L^{-1} at wavelength 225nm for Aspirin and 244nm for Paracetamol. Linear regression and correlation coefficient(r^2) were calculated using Microsoft Excel®program. Precision of the method was determined by repetitive measurements(n=6) of the UV absorbance of the pure Aspirin and Pracetamol solutions and percent relative standard deviations(% RSD) were calculated.

The validity of the methods for the analysis of Aspirin and Pracetamol was examined using the proposed procedures. Summary of analytical performance characteristics is shown in TABLE 3. The calibration graphs involved at least four experimental points for each compound and they are described by the following equations: for Aspirin at wavelength 225nm were in DDW, $y=0.0366x-0.0052$($r^2=0.9997$), in 1%HCl $y=0.0401x+0.0171$ ($r^2=0.9899$), in 2%HCl $y=0.0367x+0.0222$($r^2=0.9985$), in 5% Methanol $y=0.0371x+0.0085$($r^2=0.9998$), in 20% Methanol $y=0.0451x+0.0122$($r^2=0.9996$) and for Paracetamol at 244nm were in DDW $y=0.0675x+0.0087$($r^2=0.9989$), in 1%HCl $y=0.0455x+0.0017$($r^2=1$), in 2% HCl $y=0.067x+0.0323$ ($r^2=0.9991$), in 5% Methanol $y=0.0717x+0.0856$($r^2=0.9964$), in 20% Methanol $y=0.0745x+0.0898$($r^2=0.9958$) Precision of the method was substantiated by calculating %RSDs of the absorbance of Aspirin and Pracetamol at wavelengths of 225 and 244nm, which were less than 0.008 for all cases(n=6).

For HPLC

The validity of chromatographic procedure was established through a study of linearity, sensitivity, repeatability. Linearity was established with a series of working solutions prepared by diluting the stock solution with mobile phase to the final concentrations. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve. The calibration graphs involved at least five experimental points for each compound and they are described by the following equations: for Aspirin $y=10899x-1056.7$($r^2=0.9998$); for Paracetamol $y=25015x+1248.4$($r^2=0.9999$). Limit of detection (LOD) and quantitation(LOQ) were calculated form visual determination method of %RSD of area. The validity of the methods for the analysis of Aspirin and Pracetamol was examined. Summary of analytical performance characteristics

is shown in TABLE 4.

The results of quantitation of drugs by HPLC and UV-visible spectrometer were obtained similar for mixture of 25 ppm Aspirin and 25 ppm Paracetamol in aqueous solution.

Application of pre-concentration method for environmental samples

The developed method was used for environmental sample of treated water from sewage treatment plant. The collected sample was immediately and directly used for pre-concentration using the developed method for pre-concentration. Later the samples were analysed by the HPLC method (Chromatography procedure). The results shows that Aspirin and Paracetamol in the environmental samples where very low beyond the detection limit of the HPLC method(0.039mg L^{-1}).

CONCLUSIONS

The method developed for pre-concentration of aqueous samples containing Aspirin and Paracetamol selected using UV-Spectrometer for quantification, is accurate, sensitive and reliable and enables the determination of the target pharmaceuticals in water samples at 0.025mg ml^{-1} for Aspirin and Paracetamol., By using, easily available and less in cost macroporous polymer of PSDVB with 8% cross-linking and anion-exchanger(Amberlite IRA-93), in simple laboratory conditions an increase in concentration by a factor of 1.30 for Aspirin and 0.76 for Paracetamol can be obtained. Quantitative analysis of Aspirin and Para cetamol individual and together can be done by UV-spectrometer even at low levels.

The water sample collected from STP(Vadodara-India) after treatment does not show presence of Aspirin and Paracetamol up to the detection level of 0.03ppm. This means either the concentration of these drugs is below the levels or STP removes these drugs effectively.

Further studies are underway for determination of other pharmaceutical compounds and also use of other commercial available adsorbents for pre-concentration.

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