APPENDIX 2

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Liquid Chromatography

PRECONCENTRATION AND QUANTITATIVE DETERMINATION OF ESOMEPRAZOLE MAGNESIUM PRESENT IN WATER

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A simple, accurate, and sensitive preconcentration method for determination of esomeprazole magnesium in treated sewage water was developed based on HPLC. A preconcentration method was developed for aqueous solution containing pure drug using solid phase extraction. Macroporous beads of polystyrene divinyl benzene (PSDVB) polymer were used for preconcentration followed by chromatographic determination. Experimental parameters were optimized. This optimized method can detect esomeprazole magnesium concentration up to 0.003 mg L^{-1} after preconcentration. This method was used for determination of esomeprazole magnesium in water collected from a sewage treatment facility. Esomeprazole magnesium could not be detected in the treated sewage water sample collected for the study.

Keywords: Esomeprazole magnesium; HPLC; Preconcentration; Solid phase extraction

INTRODUCTION

Esomeprazole magnesium trihydrated, bis (5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrated is a proton pump inhibitor (PPI) developed as an optical isomer (S-Esomeprazole) for the treatment of acid related diseases (Lind et al. 2000). Esomeprazole is a potent inhibitor of gastric acid secretion and accumulates in the acidic compartment of the parietal cells where the 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

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as for its racemate, omeprazole. Esomeprazole is also excreted in an unaltered form in the same low proportion and its presence in aquatic environment has been reported (Hernando et al. 2007). Understanding the fate of such drugs in water systems 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 in water is an essential tool. 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). 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 a simultaneous assay of the two major metabolites (Grundevik et al. 1986). In recent years the combination of liquid chromatography and mass spectrometry (LC–MS) has been used for omeprazole 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). Enantioselective methods for detection of esomeprazole by liquid chromatography have also been presented that employ mass spectrometric (Wang et al. 2005) or UV-detection (Stenhoff, Blomqvist, and Lagerstrom 1999). To our knowledge, none of these methods have been applied for analysis of treated waste water for the detection of esomeprazole using HPLC.

The purpose of this work was to develop an analytical method for quantitation of esomeprazole in water samples obtained from a waste water treatment plant using a relatively simple and yet sensitive SPE method in combination with a HPLC detection method. Preconcentration of esomeprazole from aqueous solution was carried out using PSDVB beads. After adsorption the drug was recovered from the solid phase using methanol. The resultant solution was subjected to quantitation using HPLC method optimized for esomeprazole. After optimizing the preconcentration method, it was applied to a treated water sample collected from a local Sewage Treatment Plant (STP).

EXPERIMENTS

Chemicals and Reagents

Esomeprazole was obtained from local drug industry in India, where as macroporous polystyrene divinyl benzene beads (8% cross linking) was a kind gift from Doshi Ion-Exchange, Ahmedabad, India. 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 (20 μL capacity) and UV-Vis detector (SPD-10A). Data integration was done using a software package (LC-10). The column used was BDS Hypersil C8 (4.6 × 250 mm, 5 μ).

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

Stock Solutions

Stock solution of esomeprazole was prepared by dissolving 100 mg of drug in 100 mL Milli-Q water. Working standard solution was prepared by diluting stock solution with Milli-Q water to obtain the concentration of 500 mg L^{-1}. To obtain standard curve, solutions of different concentrations were prepared by diluting appropriate volumes of working standard solution. Similarly, stock solution of esomeprazole in methanol solvent was also prepared. The stock solutions were refrigerated and were consumed within three days.

Chromatography Procedure

Synthetic samples of known concentration of esomeprazole were analyzed 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 5 mL of triethyl amine and 995 mL Milli-Q water containing 1.2 g ammonium dihydrogen orthophosphate. The flow rate was 1.0 mL min^{-1}. Detection was carried out at wavelength 302 nm. All determinations were performed at room temperature. The injection volume was 20 µL. Under these conditions the retention time of Esomeprazole prepared in methanol and water was in the range of 15.0 to 15.3 min.

Pre-Concentration Studies

For 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 a synthetic sample of esomeprazole. A sample of 100 mg L^{-1} esomeprazole aqueous solution of 100 mL volume was prepared by diluting an appropriate aliquot of stock solution. The column packed with 1.0 gm of the adsorbent material (PSDVB polymer beads) was activated by passing 5 mL acetonitrile through it followed by 5 mL of acetonitrile:water (80:20) (v/v), and then by 5 mL of water. The aqueous drug sample was passed through the activated column at the rate of 0.66 mL/min. The adsorbed drug was eluted with 10 mL of methanol. Amounts of drug adsorbed and recovered in methanol were determined by HPLC analysis. To optimize the experimental conditions for preconcentration of esomeprazole, different experimental parameters where changed, one-by-one, while keeping other factors constant.

Analysis of 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 43 MLD and is located in Vadodara, Gujarat, India. A 2.5 L (volume) sample was collected from the outlet of the 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, 75 μL of 40% H_2SO_4 and a scoop of disodium ethylene diamine tetra acetate (Na_2EDTA) were added (Lindsey, Meyer, and Thurman 2001). An aliquot of the sample was then subjected to HPLC analysis as such; whereas, the other was subjected to an optimized preconcentration step, the adsorbed drug recovered by acetonitrile, and this was also analyzed using HPLC.

RESULTS AND DISCUSSION

Preconcentration Studies

We have earlier reported preconcentration studies for aspirin and paracetamol on different adsorbents from aqueous solution (Samnani et al. 2007). Optimized conditions obtained in the study viz., 1.0 gm adsorbent, 100 mL aqueous solution of drug and 5 mL solvent for recovery were used as starting set of conditions for the present work. Initially, the effect of flow rate of aqueous esomeprazole solution on adsorption was studied. With an increase in the flow rate, the adsorption of the drug on adsorbent decreased. For subsequent experiments the 0.66 mL min^{-1} flow rate was maintained, which resulted in a maximum drug adsorption up to 70%. The effect of changing the volume of aqueous solution containing the drug and the effect of changing the amount of adsorbent while keeping volume and concentration of drug solution constant were studied and optimized in the present work for esomeprazole. Studies show that more than 70% esomeprazole adsorbs on 1 gm of polymer beads when 100 mL of its aqueous solution is passed through the column. Whereas, with 50 mL of the initial drug solution, the percentage of drug adsorption increased to 87.95. With this percentage of adsorption, methanol was used for recovery. The percentage of recovery was studied with four different volumes of methanol (3, 5, 7, and 10 mL). A maximum drug recovery up to 102.91% was observed with 10 mL of methanol, which resulted in a preconcentration factor of 6.05. With the decrease in the volume of methanol for recovery, the percentage of drug recovered decreased but the preconcentration factor increased. Considering this trend, the condition for recovery of drug adsorbed on 1 gm of adsorbent was optimized to 5 mL of methanol. With these optimized conditions for recovery, preconcentration experiments were performed taking higher volumes of aqueous drug solutions while keeping the amount of drug the same. These experiments show that with an increase in volume of the initial aqueous drug solution, the percentage of amount of drug adsorbed remains almost the same; however, after their recovery with a 5 mL methanol, the preconcentration factor for the respective experiments increased. However, this trend changed with higher volumes of the initial drug solution. To study the effect of the initial volume, different sets of experiments were done with 50, 100, 150, 250, and 500 mL of initial aqueous drug solution. With 250 mL initial drug solution, a maximum of 96.51% drug was adsorbed. Results also showed that the volume of 5 mL methanol can recover 100% of the drug at lower amounts up to 1.47 mg.

The optimized conditions for maximum adsorption of drug and its recovery with a better preconcentration factor for Esomeprazole are: 250 mL of initial aqueous drug solution passed through 1 gm PSDVB beads with a flow rate of 0.66 mL per minutes, followed by 5 mL methanol used for recovery of drug adsorbed solution.

A developed optimized method for preconcentration of esomeprazole was applied to an environmental water sample collected from STP. Before preconcentration, the sample was also analyzed by HPLC. In this case, no peaks were observed in the chromatogram for esomeprazole. The samples were spiked with a known amount of drug (1 mg L^{-1}) and analyzed, but the signal enhancement for the 1 mg L^{-1} added drug was not seen. Results indicated no presence of esomeprazole in the sample collected from the STP, which was confirmed by a LC-MS method. Details of results for the LC-MS experiment for esomeprazole, along with other drugs, are being processed as a separate paper.

Accuracy of the Preconcentration Method

The optimized conditions were used to determine accuracy of the preconcentration method by fortifying known amounts of Esomeprazole to the synthesized aqueous solution at a concentration range of 30 times less than LOQ level. Thus, 0.006 mg L^{-1}, 0.014 mg L^{-1}, 0.026 mg L^{-1}, 0.053 mg L^{-1}, 0.105 mg L^{-1}, and 0.211 mg L^{-1} aqueous solutions of esomeprazole could be preconcentrated to 0.191 mg L^{-1}, 0.422 mg L^{-1}, 0.793 mg L^{-1}, 1.664 mg L^{-1}, 3.186 mg L^{-1}, and 6.512 mg L^{-1}, respectively, with a preconcentration factor of more than 30, confirming that the designed level of preconcentration was achieved in the target concentration range in synthetic aqueous sample.

Similarly, the optimized conditions were used to determine the matrix effect by fortifying a known amount of esomeprazole to the live water sample at a concentration range of 30 times less than the LOQ level. Thus, 0.007 mg L^{-1}, 0.016 mg L^{-1}, 0.03 mg L^{-1}, 0.061 mg L^{-1}, 0.12 mg L^{-1}, and 0.241 mg L^{-1} could be preconcentrated to 0.216 mg L^{-1}, 0.491 mg L^{-1}, 0.921 mg L^{-1}, 1.857 mg L^{-1}, 3.654 mg L^{-1}, and 7.283 mg L^{-1}, respectively, with a preconcentration factor of more than 30, confirming that the designed level of preconcentration was achieved in the target concentration range in live water samples.

Analytical Performance Characteristics

The validity of the 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

Table 1. Analytical Performance Characteristics

	Esomeprazole mangnesium	
Parameters	In water	In methanol
Regression Equation (y)		
Correlation Coefficient (r^2)	1	1
Slope, a	43243	43257
Intercept,	3294.5	2539.5
Limit of Quantification (mg L^{-1})	0.19	0.19
Limit of Detection (mg L^{-1})	0.09	0.09

water and methanol, individually to the final concentrations. Each concentration was injected in triplicate and the mean value of the peak area was taken for the calibration curve. The calibration graphs involved at least five experiment points for the compound, and they are described by the following equations: for esomeprazole in water: $y = 43243x + 3294.5$ ($r^2 = 1$); for esomeprazole in methanol: $y = 43257\ x + 2539.5$ ($r^2 = 1$). Limit of detection (LOD) and quantification (LOQ) were calculated from visual determination method of % RSD of area. The validity of the methods for the analysis of esomeprazole was examined. Summary of analytical performance characteristics is shown in Table (1).

CONCLUSION

The method developed for preconcentration of aqueous solutions containing esomeprazole using the HPLC method for quantification is accurate, sensitive, and reliable and enables the determination of the target drug in water sample at $0.006\,mg\,L^{-1}$. In simple laboratory conditions, aqueous solutions of esomeprazole can be preconcentrated by a factor of 30 by using commercially an 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 $0.003\,mg\,L^{-1}$ considering the preconcentration factor in optimized conditions. This means concentration of this drug is below this level or the STP is efficient in removing the drug effectively.

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