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RATIO DERIVATIVE SPECTROPHOTOMETRIC MONITORING AND PHYTOREMEDIATION OF PANTOPRAZOLE

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ABSTRACT

Recently much attention has been placed to identify the presence of emerging contaminants with special reference to pharmaceutical wastes by the development of specific techniques for detection and monitoring of environmental pollutants at the trace level. The present work aims to investigate the application of a non-destructive, selective and less expensive ratio derivative spectrophotometric method for the monitoring of pantoprazole present in the pharmaceutical binary mixture and wastewater without their prior separation. The linear calibration curve was obtained in the concentration range of 1.0-75.0 ppm for pantoprazole (r^2 = 0.994). It was found that significant amounts of pantoprazole can be recovered from laboratory prepared binary mixtures (98.73 %, 100.69 % and 103.46 %), distilled water (98.90 %, 100.13 % and 94.57 %) and wastewater (103.60 %, 98.38 % and 104.73 %) via spike method with known concentrations (40.0 ppm, 50.0 ppm and 60.0 ppm respectively) of pharmaceuticals *viz.* omeprazole and pantoprazole. Interestingly, selected macrophytes (water hyacinth and water lettuce) were capable to remove pantoprazole (~60 %) from waste water samples.

KEY WORDS: Pharmaceuticals, Ratio derivative spectrophotometric method, Binary mixture, Wastewater, Phytoremediation.

INTRODUCTION

Pharmaceuticals are considered as emerging pollutants and introduced into water bodies from various sources. They have received increasing attention in recent years as bioactive chemicals having adverse impacts on water quality, ecosystem and human health. Thus, monitoring and removal of pharmaceuticals from wastewater have received remarkable attention (Rzymski *et al.,* 2017 and Patel *et al.*, 2019).

Very few techniques are available for the estimation of drugs as impurities present in wastewater samples. In the case of low volatile, water-soluble pharmaceuticals, GC-MS involves further derivatization that creates sample preparation laborious and time taking, as well as raises the chance of errors and contamination problems (Patel *et al.,* 2019). Sometimes multicomponent analyses using absorption spectrophotometry produce incorrect results because of strong overlapping zero-order spectra (Redasani *et al.*, 2018). The literature survey revealed that numerous techniques are used for quantitative determination of pharmaceuticals (Siddiqui *et al.*, 2017 and Ahmed *et al.,* 2019) etc.

Salinas *et al.*, established a novel spectrophotometric approach, *i.e.* ratio-derivative spectrophotometric method (RDSM), which is dependent on the usage of the first derivative of the ratio spectra for the simultaneous determination of two compounds in binary mixtures (Salinas *et al.*, 1990). RDSM is an analytical approach applied for the improvement of sensitivity and specificity in qualitative and quantitative analyses of numerous compounds without the need for their prior separation (Attimarad *et al.*, 2019). This method is frequently employed as a convenient technique in pharmaceutical analysis because of its simplicity, cost-effectiveness and rapid estimation ability (Redasani *et al.,* 2018, Kamal *et al.,* 2019, Lotfy *et al.,* 2019 and Magdy *et al.,* 2020).

It is well known that omeprazole (OMZ) and pantoprazole (PAZ) inhibit H^+, K^+, ATPase pump function (proton pump inhibitors, PPIs) and used to decrease acid secretion from the gastric parietal cell (Patel *et al.,* 2019 and Rajic *et al.,*2003). The rapid advancement of dedicated analytical strategies nowadays is allowing their evaluation at trace levels, although exploration of the fate and transformation routes of these pharmaceuticals in the environment are still mostly unexplored (Patel *et al.,* 2019). Hence, it becomes a rising concern to explore eco-efficient techniques to eliminate these PPIs before their discharge into the water bodies.

The above objectives have been realized by the monitoring of various concentrations of PAZ present at trace levels in binary mixtures and wastewater without their prior separation using RDSM. An attempt has been made to study the phytoremediation ability of water hyacinth (*Eichhornia crassipes,* Pontederiaceae family, Plant **1**) and water lettuce *(Pistia stratiotes,* Araceae family, Plant **2**) to remove PAZ present in wastewater samples.

MATERIALS AND METHODS

Instruments

Absorption spectra were documented by using UV-Vis spectrophotometer (Shimadzu UV-1700 double beam). Mettler Toledo balance was used for weighing purposes. Piezo-U-Sonic ultrasonic cleaner (250 w) was used for sonication.

Reagents

Omeprazole (OMZ, Trade name; Omepron 20.0 mg) and pantoprazole (PAZ, Trade name; Pantakind 40.0 mg) were procured from the local market and used without further purification. Methanol of HPLC grade was used for spectroscopic studies.

Preparation of Stock Solutions

The drugs were used after removing their coatings. The stock solution (500.0 ppm) was prepared in aqueous methanol by dissolving powdered OMZ and PAZ followed by sonication (5.0 mins).

Preparation of Standard Solutions

Aliquots of standard stock solutions were pipetted out and suitably diluted (1.0 ppm - 100.0 ppm) with aqueous methanol to get the final concentration of standard solutions of OMZ and PAZ**.** Prepared solutions were preserved in the refrigerator at 4.0 p C.

Ratio Derivative Spectrophotometric Method

In this method, PAZ was analyzed in the laboratory prepared mixture containing OMZ and PAZ using the first derivative of their ratio spectra. The absorbances of standard solutions of PAZ, OMZ and their binary mixtures were recorded in aqueous methanol within 200-500 nm wavelength range. The ratio spectra for PAZ were obtained by dividing the absorption spectra of the mixture by the absorption spectrum of a standard solution of OMZ. Then first derivative spectra were obtained by differentiating ratio spectra using OriginPro 8.5 software. At a specified wavelength, the ratio derivative values of every single component were plotted against their concentrations. The statistical analyses of these graphs indicate good linearity. The concentrations of the desired component were calculated from their respective calibration curves (Mansour, 2017).

Sample Collection

Wastewater samples (sewage water, Sample **1** and pond water, Sample **2**) were collected from the Nadia district. Polyethylene bottles were chosen which were formerly washed by non-ionic detergent, rinsed with tap water, soaked in 8.0 N HNO_3 for one day and then rinsed with deionized water prior to their usage.

Analysis of Pharmaceuticals in Wastewater

Wastewater quality parameters were measured by APHA method (APHA/ AWWA/ WEF, 2017). To check the versatile applications of the proposed method, PAZ was determined in Sample **1** by the spike method using commercial tablets.

The recovery study was performed on the binary mixture by adding accurately weighed amounts of PAZ to the mixture and calculating the percentage recovery in each case by comparing initial and final concentrations (Altinoz *et al.,* 2003).

Analysis of Pharmaceuticals in Wastewater after Phytoremediation

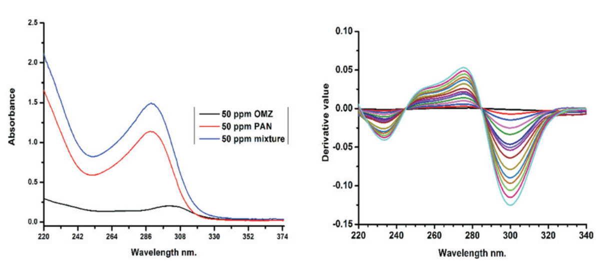
Aquatic plants were acquired from two local freshwater ponds in the Nadia district. Before use they were washed and grown in plastic containers in water for two weeks. The newly developed young plants were placed in the experimental containers (50.0 cm in length, 35.0 cm in width and 15.0 cm in depth). Phytoremediation experiments were conducted for seven days in wastewater samples using these commercial tablets (50.0 ppm) by the spike method.

Each container was filled with approximately 10.0 liter wastewater spiked with 50.0 ppm pharmaceutical mixture. The experimental study was conducted in the month of July. Mean daily temperature during the study was 25.0 ± 5.0 pC in the study area. Plastic containers were placed in a greenhouse with good ventilation and light transmission to mimic the natural conditions of ponds with aquatic plants. Physical observations of plants were conducted visually to examine the survivability of plants in the plastic containers. The observations include the freshness, color of the plants, and the level of petals survived. The percentage removal efficiency (R %) of both aquatic plants grown in spiked wastewater samples were calculated (Abbas *et al.*, 2019).

RESULTS AND DISCUSSION

Wastewater Characterization

The pH (8.3 for sample 1 and 6.8 for sample 2) values were estimated. Sample 1 exhibits high biological oxygen demand (BOD) (72.6 mg/l for sample 1 and 3.82 mg/l for sample 2) and chemical oxygen demand (COD) (409.99 mg/l for sample 1 and 130.99 mg/l for sample 2). Both the samples contain high total solids (TS) (924.0 mg/l for sample 1 and 863.0 mg/l for sample 2) and total dissolved solids (TDS) (799.0 mg/l for sample 1 and 735.0 mg/l for sample 2). Total suspended solids (TSS) were found to be 125.0 mg/l for sample 1 and 128.0 mg/l for sample 2.



(i) (ii)

Fig. 1. (i) Zero-order absorption spectra of OMZ, PAZ and their binary mixture, (ii) First derivative ratio spectra for PAZ (1.0-75.0 ppm) when 70.0 ppm OMZ using as a divisor.

Monitoring of PAZ by RDSM

The zero-order absorption spectra of OMZ, PAZ and their binary mixture were shown in Fig. 1 (i) that illustrates OMZ and PAZ exhibit overlapping spectra with absorption maxima at the wavelength of 303 nm and 290 nm respectively. The first derivative ratio spectra were obtained from the ratio spectra of PAZ as shown in Fig. 1 (ii).

In this work, monitoring of PAZ was given as a representative case in laboratory prepared binary mixture, distilled water and sample 1 by spike method. To estimate PAZ, the spectra of the mixture were initially divided by the spectrum of 70.0 ppm OMZ standard solution. The linear calibration curve was obtained in the concentration range of 1.0-75.0 ppm for PAZ with the correlation coefficient of 0.994 which indicates good linearity.

Here, Table 1 summarises the linearity range and regression coefficient obtained from the calibration graph by measuring the signals at specific wavelengths in the first derivative of the ratio spectra for PAZ at 300 nm working wavelength. Recovery studies were conducted to study the accuracy of the proposed method**.**

Each result was the average of three separate determinations; (linearity range: 1.0-75.0 ppm; regression equation: $y = -0.0077x + 0.0075$; r2: 0.994)

The results revealed that 98.73 %, 100.69 %, 103.46 % of PAZ were recovered from laboratory prepared binary mixtures, 98.90 %, 100.13 %, 94.57 % and 103.60 %, 98.38 %, 104.73 % of PAZ were recovered from spiked distilled water and sample 1 prepared by adding known concentrations of OMZ and PAZ.

Phytoremediation of PAZ followed by RDSM

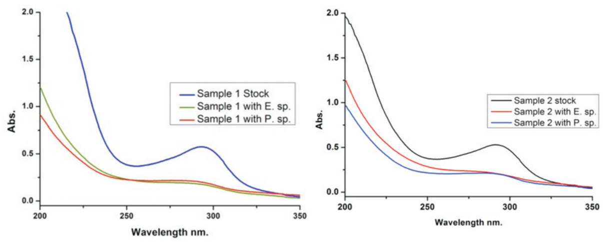
The present study aims to remediate OMZ and PAZ in wastewater samples using Plant 1 and Plant 2. After seven days, plant 1 survived well in both wastewater samples. But Plant 2 turned yellowish and died probably due to contaminant toxicity which hinders the plant growth. Plant 1 exhibits the highest survivability and adaptability in both wastewater samples.

UV-Vis absorption spectra show the decrease in PAZ concentrations (initial concentration 50.0 ppm) in sample 1 and sample 2 respectively after phytoremediation in Fig. 2 (i) and (ii).

After seven days, wastewater samples were taken from each set and absorbances were recorded in UV-Vis spectrophotometer. The PAZ concentrations

Table 1. Summary of statistical data of linear calibration curves.

Category	Amount of PAZ added (ppm)	Amount of PAZ determined (ppm)	Recovery (%)
Spiked binary mixture	40.0	39.49	98.73
	50.0	50.34	100.69
	60.0	62.08	103.46
Spiked distilled water	40.0	39.56	98.90
	50.0	50.06	100.13
	60.0	56.74	94.57
Spiked wastewater sample **1**	40.0	41.44	103.60
	50.0	49.19	98.38
	60.0	62.84	104.73



(i) (ii)

Fig. 2. (i) UV-Vis absorption spectra of sample **1** and (ii) sample **2** after phytoremediation.

were determined by RDSM and found to be 19.09 ppm and 21.31 ppm after treatment with plant 1 for wastewater sample 1 and sample 2 respectively. In the case of Plant 2, PAZ concentrations were found to be 21.44 ppm and 22.54 ppm for wastewater sample 1 and sample 2 respectively (Table 2).

Removal efficiencies of PAZ were found to be 61.82 % and 57.13 % for plant 1 in sample 1 and sample 2 respectively. Also, 57.39 % and 54.92 % removal efficiencies of PAZ were observed in the case of plant 2 for sample 1 and sample 2 respectively (Table 2).

The above study investigated the usefulness and the efficiency of selected naturally growing aquatic plants in reducing the pharmaceutical contents of two wastewater samples. It was clear that plant 1 exhibits more removal efficiency of PAZ than plant 2 in wastewater. They were capable to remove PAZ via accumulation by their efficient root system. Thus, removal efficacy of plant 1 and plant 2 through phytoremediation might be an affordable, in situ clean-up practice to reduce the pharmaceutical concentrations present in wastewaterat the laboratory, pilot as well as large scale.

CONCLUSION

The ratio derivative spectrophotometric method was simple and does not require any sophisticated technique or instrument. It was suitable for compounds having zero-order overlapping spectra. The experimental results of recovery studies obtained from the laboratory-prepared binary mixtures and spiked wastewater samples were satisfactory. This proposed method was sensitive, selective, rapid and more appropriate for the determination of pantoprazole from binary mixtures as well as in the real sample. This method has a great promise for the simultaneous determination of pharmaceuticals in mixtures and analysis of various colorants, cited drugs, without any prior separation, offering a practical potential for multicomponent analysis with its preferences of acceptable sensitivity and high selectivity. In this work, phytoremediation using water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) may be a cost-effective and eco-friendly technique to reduce pharmaceuticals from wastewater. Further degradation and detection of degraded products are in progress.

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Conflict of Interest

The authors have no conflict of interests that are directly or indirectly related to the work submitted for publication.

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Table 2. Removal percentages of PAZ in waste water samples after phytoremediation.

Plant species	Conc. (ppm) of PAZ, after 7 days in Sample **1**	Conc. (ppm) of PAZ, after 7 days in Sample **2**	Removal (%) Sample **1**	Removal (%) Sample **2**
Plant **1**	19.09	21.31	61.82	57.13
Plant **2**	21.44	22.54	57.39	54.92

Each result was the average of three separate determinations.

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