ORIGINAL ARTICLE

A RP-HPLC METHOD FOR THE ASSAY OF CEFPIROME AND ITS APPLICATION IN DRUG-METAL INTERACTION STUDIES

M. SAEED ARAYNE, NAJMA SULTANA* AND M. NAWAZ

Department of Chemistry, University of Karachi, Karachi-75270 - Email: arayne@gawab.com

**Research Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi*

ABSTRACT

An accurate, sensitive and least time consuming RP-HPLC method for the estimation of cefpirome in the presence of essential and trace metal has been developed and validated. Cefpirome was eluted from a B144A, OD-5-100, C_{18} (150 x 4.6 mm) column at room temperature with a mobile phase consisting of MeOH:H_2O (15:85, % v/v) at a flow rate of 1 ml/minute, while UV detection was performed at 265 nm. The detection limit of cefpirome was 10 ng.

Drug metal interaction studies were carried out at 37 ^oC to monitor the complexation of drug with metal ions. These studies were beneficial to determine the drug in therapeutic concentrations inside human body as well as its complexation with metal cations. The metals essential to human body like Mg(II), Ca(II), Cr(II), Mn(II), Fe (III), Co(II), Ni(II), Cu(II), Zn(II) & Cd(II) were in the form of chlorides. The carboxylic group of the dehydrothiazine ring has more binding capacity relative to other group that augments the drug complexes with essential and trace elements.

The established HPLC method is rapid, accurate, and selective, because of its sensitivity and reproducibility. The order of complexation was ferric > chromium > copper > nickel > cadmium > zinc > magnesium > manganese > calcium > cobalt.

Keywords: Cefpirome, metals, interactions, HPLC-UV.

INTRODUCTION

Cefpirome (fig. 1) is a 1-[[7-[[(2-amino-4-thiazolyl)(methoxyimino) acetyl]-amino]-2carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-en-3-yl] methyl]-6,7-dihydro-5H-1-pyrindinium hydroxide inner salt. It is a broad-spectrum semisynthetic β-lactamase resistant fourth generation cephalosporin having quaternary ammonium group at the 3 position of the cephem nucleus. It is used for the treatment of upper and lower urinary tract; lower respiratory tract, skin and soft tissue infections. Cefpirome is excreted largely unchanged in the urine with a half-life of 2 hours (Wilson and Gisvold's, 2004). Many workers reported methods for the assay of cefpirome in pharmaceutical formulations, and in human serum (Breilh *et al*., 1990; Sugioka *et al*., 1990; Ip, M. *et al*., 1998). These methods involve either complicated mobile phases, or buffers that may be corrosive to the column or flow system of HPLC. Previous investigators (Bertran *et al*., 1984, Jones et *al*., 1984, Maass *et al*., 1987, Seibert *et al*., 1983) have also involved various microbiological assay methods, including both microdilution and agar dilution, to quantitate cefpirome. Two studies (Maass *et al*., 1987, Malerczyk *et al*., 1987) reported the pharmacokinetics of cefpirome in adults after quantitation of the compound in serum by using high-performance liquid chromatography (HPLC) assay for which specific details were not provided.

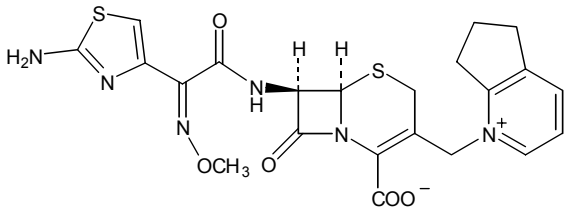

Fig. 1

Antibiotics are prone to many interactions with other drugs. There are number of interactions reported with other cephalosporins, fluoroquinolones (Bruce *et al*., 1996), aminoglycosides (Barbhaiva *et al*., 1992) and antacids containing di and trivalent metal cations (Arayne *et. al*., 2005). The availability of cephalosporins can be affected by the concurrent ingestion of drugs containing multivalent cations. It is imperative to be aware of these interactions because they may so greatly affect the antibiotic availability that it may compromise the patient's outcome. In present paper we present an accurate and fast gradient method for the quantitation of cefpirome and its metal complexes with essential and trace elements, which may either be present in low concentrations in human body or may be ingested as a result of multiple drug therapy. The proposed method has been validated and has low LOD (10 ng) and LOQ (20 ng).

Corresponding author: Tel.: +92-21-4610136; email; arayne@gawab.com

Table 1: Percent accuracy/recovery of the method for cefpitrome analysis

Conc. injected (μg.ml$^-$)	<— Cefpirome (Retention time = 6.90 minutes)—>			
	Recovery	S.D	RSD	% accuracy
1.00	0.999	0.001	0.001	99.90
2.00	1.997	0.002	0.001	99.85
4.00	4.017	0.012	0.003	100.43
5.00	5.008	0.006	0.001	100.16
10.00	10.033	0.023	0.002	100.33
50.00	49.085	0.647	0.013	98.17

Table 2: Inter-day recovery and regression characteristics of proposed method for cefpirome

Concentration	<——— Recovered concentration ———>			
Injected (μg.ml$^-$)	Day1	Day 2	Day 3	Day 4
1.00	0.99	0.99	1.00	1.00
2.00	1.96	1.99	2.01	2.01
4.00	3.99	4.00	4.00	4.00
5.00	4.97	5.00	4.98	4.99
10.00	10.01	10.00	10.01	10.00
50.00	49.96	50.00	50.00	50.00
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Standard error	0.0015	0.0020	0.0038	0.0024
Std Error of estimate	0.0040	0.0053	0.0100	0.0064
Intercept	0.0025	-0.0035	-0.0009	0.0014
Slope	1.00	1.00	1.00	1.00
P value	0.0000	0.0000	0.0000	0.0000

*Mean recovery values represent "concentration mean" of ten different samples for each concentration.

EXPERIMENTAL

Material and reagents

Reference standard of cefpirome was obtained from Pantheon UK Limited, England. All reagents used were of HPLC grade. Methanol gradient grade (Merck, USA) and HPLC grade water were used to prepare the mobile phase. All other reagents used were of spectroscopic grade or analytical grade and obtained from BDH Laboratories Supplies (BDH Chemical Ltd., Poole, U.K.).

Apparatus

The liquid chromatographic system consisted of Shimadzu model LC-10AT VP gradient pump, a Shimadzu model SPD-10AT VP, variable wavelength UV-Visible detector (Shimadzu Corporation, Kyoto, Japan). Chromatographic system was integrated via Shimadzu model CBM-102 Communication Bus Module. Analysis was conducted on a B144A, OD-5-100, C_{18} (150 x 4.6 mm) analytical reverse-phased column at 37^{o}C.

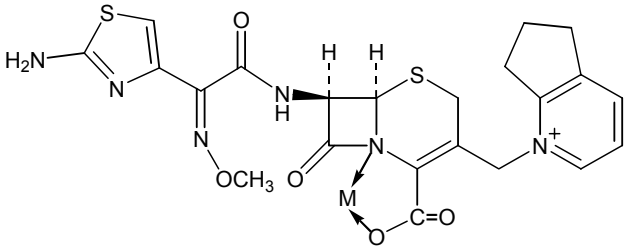

Fig. 2: Proposed Cefpirome-monodentate complex

Chromatographic conditions

The mobile phase consisted of methanol-water (15:85 %v/v). Before delivering into the system, it was filtered through a 0.45μm millipore filter and degassed in an ultrasonic bath. The samples were introduced through an injector valve with a 20-μL sample loop at a flow rate of 1 ml/min at 37^{o}C using gradient pump system. Chromatograms were recorded at 265nm using SPD-10AT VP Shimadzu UV-Visible detector.

Table 3: Quantitation of cefpirome metal complexes by the proposed method

Drug/Metal complex	Retention time	AUC	% Drug available	Retention time	AUC	% Drug complexed
Cefpirome	5.90	294597	100	–	–	–
Fe(III)	6.08	2743	0.93	1.31	17682845	99.06
Cr(III)	6.39	31224	10.60	1.29	1570124	89.41
Cu(II)	6.27	137790	46.77	1.31	6059736	53.23
Ni(II)	6.1	229005	77.73	1.29	129568	22.23
Cd(II)	5.68	270179	91.71	Complex not detected		
Zn(II)	5.84	283688	96.30	Complex not detected		
Mg(II)	5.85	286034	97.09	2.68	5734	2.91
Mn(II)	5.82	286703	97.32	Complex not detected		
Ca(II)	5.84	293444	99.60	1.35	2526	0.40
Co(II)	6.29	321597	100.16	Complex not detected		

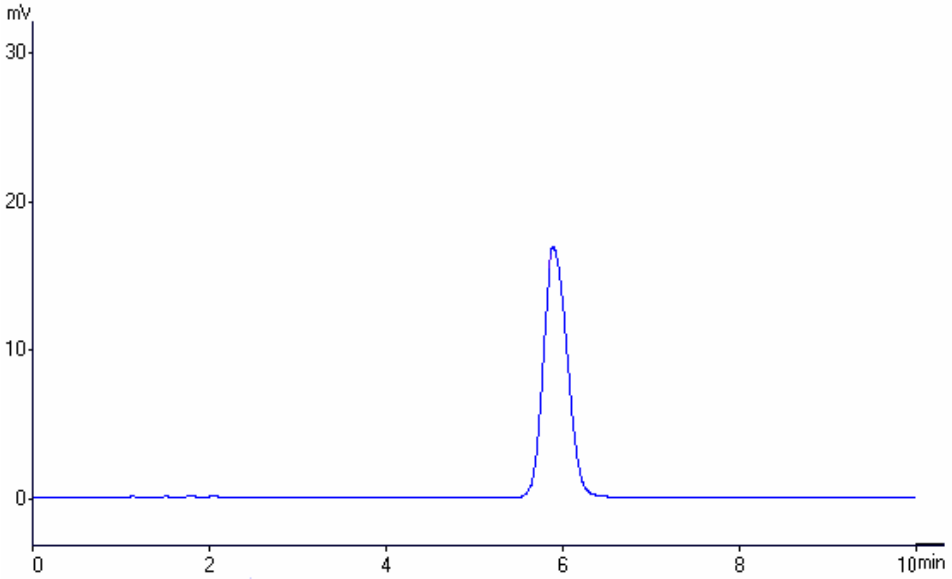

Fig. 3: A typical chromatogram of cefpirome.

Assay procedure

An amount equivalent to 5.00 mg of cefpirome was accurately weighed and dissolved in freshly prepared deionized water in a 100ml volumetric flask to produce a standard solution of 50 µg/ml. Aliquots were diluted to 10.0, 5.0, 4.0, 2.0 and 1.0 µg/ml of working solutions.0.5g of metal salt was weighed accurately in 25ml Erlenmeyer flask. Aqueous solution of cefpirome (10ml from 50 µg/ml solution) was added to each flask. These flasks were kept in a constant temperature bath at 37^{o}C for half an hour with constant stirring. The contents of the flask were filtered through a millipore filter (0.45µ) and injected through a rheodyne of 20-µL loop to HPLC system as described above.

RESULTS AND DISCUSSION

The mobile phase consisted of methanol and water (15:85) but various ratios (20:80, 15:85, 30:70 v/v) was also tested as starting solvent for system suitability study. The variation in the mobile phase leads to considerable changes in the chromatographic parameters, like peak symmetry and retention time. However, the ratio of 15:85 (v/v) yielded best results. The inter and intra day results shown in tables 1

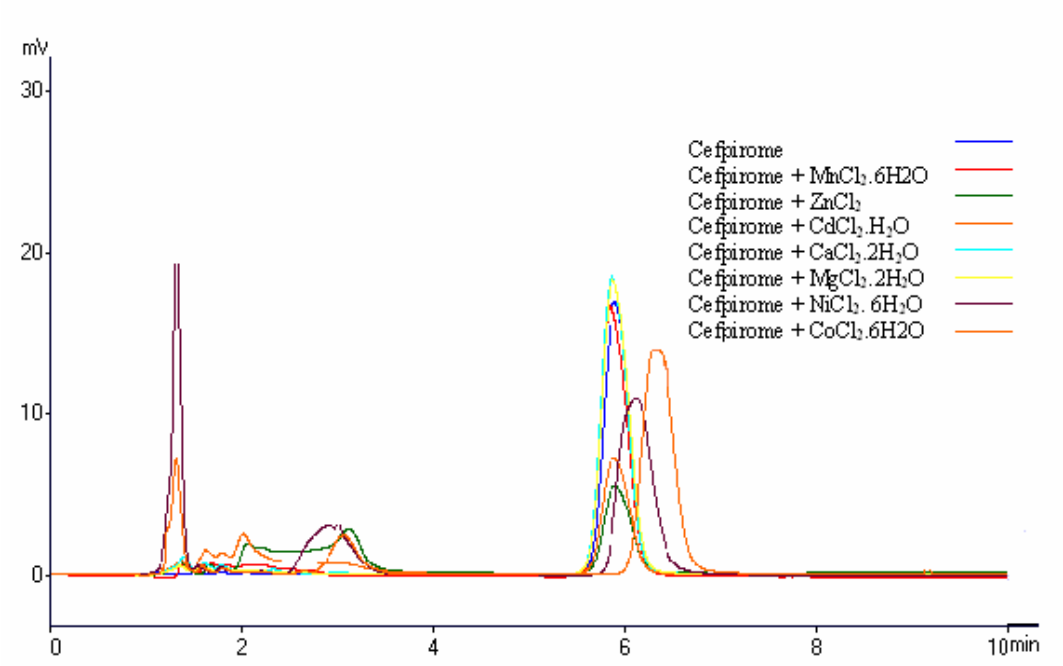

Fig. 4: Chromatograms showing interactions of cefpirome with metals.

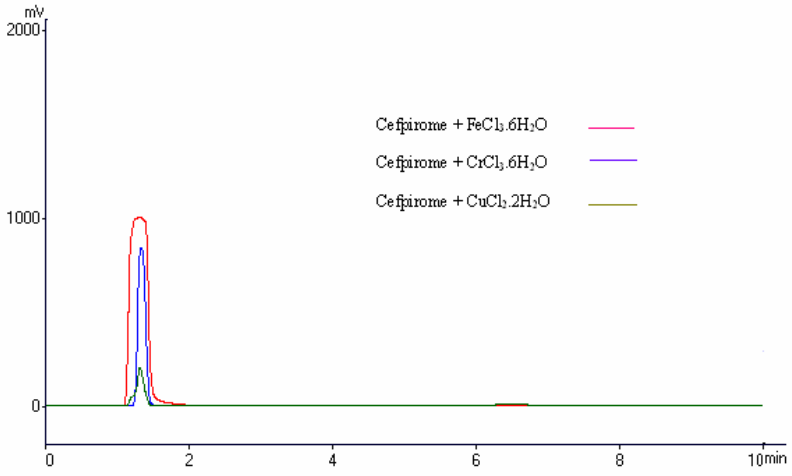

Fig. 5: Chromatograms showing interactions of cefpirome with metals.

and 2 are correlated well with a correlation coefficient of 0.999 and a standard deviation of >0.0038.

Cefpirome is broad-spectrum antibiotic. Like other antibiotics, the interaction between cefpirome and metals is a chelation reaction. During these reactions, cefpirome forms metal chelate with the carboxylate group of dehydrothiazine ring and β-lactam nitrogen (figure 2). Before metal interaction cefpirome appears at retention time of 5.9 minutes (figure 3), while after interactions, due to increase in molecular weight, the metal complexes appeared at 1 – 3 minutes as shown in figures 4 and 5. The observed changes in retention times after interactions with each metal are shown in table 3. This table also shows the drug complexed and drug left in each case. The maximum complexation occurred with ferric and chromium chloride and complex appeared at retention time of 1.3 and 1.29 minutes respectively. In case of iron 99.06 % drug-metal

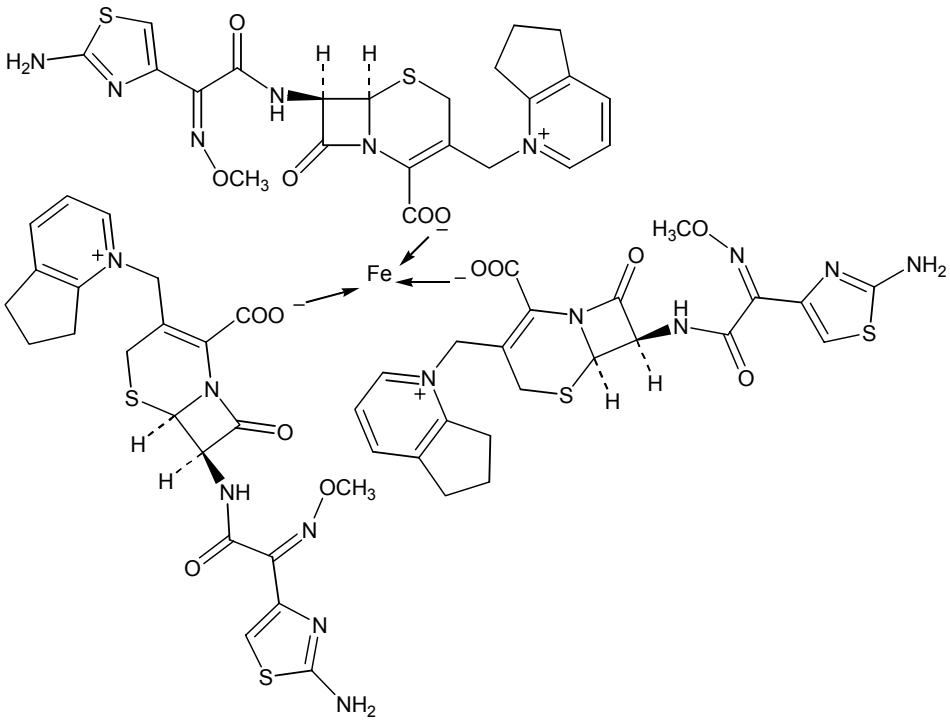

Fig. 6: Proposed cefpirome-Fe complex.

complex is formed and 0.93 % of the drug is left .The proposed structure of Fe-cefpirome complex is shown in figure 4. In case of chromium 89.11% drug was complexed, while copper reduced 53.23 % of the drug. Nickel formed only 22.23 % complex. In case of magnesium and calcium 97-99% drug was available. While in case of cadmium, zinc and manganese, the decrease in the amount of drug indicates complexation, but the resulting complex could not be detected. However, in case of cobalt chloride, the complex was not formed and the entire drug was available.

Cephalosporins form metal complexes with di- and trivalent metal both at carboxylic group of dehydrothiazine ring as well as β-lactam nitrogen (Sultana *et al.,*2005), which depends upon the reaction conditions. When the cefpirome acts as a monodentate ligand, a complex as shown in figure 6 is formed, whilst the drug acts as a bidentate ligand, β-lactam nitrogen is also involved in the coordination and a complex as shown in figure 2 may be formed. In our reactions it is anticipated that in case of iron and chromium, cefpirome acted as a monodentate whereas with all other metals acted as bidentate. The stoichiometry of these cefpirome metal complexes is under investigation.

CONCLUSION

The present work describes a new RP-HPLC method for cefpirome-metal interactions. The proposed HPLC method enables simultaneous determination and quantitation of cefpirome and its metal complexes with a good separation and resolution of the chromatographic peaks. The proposed method is rapid, precise, specific, precise and accurate and is suitable for quantitative analysis of cefpirome and its metal complex.

REFERENCES

Arayne MS, Sultana N and Hussain F (2005). Interaction between ciprofloxacin and antacids – Dissolution and adsorption studies. *Drug Met. & Drug Interac.,* **21**(2): 117-129.

Bruce G Charles, Steven C Wallis, Lawrence R Gahan, Lucio J Filippich, Megan G Bredhauer and Paul A Duckworth (1996). Interaction of norfloxacin with divalent and trivalent pharmaceutical cations. *In vitro* complexation and *in vivo* pharmacokinetic studies in the dog. *Journal of Pharmaceutical Sciences,* **85**(8): 803-809.

Breilh D, Lavallee C, Fratta A, Ducint D, Cony-Makhoul P and Saux MC (1999). Determination of cefepime and cefpirome in human serum by high-performance liquid chromatography using an ultrafiltration for antibiotics serum extraction. *J. Chromatogr. B. Biomed. Sci. Appl.,* **734**(1):121-7.

Barbhaiya RH, Knupp CA, Pfeffer M and Pittman KA (1992). Lack of pharmokinetics interaction between

cefepime and amikacin in humans. *Antimicrob. Ag. Chemother*., **36**: 1382-6.

Bertran MA, Bruckner DA and Young LS (1984). *In vitro* activity of HR 810, a new cephalosporin. *Antimicrob. Agents Cemother*., **26**: 277-279.

Ip M, Au C, Cheung SW, Chan CY and Cheng AFB (1998). A rapid high-performance liquid chromatographic assay for cefepime, cefpirome and meropenem. *J. Antimicrob. Chemother.,* **42**(1): 121-123.

Jones RN, Thornsberry C, Barry AL, Ayers L, Brown S, Daniel J, Fuchs PC, T.L.Gavan TL, Geriach EH, Mastsen JM, Reller LB and Sommers HM (1984). Disk diffusion testing, quality control guidelines, and antimicrobial spectrum of HR 810, a fourth-generation cephalosporin, in clinical microbiology laboratories. *J. Clin. Microbiol.,* **20**: 409-412.

Maass L, Malerczyk V, Verho M, Hajdu P, Seeger K and Klesel N (1987). Dose linearity testing of intravenous cefpirome (HR 810), a novel cephalosporin derivative. *Infection,* **15**: 202-206.

Maass L, Malerezyk V and Verho M (1987). Pharmacokinetics of cefpirome (HR 810), a new cephalosporin derivative administered intramuscularly and intravenously to healthy volunteers. *Infection,* **15**: 207-210.

Malerczyk V, Maass L, Verho M, Hajdu P, Klesel N and Rangoonwala R (1987). Single and multiple dose pharmacokinetics of intravenous cefpirome (HR 810), a novel cephalosporin derivative. *Infection,* **15**: 211-214.

Seibert G, Limbert M, Winkler I and Dick T (1983). The antibacterial activity "*in-vitro*" and β-lactamase stability of the new cephalosporin HR 810 in comparison with five other cephalosporins and two aminogycosides. *Infection,* **11**: 275-279.

Sultana N, Arayne MS and Afzal M (2005). Synthesis and Antibacterial Activity of Cephradine Metal Complexes: Part II Complexes with Cobalt, Copper, Zinc and Cadmium. *Pak. J. Pharm. Sci*., **18**(1): 36-42.

Sugioka T, Asano T, Chikaraishi Y, Suuki E, Sano A, Kuriki T, Shirotsuka M and Saito K (1990). Stability and degradation pattern of cefpirome (HR810) in aqueous solution. *Chem. Pharm. Bull.,* **38** (7): 1998-2000.

Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th edition, 2004, pp.332-333.

Received: 26-1-2006 – Accepted: 28-2-2006