Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2010, 2(5): 21-28

A practical HPLC approach for the determination of trace level drug related substances in effluent

N. Sheetal Anand[1], M.V. Suryanarayana[2], V. Hima Bindu[3], P. Madhavan[4*], Pravin Bhandare[4]

Regulatory Affairs, Integrated Product development, Dr. Reddy's Laboratories Ltd.[1]
Analytical Research, Matrix Laboratories Ltd.[2]
Jawaharlal Nehru Technological University, Kukatpally, Department of Environmental Chemistry, Hyderabad, India[3]
Custom Pharmaceutical Services, Miyapur, Dr. Reddy's Laboratories Ltd.[4]

ABSTRACT

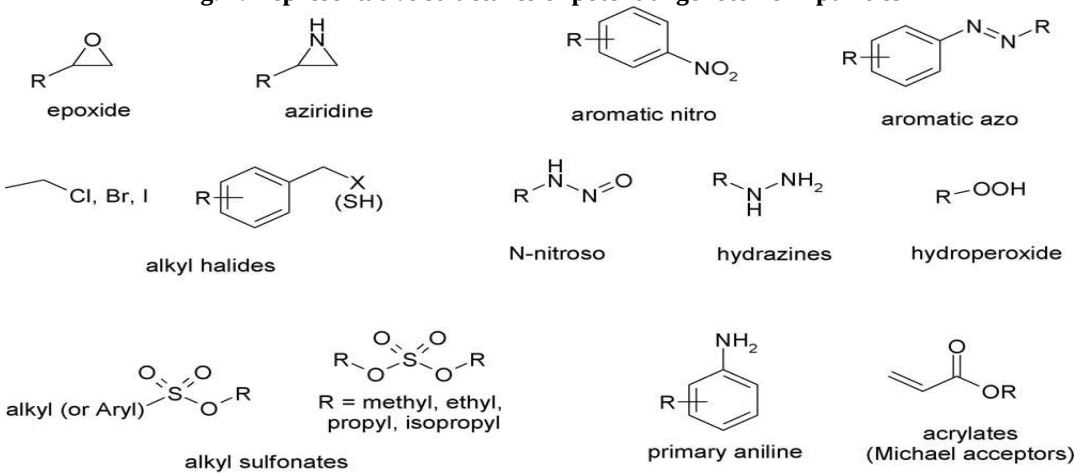
A gradient High performance liquid chromatography (HPLC) method with UV detector was developed for the determination of drug related substances and which find their origin during the manufacture of the active pharmaceutical ingredients like, Omeprazole, Rabeprazole and Pantoprazole. The selection of these active pharmaceuticals was based on usage estimates. Such drug related substances in effluents are of increasing concern in the regulatory realm due to their potential therapeutic action in humans. A validated HPLC method was developed for the study of the selected drug related substances. Good chromatographic separation was achieved for all the substances owing to their structural similarities thereby resulting in acceptable resolution. The HPLC method employs market available Xterra RP 18 column. The constitution of the mobile phase solutions A and B are as follows: The solution A contains 10 mM Ammonium acetate buffer with pH 8 and solution B contains pure Acetonitrile. The detection wavelength used is 210 nm. The resolution between the closely eluting analytes, Pantoprazole N-Oxide and Rabeprazole N-Oxide was found to be greater than 2. The limit of quantification (LOQ) and Limit of detection (LOD) of the proposed method is in the range of 0.16 ppm – 0.3 ppm and 0.053 ppm – 0.1 ppm respectively. Recovery at limit of quantification of the pharmaceuticals were higher than 80% and the precision at limit of quantification calculated as relative standard deviation (RSD), ranged from 7.13% to 14.76%. The validated HPLC method is further employed to study the concentrations of these drug related substances in effluents pre and post treatment.

Key words: Omeprazole, Rabeprazole Pantoprazole Pharmaceutical effluents

INTRODUCTION

With growing demand for pharmaceutical products, industries are seamlessly engaged in producing voluminous drugs. As a result, the governmental agencies and educational academies in the role of the watch dog are monitoring the environmental loads of therapeutic drugs [1]. However responsibility lies with the pharmaceutical industries to follow guidance provided by various regulatory and environmental bodies [2,3]. Environmental protection agency (EPA) establishes effluent limitations guidelines and standards to require a minimum level of treatment for industrial point sources [2]. The occurrence of numerous pharmaceuticals in municipal waste water and in surface waters that receive waste water effluent have been reported [4, 5, 6, and 7]. Although effluent limitation guidelines ensure overall quality of effluents, very few studies have been carried out on the effluents toxicity [8]. For difficult-to-treat waste waters, many industries are prohibited from discharging any liquid waste originating from their facilities. More companies and industries have to treat or eliminate waste streams to a much higher standard than ever before. The usage of certain reactive reagents leading to the formation of intermediates and byproducts may pose a risk of damaging the genetic material, DNA of an organism. Such impurities are termed, Genotoxic impurities. The study on deciding whether or not a given impurity possesses a genotoxicity risk was published by Mueller et al. [9]. Some of the commonly encountered potentially genotoxic structural motifs are exemplified in fig. 1 [9]. Therefore, elimination of drug related substances through good treatment techniques is a necessity to ensure the environmental quality.

Fig. 1. Representative structures of potential genotoxic impurities



The specific objectives include:

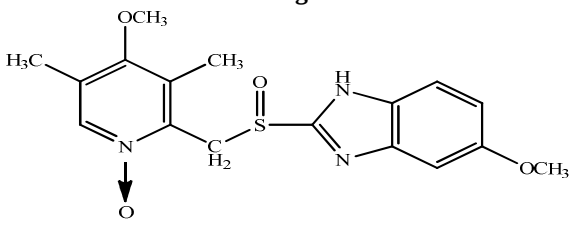
- To develop a sensitive validated HPLC method that is capable of retaining and separating the chosen analytes with close structural similarities.
- Investigating the effect of effluent treatment system on these analytes through the newly established validated HPLC method.

EXPERIMENTAL SECTION

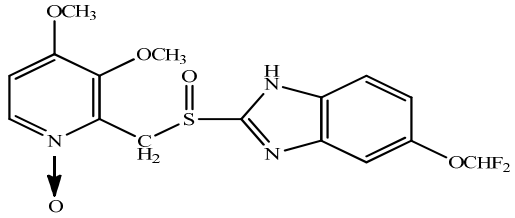
2.1 Chemicals and reagents

Samples of 5- Methoxy-2-[[(4-methoxy-3, 5-dimethyl-pyridin-2-yl)-1-oxide methyl]- sulphinyl]-1*H*-benzimidazole (Omeprazole N-Oxide), 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole-1-oxide (Pantoprazole N-Oxide), 2-[[[4-(3-methoxy propoxy)-3-methyl-2-pyridinyl-1-oxide] methyl] sulphinyl] -1*H*-benzimidazole (Rabeprazole N-Oxide), 2-chloromethyl-3, 5-dimethyl-4-methoxy pyridine hydrochloride (Cc-993) and 4-(3-methoxypropoxy)-3-methyl-2-chloromethyl pyridine hydrochloride (C-686) (Fig.2) were received from business unit of Dr. Reddy's Laboratories Ltd., Hyderabad, India. C-686 and Cc-993 find their origin from Rabeprazole and Omeprazole. HPLC grade Acetonitrile was purchased from Rankem, India. HPLC grade Methanol was purchased from SD – Fine Chemicals limited, India and analytical reagent grade Ammonium acetate was purchased from Qualigens fine chemicals, India. Dichloromethane was purchased from Sigma Aldrich, USA. High pure water was prepared by using Millipore Milli Q plus purification system.

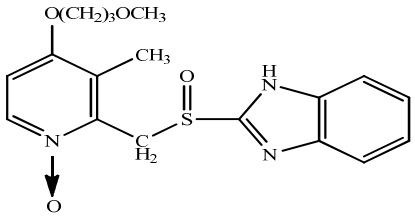
Fig. 2: Chemical structures of the drug related substances



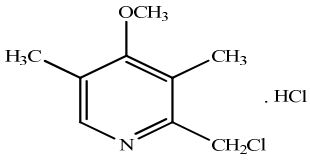
5-methoxy-2-[[(4-methoxy-3,5-dimethyl pyridin-2-yl)-1-oxide)methyl]-sulphinyl]-1H-Benzimidazole (Omeprazole N-Oxide)



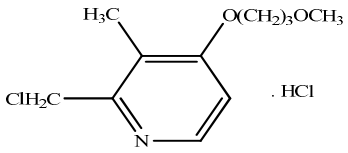
5-Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulphinyl]-1H-benzimidazole-1-oxide (Pantoprazole N-Oxide)



2[[[4-(3-Methoxypropoxy)-3-methyl-2-pyridinyl-1-oxide]methyl]sulphinyl]-1H-benzimidazole (Rabeprazole N-Oxide)



2-Chloromethyl-3,5-dimethyl-4-methoxypyridine hydrochloride (Cc-993)



4-(3-Methoxypropoxy)-3-methyl-2-chloromethyl pyridine hydrochloride (C-686)

2.2 Instrumentation

The HPLC system used for the method development was Agilent 1100 series manufactured by Agilent Technologies, Waldbronn, Germany. The system is equipped with a photo diode array detector. The output signal was monitored and processed using Chemstation software designed by Agilent Technologies, Waldbronn, Germany on Lenovo computer.

2.3 Method

The Xterra column with Hybrid Particle Technology is based on an organic /inorganic particle that combines all the advantages of silica and polymeric chromatographic supports for basic compounds was selected for method development. The analytes are quantified at detector wavelength 210 nm. The dimensions of Xterra RP18 column used are 150 mm length, 4.6 mm internal diameter, with 5 µ particle size. The gradient Mobile phases comprise of Mobile phase A:10 mM ammonium acetate (pH 8); Mobile phase B: Acetonitrile. The column temperature was maintained at 25°C throughout the experiments. The flow rate of the mobile phase was maintained at 0.8 mL/ min with 20 µL injection volume. Mobile phase A, Acetonitrile and Methanol in the ratio 70:20:10 was used as the Diluent. The gradient has been programmed as: Time (min) / %B: 0/10, 15/25, 20/35, 25/35 and 30/10.

2.4 Preparation of standard solutions

Stock solution of Omeprazole N-Oxide, Rabeprazole N-Oxide, Pantoprazole N-Oxide, Cc-993 and C-686 was prepared by dissolving appropriate quantity in the diluent. Stock solution was further diluted with diluent to obtain a standard solution of 1 mg/mL for the determination.

2.5 Sample pretreatment

The effluent samples were pretreated before injecting into the HPLC system by neutralizing the pH to 7 followed by centrifugation. The upper layer is extracted and treated with dichloromethane to obtain two layers. The aqueous layer is extracted and mixed with diluent in 1:1 ratio, filtered through 0.45 µ filters and injected.

RESULTS AND DISCUSSION

3.1 Method development and optimization

Numbers of attempts were made to develop a sensitive stability indicating, precise and accurate HPLC method for the selected analytes namely, Omeprazole N-Oxide, Pantoprazole N-Oxide, Rabeprazole N-Oxide, C-686 and C-993. Owing to the structural similarities it was difficult to initially resolve Omeprazole N-Oxide, Pantoprazole N-Oxide and Rabeprazole N-Oxide, from each other. C-686 and Cc-993 originate from Rabeprazole and Omeprazole respectively. Since the molecules chosen for study are basic in nature, the method development trial started with columns that could withstand high basic conditions. Hence various columns were screened which include the Zorbax eclipse XDB, Polaris and X-bridge columns. Similarly various buffers like ammonium formate and phosphate were screened before finally concluding on the Ammonium acetate. Combination of Ammonium acetate buffer and Acetonitrile resulted in better resolution. To achieve resolution, initially isocratic method was used which resulted in the merging of the closely eluting analytes namely, Pantoprazole N-Oxide, Omperazole N-Oxide and Rabeprazole N-Oxide. Hence gradient elution was preferred. For this pure Acetonitrile was

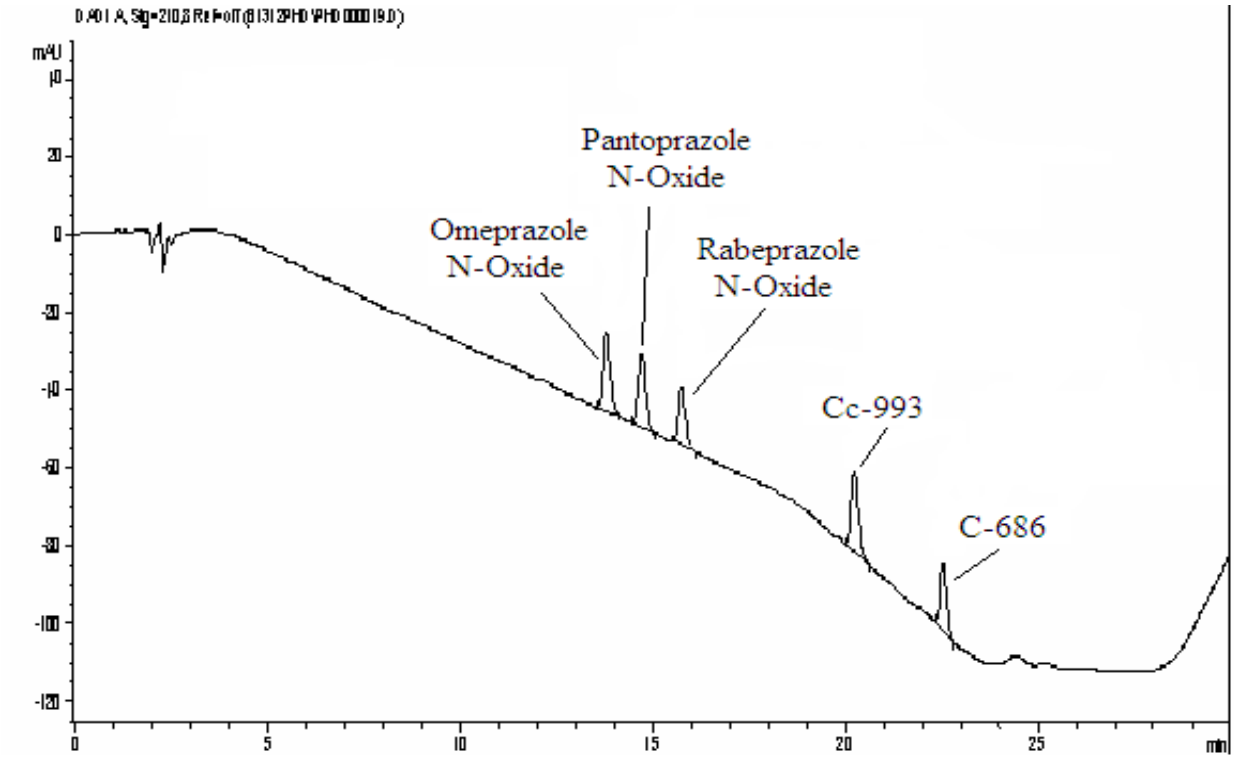
used as Mobile phase B and the compositions for better separation was studied before concluding at 10 mM strength and pH 10. With combination of Acetonitrile, it was found that the retention time as well as the tailing factor was fantastic. pH of the buffer has played a significant role in achieving a good resolution.

The chromatographic separation was achieved on X-Terra RP18, 150 mm x 4.6 mm, 5 µm column by using solutions A and B as mobile phase. The solution A contains 10 mM Ammonium acetate buffer, pH 10 and solution B contains pure Acetonitrile. The column temperature (25°C) has improved the peak shape of all the analytes. Under optimized conditions, excellent resolution was achieved with a resolution greater than 1.5 (Fig.3). The system suitability results are presented in Table – 1 and the developed HPLC method was found to be specific for all the analytes. The tailing factor ranged from 1.10 – 1.15 and the retention time ranging from 15 minutes to 24 minutes.

Table 1: System suitability results in standard blend solution

System suitability	Omeprazole N-Oxide	Rabeprazole N-Oxide	Pantoprazole N-Oxide	Cc-993	C-686
USP Tailing factor (T)	1.12	1.102	1.15	1.145	1.152
No. of theoretical plates (N) Tangent method	35574	16338	16338	47779	65340
USP Resolution (Rs)	-	2.26	2.26	9.34	5.93

Fig. 3: Typical impurities spiked chromatogram



3.2 Optimized Chromatographic conditions

X-Terra RP18 column with dimensions 150 mm length, 4.6 mm ID and 5 µm particle size has been finalized. The gradient HPLC method employs solutions A and B as mobile phase at a flow rate of 0.8 mL/min. The solution A contains Ammonium acetate buffer at pH 8 and solution B contains pure Acetonitrile. The HPLC gradient program of mobile phase composition was set as Time (min) / % solution B: 0/10, 15/25, 20/35, 25/35 and 30/10. The column temperature was maintained at 25°C and the detection was monitored at wavelength 210 nm. The injection volume is 20 µL. A mixture of Mobile phase A, Acetonitrile and Methanol in the ratio 70:20:10, v/v was used as the Diluent.

3.3 Method Validation

3.3.1 Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit [11]. The Limit of detection has been established for individual analytes and the results are tabulated in Table – 2.

3.3.2 Limit of Quantification (LOQ)

Limit of quantification is the minimum concentration at which the analyte can be reliably quantified with acceptable accuracy and precision, where the determination of signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. A typical signal-to-noise ratio is 9 – 10.4 [11]. The Limit of quantification has been established for individual analytes and the results are tabulated in Table – 2.

Table 2: Limit of detection and Limit of Quantification

Name of the Analyte	LOD	LOQ
Omeprazole N-Oxide	0.076 ppm	0.23 ppm
Rabeprazole N-Oxide	0.06 ppm	0.18 ppm
Pantoprazole N-Oxide	0.056 ppm	0.17 ppm
Cc-993	0.1 ppm	0.3 ppm
C-686	0.053 ppm	0.16 ppm

3.3.3 Precision at Limit of Quantitation

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the same homogenous sample under prescribed conditions [10-11]. The precision of the method has been performed by injecting six preparations of each analyte at the limit of quantitation. The percentage relative standard deviation (%RSD) of the peak area of individual analyte at LOQ level was found to be below 10%.

3.3.4 Accuracy at Limit of Quantification

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy solution was prepared by spiking standard solution at their respective limit of Quantitation level in triplicate and injected each preparation once into chromatographic system. The % recovery for each analyte was found higher than 80%.

3.3.5 Effluent treatment and Results

The effluents were collected from a pharmaceutical company which produces large volumes of active pharmaceuticals namely Rabeprazole, Omeprazole and Pantoprazole on a regular basis. The facility is equipped with Zero liquid discharge to treat the generated effluents. The Zero liquid discharge technique involves seven stages of effluent treatment namely Flocculation, Aeration, Filtration, Thermal destruction through Multiple effect evaporator (MEE), spray drying, forced combustion and scrubbing. The untreated effluent samples were analyzed for the key parameters recommended by the Pollution control board (PCB). Very high Chemical oxygen demand (COD), Biological oxygen demand (BOD) values were reported (Ref: Table: 3). Following the treatment through MEE, the obtained condensate is collected, analyzed for COD, BOD and few key parameters (Table: 4). Thus obtained condensate is directed to the cooling towers and the concentrate is fed in to the Spray drier where the temperature maintained is 100 °C. The vapors from the spray drier are passed into the forced combustion chamber where 800°C temperature is maintained and then to the scrubber where the acid vapors are scrubbed with alkaline to form salts. The water vapors escape into the atmosphere. The residue from spray drier is directed to the landfills.

The pre and post treated effluent including sludge was analyzed using the newly developed and validated HPLC method in order to study the effectiveness of the treatment system in removing the drug related substances. The results are tabulated in Table 5.

Calculation:

Concentration of the analyte = sample area / standard area * concentration of standard/1 *100.

The analytes that were seen in the pre-treated effluents were below detection levels post treatment.

Table 3: Analysis of pretreated effluent

S. No	Parameters	Pantoprazole	Rabeprazole	Omeprazole
1	pH	7.42	8.62	7.82
2	* Total Suspended Solids	893	23,164	136
3	* Biochemical Oxygen demand (5 days at 20 °C)	5839	46,508	80,592
4	* Chemical Oxygen demand	13,610	1,72,106	4,21,638
5	* Total dissolved solids	5,596	4,52,630	40,346

** All the values are expressed in mg/ L*

Table -4 : After treatment (Outlet waste water)

S. No	Parameters	Outlet waste water	Limits Prescribed by **CETP
1	Biochemical Oxygen demand (5 days at 20 °C)	273	---
2	Chemical Oxygen demand	1204	---
3	Total dissolved solids	1452	5,000

** All the values are expressed in mg/ L*

*** CETP – Common Effluent Treatment Plant*

Table 5: HPLC analysis results

Effluent name	Concentration in ppm Omeprazole N-Oxide	Concentration in ppm Rabeprazole N-Oxide	Concentration in ppm Pantoprazole N-Oxide	Concentration in ppm C-993	Concentration in ppm C-686
Condensate	Less than *LOD	Less than LOD	Less than LOD	Less than LOD	Less than LOD

** LOD – Limit of Detection*

CONCLUSION

The validated method for the determination of the analytes content in effluents thus proved that the zero liquid discharge is effectively able to eliminate the drug related substances that generated in production of Omeprazole, Rabeprazole and Pantoprazole, from effluent.

REFERENCES

[1] S.Castiglioni, R.Bagnati, R.Fanelli, F.Pomati, D.Calamari and E.Zuccato , *Environ. Sci. Technol*, **2006**, 40: 357-363.
[2] U.S. Environmental Protection Agency Office of Water Engineering and Analysis Division, *Permit Guidance Document: Pharmaceutical Manufacturing Point Source Category*, **2006,** 40 CFR Part 439.
[3] U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), *Guidance for Industry Environmental Assessment of Human Drug and Biologics Applications*, **1998**.
[4] Buser, H.R., M.D. Muller and N. Theobald, *Environ. Sci. Technol.*, **1998**, 32: 188-192.
[5] Buser, H.R., T. Poiger and M.D. Muller, *Environ. Sci. Technol.*, **1998**, 32: 3449-3456.
[6] Hartmann, A., A.C. Alder and T. Koller R.M. Widmer, *Environ. Toxicol. Chem.*, **1998**, 17: 371-382.
[7] Hirsch, R., T. Ternes, K, Habener and K.L, Kratz, *Sci. Total Environ.*, **1999**, 225: 109-118.
[8] A. Akintonwa, O. Awodele, A.T. Olofinnade, C. Anyakora, G.O. Afolayan and H. A. B. Coker, *American J. Pharmacology and Toxicology*, **2009**, 4 (4): 142-148.
[9] L. Mueller, R.J. Mauthe, C.M. Riley, M.M. Andino, D. De Antonis, C. Beels, J. De George, A.G.M. De Knaep, D. Ellison, J.A. Fagerland, R. Frank, B. Fritschel, S. Galloway, E. Harpur, C.D.N. Humfrey, A.S. Jacks, N. Jagota, J. Mackinnon, G. Mohan, D.K. Ness, M.R. O'Donovan, M.D. Smith, G. Vudathala, L. Yotti, Regul, *Toxicol. Pharmacol*, **2006**, 44: 198–211.
[10] United States Pharmacopoiea, USP 32. The United States Pharmacopoeial Convention, Maryland, USA, **2009**. General Chapters <621>, <1225>.
[11] International Conference on Harmonization tripartite guideline, ICH Secretariat, Geneva, Switzerland, **2005**. Q2 R1.