HPLC method development and validation for the determination of Cefaclor in human plasma

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Abstract: Cefaclor was analyzed in the human plasma by developing a simple, precise and accurate assay method which was then validated for its accuracy, specificity and precision. The mobile phase comprised of a mixture of sodium 1-pentanesulfonate, water, triethylamine and methanol. Phosphoric acid was used to adjust the pH to 2.5±0.1. The flow rate was maintained at 1.5ml/min and the wavelength was set at 265 nm. A C-18 HPLC, column 5um particle size, L x 1.D. 25cm x 4.6mm (Supelcosil) was utilized for chromatographic separation. The retention time of Cefaclor was found to be 17min. This method was validated for selectivity, accuracy, precision, repeatability, reproducibility, recovery, linearity, and stability. Calibration curves were found linear were in the range of 0.39µg/ml to50µg/mland the coefficient of correlation (R^2) was found to be 0.999. Hence, this method has been found useful for the determination of Cefaclor in plasma.

Keywords: Cefaclor, HPLC, Plasma.

INTRODUCTION

Cefaclor ($C_{15}H_{14}ClN_3O_4S•H_2O$) (Dollery, 1991) is second generation (Flores & Aguilar *et al*, 2001) cephalosporin which is absorbed orally and is semi synthetic in nature. It was developed in the Lilly laboratories (Bill & Washington, 1977). It is indicated for the use against different infections in human body like pneumonia, ear, lung, skin, throat and urinary tract infections (Basaveswara & Majti *et al*, 2011). The structural formula of Cefaclor is given in fig. 1.

After 30 minutes to 1 hour, the oral doses of 250mg, 500 mg and 1g of Cefaclor generate peak plasma concentrations of approx. 7, 13 and 23µg/ml respectively but there may be delay in the absorption due to the presence of food but total amount absorbed is unchanged. It has reported plasma half-life of 0.5-1hr and almost 25% is bound to plasma proteins. High concentrations of the drug are achieved in urine within 8 hours of a dose and is rapidly excreted by the kidneys (Sweetman, 2009).

Several analytical methods have been reported for determination of Cefaclor in different pharmaceutical dosage forms and in human plasma. (Basaveswara & Majti *et al*, 2011; Sivanadh & Subhashini *et al*, 2016; Mohammad, 2016; Manna & Valvo, 2004).

The objective of this study was to develop and validate a simple, effective and reproducible HPLC method for the determination and quantification of Cefaclor in human plasma.

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MATERIALS AND METHOD

Cefaclor was obtained from Elli Lilly Pharma Pakistan. Sodium 1-pentanesulfonate, triethylamine, methanol HPLC Grade (Merck, Darmstadt, Germany) and water (Milli-Q grade).

The equipments used in this study were High performance Liquid Chromatography Pump, LC-10AT VP (Shimadzu Corporation, Kyoto, Japan) with UV Detector, Ultra Sonic bath (Clifton, Nickel Electro Ltd, Somerset, England), SPD-10 A (Shimadzu Corporation, Kyoto, Japan) and 0.45 micron Millipore filters.

Chromatographic condition

For the mobile phase 1g of sodium 1-pentanesulfonate was dissolved in a mixture of 780ml of water and 10ml of triethylamine; pH was adjusted to 2.5±0.1 with phosphoric acid and 220ml of methanol was mixed. The mobile phase was then filtered using 0.45 micron Millipore and degassed for 10 min in ultrasonic bath. The flow rate was maintained at1.5ml/min and the wavelength was kept at 265nm.

Preparation of standard solution

To obtain the concentration of 50µg/ml, the standard stock solution of Cefaclor was prepared by dissolving 5 mg of the drug in 100ml of the mobile phase and in plasma. The serial dilutions of the stock solution were

prepared in the concentrations of 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.563µg/ml, 0.782µg/ml, 0.390 µg/ml, and 0.195µg/ml.

Preparation of plasma sample solution

For the preparing samples, 1ml of plasma from stock solution was taken with same quantity of acetonitrile in 1:1 proportion. Samples were vortexed for 5 minutes and centrifugation was done using Centrifuge machine (Hereues, Osterode, Germany) for 10 minutes, at 4000 rpm. The supernatant was collected and filtered using 0.45 µm membrane filters with the help of Swinney Filter Assembly (Millipore, England). Using a microliter syringe, 50 µl of the sample was injected in HPLC. The flow rate was maintained at 1.5 ml/min and the sample was detected at 265 nm.

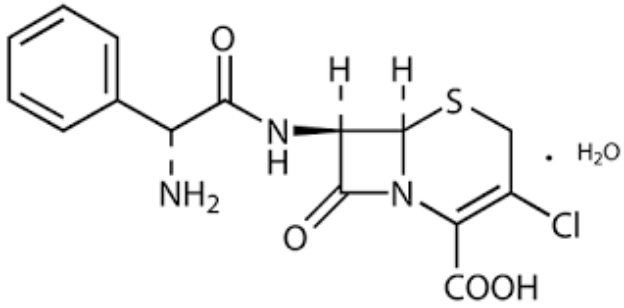

Fig. 1: Chemical structure of Cefaclor

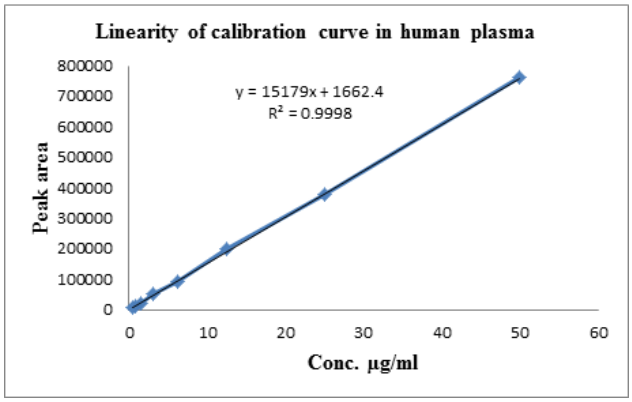

Fig. 2: Linearity of Calibration curve in human plasma

Selectivity

Selectivity was investigated by extracting blank plasma from six different sources and were analyzed as per proposed method. The chromatograms were assessed for any endogenous compound at the retention time of analyte at the lowest concentration (LLOQ) (FDA, 2001).

Linearity and calibration curve

Calibration curve was constructed from different concentrations of Cefaclor ranging from 50µg/ml to 0.39 µg/ml in both mobile phase and in plasma and fitted and subjected to linear regression analysis. Linearity was observed by determining the coefficient of correlation (R^2).

Precision and accuracy

The precision and accuracy were observed within the acceptable limit of i.e. ±15%. In order to evaluate the accuracy and precision (intraday) of the method, five injections on the same day of the concentrations i.e. 25 µg/ml, 6.25µg/ml, 3.125µg/ml and 0.782µg/ml of Cefaclor were injected.

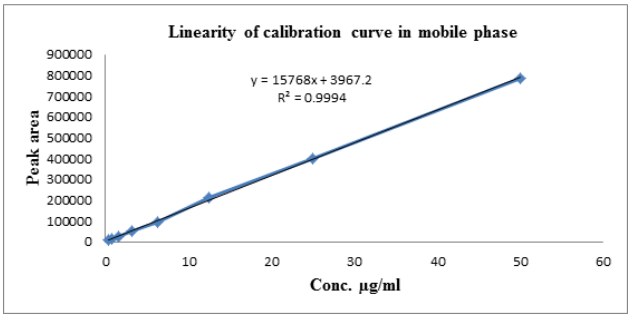

Fig. 3: Linearity of Calibration curve in mobile phase

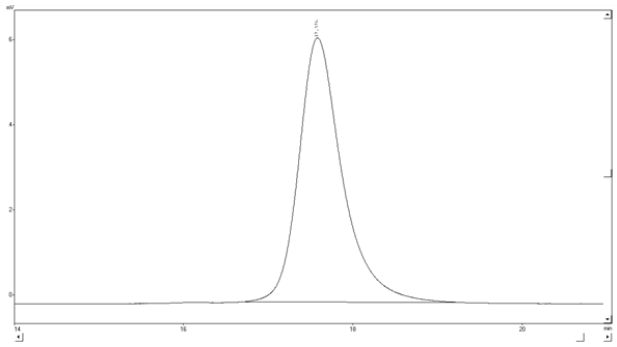

Fig 4(a): Chromatogram showing Cefaclor in Mobile Phase

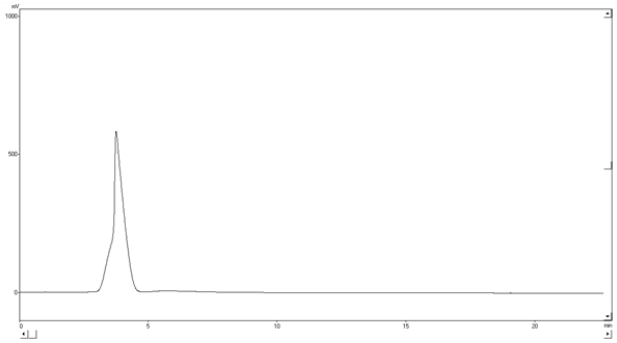

Fig 4(b): Chromatogram of blank plasma

Interday precision

Triplicate injections of the identical concentrations of 25 µg/ml, 6.25µg/ml and 0.782µg/ml were injected for 3 consecutive days for determining inter-day precision. Fresh samples were prepared each day for this purpose.

Recovery

Three different concentrations of Cefaclor (25µg/ml, 6.25 and 0.782µg/ml) in plasma were analyzed using triplicate injections and these were compared with the respective concentrations of standard solutions and percentage recovery was calculated.

Table 1: Intraday and Interday Accuracy and Precision in Plasma

Measured Conc. (µg/ml) during different timings of the day						
Conc. (µg/ml)	Mean	SD	CV%	Accuracy %		
	n=5					
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		
	Day 1		Day 2		Day 3	
	Accuracy		Accuracy		Accuracy	
	Mean ± SD	%CV and	Mean ± SD	%CV and	Mean ± SD	%CV and
Conc. (µg/ml)	(n=5)	% Accuracy	(n=5)	% Accuracy	(n=5)	% Accuracy
25	24.94± 0.11	0.45 and 99.78	24.77±0.62	2.49 and 99.10	25.19±0.48	1.91 and 100.76
6.25	6.24±0.06	0.93 and 99.84	6.25±0.12	1.91 and 99.97	6.32±0.25	3.89 and 101.06
3.125	3.13± 0.09	2.93 and100.19	3.14±0.14	4.50 and100.56	3.12±0.12	3.98 and 99.78
0.782	0.77± 0.04	5.54 and 97.88	0.76±0.04	5.16 and 97.47	0.76±0.04	5.87 and 97.70

Table 2: Absolute Analytical Recovery

	Peak Area of Cefaclor in Plasma				
	Conc. µg/ml	Mean n=5	SD	%CV	
	25.00	390201.80	1876.26	0.48	
	6.25	92566.40	388.77	0.42	
	0.78	12845.40	168.67	1.31	
	Absolute Analytical Recovery				
	Peak Area of Cefaclor in Mobile Phase				
	Conc. µg/ml	Mean n=5	SD	%CV	
	25.00	403606.20	3840.64	0.95	
	6.25	94455.80	192.94	0.20	
	0.78	14752.60	100.56	0.68	
	Mean Absolute Recovery				
	Conc. µg/ml	Plasma Samples		Mobile Phase	Recovery
	25.00	390201.80		403606.20	96.68
	6.25	92566.40		94455.80	98.00
	0.78	12845.80		14752.60	87.07
Mean					93.92
SD					5.96
%CV					6.35

LLOQ

The lower limit of detection and limit of quantifications were assessed by injecting multiple concentrations of the drug (0.782µg/ml, 0.390µg/ml, and 0.195µg/ml) into the HPLC column. Then, calculations were made for evaluating SD, precision, % accuracy and % CV (Guideline, 2005).

Stability

In short-term temperature stability testing, the low and high concentrations, (3 aliquots) of 25µg/ml and 0.782 µg/ml were stored at -20ºC for 24 hours and thawed completely at room temperature without any aid, and then restored at same freezing temperature for 12-24hours. This freeze and thaw cycles were repeated thrice and then Cefaclor was analyzed by the proposed method. The long-term temperature stability samples of same concentrations were kept under same storage conditions for the estimated period of first sample collection and sample concentrations were compared to the mean of back-calculated values. Stability of the stock solution was

Table 3: Long Term Stability of Cefaclor 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	0.695	0.704	0.69	24.795	24.778	24.708
SD	0.02	0.01	0.02	0.03	0.03	0.04
%CV	2.54	1.91	3.4	0.1	0.11	0.17
Accuracy %	88.874	90.025	88.235	99.18	99.112	98.832

investigated for three weeks by comparing the peak area of nominal concentrations with freshly prepared samples (Guideline, 2005).

RESULTS

The method was found selective when investigated as per FDA guidelines (fig. 5). The linearity was found excellent at the retention time of 17min., in the concentration range 50µg/ml to 0.195µg/ml (R^2 0.999) at 265nm both in mobile phase and plasma (see fig. 2 and 3). The result of accuracy and precision were within 15% limit, table1, showing % CV in the range of 2.59%-4.72% and accuracy 86.86%-98.11% (intraday accuracy and precision). Whereas the values for interday accuracy and precision were found in the range of 1.91%-5.87% and 97.07%-101.06% respectively. table 2 exhibits the absolute analytical recovery of the method in the range of 87.07%-98.00%. The LLOQ was found to be 0.39µg/ml and was within acceptable accuracy limit (see fig. 4) (Guideline, 2005) and the limit of detection observed was 0.195µg/ml. The analyte was found stable when subjected to freeze and thaw stability with the accuracy of 88.23%-91.02% (0.782µg/ml) and 98.83%-99.18% (25µg/ml). Table 3 shows the long term stability of Cefaclor with the percentage accuracy of 88.235%-90.025% (0.782µg/ml) and 98.832%-99.18% (25µg/ml). Percent coefficient of variance was also found within 15% limit after stability testing.

DISCUSSION

For the detection of Cefaclor in the human plasma various methods have been reported (Samanidou & Hapeshi *et al*, 2003, Shah & Rasul *et al*, 2013). In the present study USP 32, NF 27 (Pharmacopeia, 2009) method was modified and validated for the quantification of Cefaclor in human plasma. Since compendial procedures are focused on quantitative analysis of drugs in pharmaceutical dosage forms, the currently reported method was modified in terms of pretreatment procedure of biological samples and lower LLOQ which is essential for obtaining a comprehensive pharmacokinetic profile. The study involved a mobile phase containing a mixture of sodium 1-pentanesulfonate, water, triethylamine and methanol. Satisfactory separation between endogenous plasma components and drug was obtained. Bioanalytical method validation was carried out as per FDA bioanalytical method validation guidelines. Linearity of an analytical method should be established within analytical range intended to cover the maximum concentration versus time profile in a Bioavailability or Bioequivalence study. The proposed method showed excellent linearity in dynamic range 50µg/ml to 0.195µg/ml. The other essential parameters like Selectivity, Sensitivity, Stability, Analytical recovery, Accuracy and Precision were investigated to determine if the analytical method was fit for purpose. The findings of the validation experiments were in concordance with previously reported results. (Shah *et al*, Basaveswara *et al*).

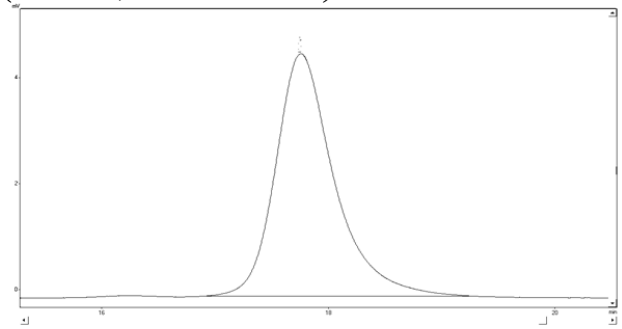

Fig. 4(c): Chromatogram showing Cefaclor in human Plasma

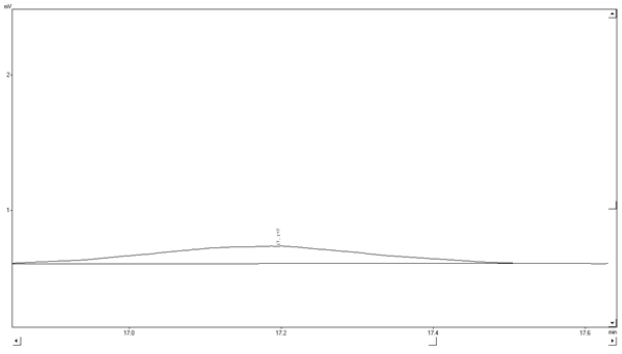

Fig. 4(d): Chromatogram showing Lower Limit of Quantification (LLOQ) in human plasma

CONCLUSION

The developed and validated, RP-high performance liquid chromatographic method for the detection and quantification of Cefaclor in human plasma was found to be accurate, precise, simple and sensitive, with excellent

linearity. Therefore this method was found an adequate method for the plasma analysis of Cefaclor in pharmacokinetic and bioequivalence studies.

ACKNOWLEDGEMENT

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