Development and validation of HPLC method for the determination of Cefpodoxime Proxetil in human plasma

Lubna Bashir, Muhammad Harris Shoaib, Shazia Naz, Rabia Ismail Yousuf, Sabahat Jabeen, Fozia Israr and Fahad Siddiqui*
Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

Abstract: A new, simple, accurate, precise and specific method has been developed for the analysis of Cefpodoxime Proxetil in human plasma. The proposed method was developed and validated with the aim to be used in Bioavailability/Bioequivalence studies for quantification of drug in human plasma. The mobile phase components were acetonitrile, methanol, and water in the ratio of 20:50:30. *Ortho* phosphoric acid was used to adjust at pH5.0. Flow rate and wavelength were kept 1ml/min and 247nm respectively. The column was C-18 HPLC column 5um particle size, L x 1.d. 25cm x4.6mm. (Supelcosil). Retention time of Cefpodoxime Proxetil was found to be 10.967min. The developed method was validated for selectivity, recovery, accuracy, precision, repeatability, reproducibility, stability and linearity in the range of 0.195mcg/ml to 50mcg/ml. The accuracy and Precision of the proposed method were well within the predefined limits i.e. ±15% for all the calibration standards other than LLOQ (Lower Limit of Quantification) where it was well within ±20% of the nominal value. The analytical recovery was always above 89% showing satisfactory recovery. The coefficient of correlation (R^2) was 0.999. The developed method was found suitable for the estimation of Cefpodoxime Proxetil in plasma.

Keywords: Cefpodoxime Proxetil, HPLC, validation, human plasma.

INTRODUCTION

Cefpodoxime Proxetil is a semi-synthetic antibiotic of the cephalosporin, administered orally having extended spectrum of activity. Its chemical name is RS)-1(isopro poxycarbonyloxy) ethyl (+)-(5-thia-1-azabicyclo [4.2.0] oct-2-ene- 2-carboxylic acid, 7-[2-(2-amino-4-thiazolyl)-2-{(Z) methoxyimino} acetyl)amino)]-3-methoxymethyl-8-oxo-1-(1-methylethoxy) carbonyl)oxy) ethyl ester, (6R,7R)- and its molecular formula is $C_{21}H_{27}N_5O_9S_2$ with the molecular weight of 557.6g/mol (Sittig, 2015). The structural formula is given below in fig. 1.

It is a β- lactum bactericidal agent, which after oral absorption de-esterifies to its active metabolite cefpodoxime (O'Neil, 2013, Rockville, 1942). It inhibits the cell wall synthesis by passing through porin canal and attaches itself to a penicillin binding proteins, resulting in the reduction of the synthesis of peptidoglycans, thereby damaging the cell wall (Bhandari, 2012).

The drug is found to be only 50% bio available at the doses of 100 to 400mg. The half-life is about 2-3 hours. The presence of beta-lactamase enzyme enhances its stability.

It is a third generation antibiotic used for the treatment of certain mild to moderate respiratory tract infections **(**tonsillitis, pharyngitis, community acquired pneumonia and acute bacterial exacerbation of chronic bronchitis), urinary tract infections**(**acute uncomplicated urethral gonorrhea), acute uncomplicated ano-rectal infections in women, uncomplicated skin and skin structure infections, acute maxillary sinusitis and uncomplicated urinary tract infections (cystitis) (Beale *et al*., 2010, Bergogne-Berezin, 1991, Borin, 1991, Chocas *et al*., 1993, Geddes, 1991, Kakumanu *et al*., 2006).

Literature survey has shown that there are several reported methods for the determination of Cefpodoxime Proxetil with other drugs like Ambroxol and Ofloxacin and ClavulanicAcid in bulk as well as in tablet form (Kotkar *et al*., 2012, Kumar *et al*., 2012, Malathi *et al*., 2009). Rote in 2011 evaluated HPTLC method for Cefpodoxime Proxetil and AmbroxolHydrochloride in human plasma (Rote and Kande, 2011). Dubala *et al*., has reported simultaneous determination of Cefpodoxime and Clavulanic Acids in human plasma through HPLC technique (Dubala *et al*., 2013). The proposed method is suitable, precise, accurate, simple as well as effective method and less time consuming for the determination and validation of CefpodoximeProxetil in human plasma.

MATERIALS AND METHOD

Cefpodoxime Proxetil was provided as a gift by S. J. & G. Fazul Ellahie (PVT) LTD. The mobile phase composed of Methanol, Acetonitrile HPLC Grade and *Ortho* phosphoric acid (Merck, Darmstadt, Germany) and HPLC grade water. Analysi

**Corresponding author:* e-mail: Fahadbbrf@gmail.com

s was performed through High Performance Liquid Chromatography Pump, LC- 10AT VP (Shimadzu Corporation, Kyoto, Japan) with UV Detector**,** SPD-10 A (Shimadzu Corporation, Kyoto, Japan). Samples were filtered through 0.45 micron Millipore filters, Ultra Sonic bath (Clifton, Nickel Electro Ltd, Somerset, England).

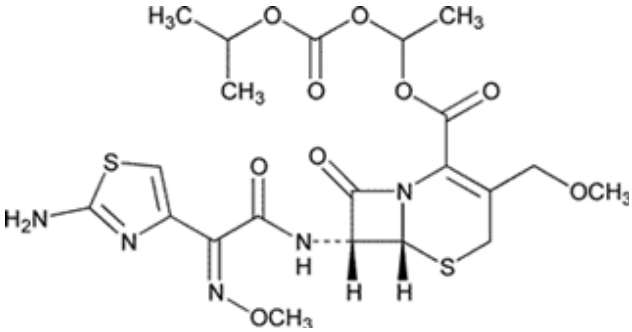

Fig. 1: Chemical structure of Cefpodoxime Proxetil

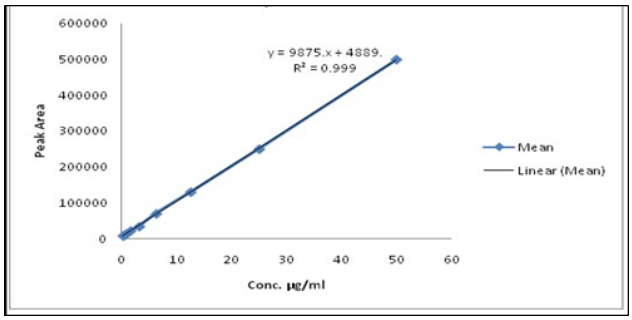

Fig. 2: Linearity of calibrators prepared in mobile phase

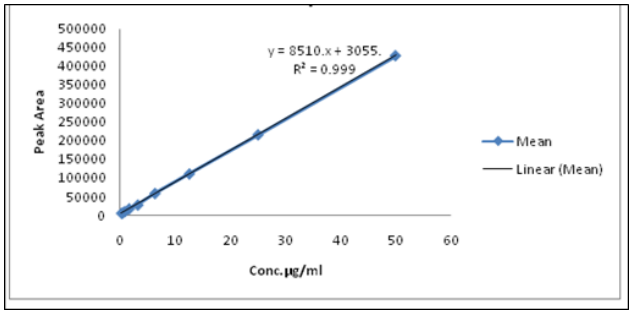

Fig. 3: Linearity of calibrators prepared in plasma

Chromatographic conditions

Mobile phase comprised of acetonitrile: methanol: water in the ratio of 20: 50:30 v/v/v adjusted to pH5.0 with 10% *Ortho* phosphoric acid at the flow rate of 1ml/min. The detection was carried out at 247nm using UV detector. The column was C-18 HPLC column 5um particle size, L x 1.d. 25cm x 4.6mm (Supelcosil). The filtration of mobile phase was through 0.45 micron Millipore filters and degassed by using Ultra Sonic bath. The sample injection volume was 50ul.

Preparation of standard solution

Standard stock solution of Cefpodoxime was prepared by dissolving 5mg of the drug in 100ml of the mobile phase in order to achieve the concentration of 50mcg/ml. Stock mixture was serially diluted to prepare concentrations of 50mcg/ml, 25mcg/ml, 12.5mcg/ml, 6.25mcg/ml, 3.125 mcg/ml, 1.563mcg/ml, 0.782mcg/ml, 0.390mcg/ml, 0.195 mcg/ml, and 0.09 mcg/ml.

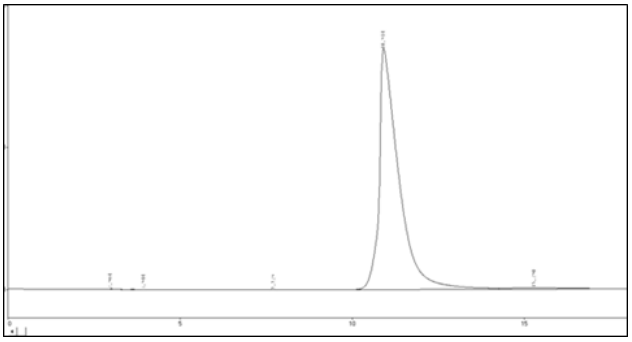

Fig. 4: Drug in mobile phase

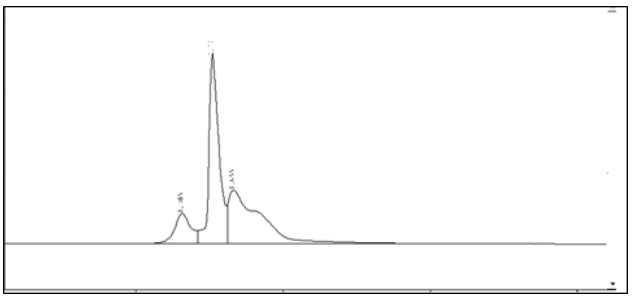

Fig. 5: Chromatogram of blank plasma

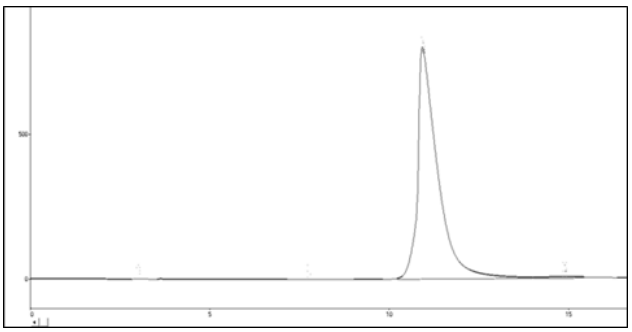

Fig. 6: Drug in plasma

Preparation of plasma sample solution

Stock solution of 50mcg/ml of drug was prepared in plasma then serial dilutions were prepared by taking 1ml from 50mcg/ml and acetonitrile was added in the ratio of 1:1. The sample of stock solution was mixed in a Vortex mixer (Whirl mixer, England) for at least five minutes and then centrifuged (Centrifuge machine, Hereus, Osterode, Germany) at 3000rpm for ten minutes. The resilient was collected and filtered with the help of 0.45µ membrane filter in a swinny filtration assembly (Millipore, England). Then the 50µl of the sample was injected with the help of microliter syringe at wavelength of 247 nm and the flow rate was 1ml/min.

Calibration curve

Calibration curve was prepared by taking different concentrations of 50mcg/ml to 0.195mcg/ml of

Cefpodoxime Proxetil in mobile phase as well as in plasma and fitted through the linear equation for the determination of coefficient of correlation (R^2).

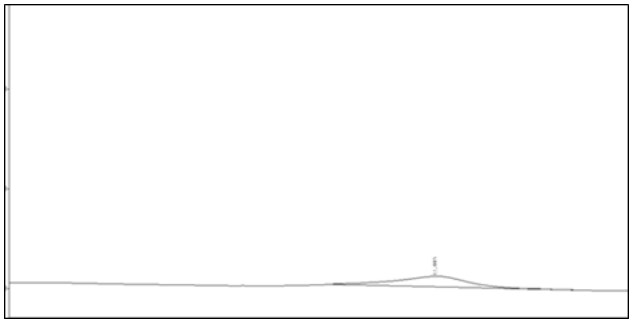

Fig. 7: LLOQ sample in plasma

Recovery

In order to determine recovery of the method, triplicate injections of three different concentrations of Cefpodoxime Proxetil in plasma i.e. 25 mcg/ml, 6.25 and 0.782mcg/ml were analyzed and compared with the respective concentrations of standard solutions.

Accuracy and precision

The accuracy was observed within ±15% of the actual values. Similarly, precision (RSD) was also found within the acceptable limit of 15%. In order to evaluate the intraday precision of the method, five injections of25 mcg/ml, 6.25mcg/ml, 3.125mcg/ml and 0.782mcg/ml of Cefpodoxime Proxetil were analyzed. For interday precision the triplicate injections having the concentrations i.e. 25mcg/ml, 3.125mcg/ml, 0.39mcg/ml were analyzed on three consecutive days, fresh samples were prepared before analysis.

Stability

The stability was assessed by analyzing three samples of two concentrations i.e. 0.782mcg/ml and 25 mcg/ml. The samples were stored frozen at -20˚C for 24 hours then thawed untreated at room temperature. The thawed samples were then re frozen at same temperature for 12-24h (FDA, 2001). The stability of the stock solution was investigated for 2 and 3weeks.

Limit of detection (LOD)

Signal to noise ratio is applicable for the analytical procedures having baseline noise. It is basically the comparison of the observed signals from the samples with low concentrations of measure and with that of the blank samples and is also used to measure the lowest concentrations at which the analyte can be detected reliably. For the limit of detection, the ratio 2:1 or 3:1 is considered as acceptable (ICH, 2005).

Lower limit of quantification (LLOQ)

10:1 ratio (signal to noise) is used to measure the lowest concentrations at which the analyte can be quantified reliably. Multiple concentrations were made and injected into the HPLC column for the analysis of lower limit of detection and limit of quantifications, i.e. 50mcg/ml, 25 mcg/ml, 12.5mcg/ml, 6.25mcg/ml, 3.125mcg/ml, 1.563 mcg/ml, 0.782mcg/ml, 0.390mcg/ml, 0.195mcg/ml, and 0.097mcg/ml. After that SD, %accuracy as well as % CV were obtained (ICH, 2005). Multiple concentrations of 0.390mcg/ml, 0.195mcg/ml and 0.097mcg/ml were made.

Selectivity

It was assessed by taking six blank samples of plasma. In order to assure no interference, selectivity was determined at lower limit of quantification (0.195mcg/ml) (FDA, 2001).

RESULTS

The method validation was according to the FDA guidelines for linearity, selectivity, recovery, accuracy, precision, stability, and sensitivity. The coefficient of correlation for the concentration range of 50mcg/ml to 0.195mcg/ml, was determined and found to be R^2 0.999 in plasma as well as in mobile phase. Results are given in fig. I and II. The analytical recovery results as investigated on three selected concentrations were found consistent and reproducible. The % i.e. 25mcg/ml, 6.25 and 0.782mcg/ml recovery was found to be within 89.82% to 101.62%. See table I. The linearity was statistically confirmed. The intraday accuracy was found in the range of 86.86% to 98.11% where as interday accuracy of three consecutive days were 92.88%-100.16%, 91.86%-99.68% and 92.52% 99.84% respectively. The results are given in table II. The analyte was found stable when subjected to the proposed method. The percentage accuracy was 88.85% and 90.76% of 0.782mcg/ml, after two and three weeks respectively. Similarly for the concentration of 25mcg/ml the consecutive percentage accuracy values were 98.5% and 98.64% after two and three weeks, which were satisfactory i.e. within 15%, table III exhibits the stability results. Selectivity is the capacity of an analytical method to distinguish and to compute the drug (analyte) when other component is also present [FDA, 2001]. The method was found selective at LLOQ i.e. 0.195mcg/ml**.** See figs. III, IV, V and VI. The limit of detection was 0.097mcg/ml.

DISCUSSION

Different methods for the detection of Cefpodoxime Proxetil in human plasma were tried. A mobile phase comprised of acetonitrile: Methanol: water in the ratio of 20: 50: 30 was found to be optimized. This method was then validated in human plasma. The calibration curve (range from 50mcg/ml to 0.195mcg/ml) was found to be linear with R^2 0.999 for both the plasma as well as mobile phase. The linearity was statistically confirmed. The % recovery was found to be within 89.82% to 101.62%.

Table 1: Absolute Analytical Recovery

Peak Area of Cefpodoxime Proxetil in Plasma			
Conc. µg/ml	Mean	SD	%CV
25	229710	1516.29	0.66
6.25	61545.4	553.78	0.90
0.782	10535	504.85	4.79

Peak Area of Cefpodoxime Proxetil in mobile Phase			
Conc. µg/ml	Mean	SD	%CV
25	248603.8	515.87	0.21
6.25	68553.8	843.11	1.23
0.782	10911.2	342.42	3.14

Mean Absolute Recovery				
	Conc. µg/ml	Plasma Samples Mean (n=5)	Mobile Phase Mean (n=5)	% Recovery
	25	229710	248603.8	92.40
	6.25	61545.4	68553.8	89.78
	0.782	10535	10911.2	96.55
Mean				92.91
SD				3.42
%CV				3.68

Table 2: Intraday and Interday Accuracy and Precision in Plasma

Measured Conc. (µg/ml) during different timings of the day						
Conc. (µg/ml)	Mean n=5	SD	CV%	Accuracy %		
25	24.53	0.64	2.59	98.11		
6.25	5.86	0.28	4.72	93.74		
3.125	2.76	0.1	3.79	88.28		
0.782	0.68	0.02	3.51	86.86		
Conc. (µg/ml)	Day 1		Day 2		Day 3	
	Accuracy		Accuracy		Accuracy	
	Mean ± SD (n=5)	%CV and % Accuracy	Mean ± SD (n=5)	%CV and % Accuracy	Mean ± SD (n=5)	%CV and % Accuracy
25	23.22±0.73	3.12 and 92.88	22.95 ± 0.42	1.83 and 91.8	23.13± 0.22	0.95 and 92.52
6.25	6.26 ± 0.27	4.32 and 100.16	6.23 ± 0.32	5.16 and 99.68	6.24 ± 0.09	1.45 and 99.84
3.125	2.97 ± 0.26	8.81 and 95.04	2.99 ± 0.15	4.88 and 5.68	2.98 ± 0.24	7.93 and 95.36
0.782	0.73 ± 0.01	1.08 and 93.93	0.73 ± 0.02	3.34 and 3.35	0.74 ± 0.04	5.65 and 94.6

Table 3: Long Term Stability of Cefpodoxime Proxetil in Plasma

Samples	Low Concentration 0.782 (µg/ml)			High Concentration 25 (µg/ml)		
	Fresh Plasma	After two weeks at -20˚C	After three weeks at -20˚C	Fresh Plasma	After two weeks at -20˚C	After three weeks at -20˚C
Mean n=5	0.702	0.695	0.71	24.979	24.624	24.66
SD	0.02	0.02	0.01	0.01	0.39	0.31
%CV	3.08	2.3	1.33	0.03	1.59	1.24
Accuracy %	89.82	88.85	90.76	99.91	98.5	98.64

Precision and accuracy were satisfactory i.e. within 15%. The stability of analyte was found good for the concentrations of 0.782mcg/ml to 25mcg/ml. The limit of quantification and detection were 0.195mcg/ml and 0.097 mcg/ml respectively.

CONCLUSION

The proposed method is an accurate, simple and less time consuming for the estimation of Cefpodoxime Proxetil in human plasma. The method exhibited good stability of Cefpodoxime Proxetil with the accuracy within 15%. Since the method showed good selectivity for LLOQ, so can be easily adopted for the analysis of Cefpodoxime Proxetil in plasma.

ACKNOWLEDGEMENT

The author is grateful to S. J. & G. Fazul Ellahie (PVT) LTD.

REFERENCES

Beale JM, Block J and Hill R (2010). *Organic medicinal and pharmaceutical chemistry*, Lippincott Williams & Wilkins Philadelphia.

Bergogne-Berezin E (1991). Cefpodoxime proxetil in upper respiratory tract infections. *Drugs,* **42**: 25-33.

Bhandari S (2012). Development and Validation of a denstometric HPTLC method for quantitative analysis of cefpodoxime proxetil in human plasma. I*nt J Pharm Pharm Sci.*, **4**: *Suppl* 1, 100-103.

Borin MT (1991). A review of the pharmacokinetics of cefpodoxime proxetil. *Drugs,* **42**(Suppl 3): 13-21.

Chocas EC, Paap CM and Godley PJ (1993). Cefpodoxime proxetil: A new, broad-spectrum, oral cephalosporin. *Ann. Pharmacother.,* **27**: 1369-77.

Dubala A, Nagarajan JSK, Vimal CS and George R (2013). Simultaneous quantification of cefpodoxime proxetil and clavulanic acid in human plasma by LC-MS using solid phase extraction with application to pharmacokinetic studies. *J Chromatogr B Biomed Sci App.* **921**: 49-55.

FDA C (2001). Guidance for industry: Bioanalytical method validation. US Department of Health and Human Services. *Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CV).*

Geddes A (1991). Cefpodoxime proxetil in the treatment of lower respiratory tract infections. *Drugs,* **42**: 34-40.

ICH I Q2 (R1): Validation of analytical procedures: text and methodology. International Conference on Harmonization, Geneva, 2005.

Kakumanu VK, Arora V and Bansal AK (2006). Investigation on physicochemical and biological differences of cefpodoxime proxetil enantiomers. *Eur. J. Pharm. Biopharm.,* **64**: 255-259.

Kotkar R, Shirkhedkar A and Surana S (2012). Development and validation of RP-HPLC method for simultaneous estimation of Cefpodoxime Proxetil and Ambroxol hydrochloride in bulk and in tablets. *Int. J. Res. Pharm. Bio. Sci.,* **3**: 156-63.

Kumar KS, Srinivas R, Rao VJ, Rani SS, Kumar DK and Babu KBR (2012). Development and validation of a RP-HPLC method for simultaneous estimation of cefpodoxime proxetil and ofloxacin in bulk drugs and in pharmaceutical dosage forms. *J Pharm Res.*, **5**: 3904.

Malathi S, Dubey R and Venkatnarayanan R (2009). Simultaneous RP-HPLC estimation of cefpodoxime proxetil and clavulanic acid in tablets. *Indian J Pharm Sci.,* **71**: 102.

O'Neil MJ (2013). *The Merck index: an encyclopedia of chemicals, drugs and biologicals*, RSC Publishing.

Rockville M (1942). United States Pharmacopeial Convention 2009. *The United States Pharmacopoeia,* 24.

Rote AR and Kande SK (2011). Development of HPTLC method for determination of cefpodoxime proxetil and ambroxol hydrochloride in human plasma by liquid-liquid extraction. *Pharmaceutical Methods,* **2**: 242-246.

Sittig M (2015). *Pharmaceutical manufacturing encyclopedia*, William Andrew Pub.