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Research Article

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DETERMINATION OF CEFDITOREN PIVOXIL IN HUMAN PLASMA BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD

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ABSTRACT
A simple, rapid, sensitive high performance thin layer chromatography method for cefditoren pivoxil in human plasma using cephalexin as an internal standard has been developed and validated. The method enables to determine cefditoren pivoxil with minimum quantification limit 0.1µg ml^{-1} to 0.6 µg ml^{-1} having retention factor of 0.74±0.03. The sample preparation involves the simple protein precipitation technique using methanol as a precipitating agent. The determination was carried out on silica gel 60 F_{245} TLC plate with a mobile phase consisted of toluene: methanol: triethylamine (5:3.5:0.09 v/v/v). The wavelength selected was 250nm.
KEYWORDS: Cefditoren pivoxil, HPTLC, human plasma

INTRODUCTION

Cefditoren pivoxil is a third generation cephalosporin used to treat uncomplicated skin and skin structure infections, community-acquired pneumonia, acute bacterial exacerbation of chronic bronchitis, pharyngitis, and tonsillitis[1,2]. It is effective against haemophillus influenza, moraxella catarrhalis and beta lactamase strains[3]. The cefditoren pivoxil has not yet published as an official monograph but the drug has been approved by FDA[4] and available in a USP pending monograph[5], Martindale[6], Merck index[7]. It is chemically (-)-(6R,7R)-2,2 dimethylpropionyl-oxymethyl 7-[(Z)-2-(2- aminothiazol-4-yl)-2 methoxy iminoacetamido]-3-[(Z)-2- (4-methylthiazol-5-yl) ethenyl]-8-oxo-5-thia-1 azabicyclo[4.2.0] oct-2-ene-2-carboxylate. The Cmax is 3.1-3.6mg/ml[8].

There are spectrophotometric methods reported for cefditoren pivoxil individually as well as in combination with other drugs[9,10]. The RP-HPLC method has been available for its determination in bulk, in presence of degradation products[11]. The bioequivalence and pharmacokinetic study on rat has been done by HPLC method[12]. The pharmacokinetic/ pharmacodynamic serum and urine profile has been done by HPLC method[13].

No references found for quantitative determination of cefditoren pivoxil in human plasma by HPTLC method.

In this work we report simple, accurate, precise and sensitive High Performance thin layer chromatography method for quantitative determination of cefditoren pivoxil in Human plasma. The proposed method is optimized and validated according to EMEA guidelines[14].

MATERIALS AND METHOD

Instrumentation

Chromatographic separation was performed on a Merck TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 µm thickness, E. Merck, Darmstadt, Germany, purchased by Anchrom Technologies, Mumbai, India). The samples were applied onto the plates using Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe as a band with 6 mm width using a Camag Linomat 5 applicator (Camag, Muttenz, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm). Densitometric scanning was performed on Camag TLC scanner 3 at 235 nm for all measurements and operated by winCATS software (V 1.4.2, Camag).

Reagents and Solvents

Cefditoren pivoxil was obtained as a gift sample from Maxim pharmaceuticals, Pune. All other chemicals and reagents were of analytical grade.

For validation of the method, human plasma was obtained from Akshay blood bank, pune.

Working standard solution

25mg of Cefditoren pivoxil was dissolved in 25ml of methanol to obtain 1000µg/ml of standard solution. From this solution 0.08, 0.16, 0.24, 0.32, 0.40, 0.48ml was taken and made the volume to 10ml so as to obtain the concentrations of 8, 16, 24, 32, 40, 48µg/ml which were used for preparing the plasma samples. For internal standard, Cephalexin was dissolved in methanol and diluted to get concentration of 200µg/ml.

Sample preparation

Each of above working standard solutions of Cefditoren pivoxil 0.5ml was used to spike 1ml of plasma. Then 0.2ml of internal standard and 0.3ml of methanol was added to precipitate the plasma proteins. Then these mixtures were vortex mixed on Remi mixer and centrifuged for 15min at 6000rpm. The final plasma solution was obtained in concentrations of 2, 4, 6, 8, 10, 12µg/ml. From these solutions 50µl was spotted to yield 100, 200, 300, 400, 500, 600ng/spot. The QC samples were prepared in plasma in concentration range of 200, 400, and 600 to design the QC samples as LQC, MQC, and HQC.

Selectivity

Blank human plasma from six different sources was assessed by above developed method to evaluate selectivity of the method.

Lower limit of Quantification

The Lower limit of quantification was decided on the basis of lowest concentration on calibration curve.

Calibration curve

For plotting calibration curve, the selected range of Cefditoren pivoxil (100ng/spot to 600ng/spot) and Cephalexin (500ng/spot) was spotted and areas were taken. The calibration curve was plotted as response factor versus concentration and linear over the range 100ng/spot to 600ng/spot for Cefditoren pivoxil. The response factor was calculated as the ratio of area of drug to the area of internal standard.

Precision and Accuracy

The precision of the method were examined by using quality control samples. Four concentration levels selected for assessment were 4, 8, 10, 12µg/ml. 4µl of each was spotted to obtain concentration levels of 100, 200, 400 and 600ng/ml.

Accuracy

The accuracy of the assay was calculated as the absolute value of the ratio of the calculated mean values of the quality control samples to their respective nominal values, expressed as percentage. Accuracy

was measured using minimum five determinations per four concentrations (100, 200, 400, 600ng/spot).

Extraction recovery

The % mean recoveries were determined by measuring the responses of the extracted plasma quality control samples against unextracted quality control samples at LQC, MQC and HQC levels. Recovery from human plasma samples was evaluated in triplicate for each three concentrations of Cefditoren pivoxil (200, 400, 600ng/spot).

Stability

The stability of stock solution of cefditoren pivoxil was carried out the by storing the stock solution throughout the validation of method at -5^{0}C. The freeze thaw stability was carried out by performing three cycles of freezing and thawing of quality control samples. Bench top stability carried out by storing the samples at room temperature for 24hr. The long term stability was determined by storing the spiked plasma in refrigerator for 12 days. In post-preparative stability was assessed by keeping the processed samples for 5hrs. These stability samples was spotted alongwith the freshly prepared plasma samples for comparison.

RESULT AND DISCUSSION

Method development

Precipitation Method Optimization

Different solvents such as methanol, acetonitrile, trichloroacetic acid were tried alone and in combination for the precipitation of human plasma protein. Among the various trials, use of methanol provided acceptable recovery and therefore it was selected as precipitating agent.

Optimization of chromatographic conditions

For selection of mobile phase solvents like methanol, toluene, hexane, ethyl acetate and Dichloromethane were tried in various combinations. Toluene and methanol in the ratio of (5:3.5 v/v) provided acceptable Rf values and resolution. Addition of 0.09 ml of triethylamine in above mobile phase improved peak shapes. Hence mobile phase consisting toluene: methanol: triethylamine (6.5:3.5:0.07, v/v/v) was optimized. Detection wavelength selected was 250 nm as cefditoren shows absorption at this wavelength. The Rf value for cefditoren pivoxil is 0.75±0.03. Well defined spots were obtained when plate was activated at 60 ◦C for 15 min and the chamber was saturated with the mobile phase for 20 min at room temperature.

Method validation

Selectivity

The selectivity of the method was determined and there were no plasma interferences observed at the retention factor of drug.

Lower limit of Quantitation

The lower limit of quantitation selected from the calibration curve was 100ng/spot.

Calibration curve

The linearity of the method was statistically confirmed having correlation coefficient of 0.996±0.002. The regression equation was y= 0.001x + 0.244. The coefficient of variance (%CV) values of slope was less than 3%.

Precision

For Intraday precision, the % CV of calculated response factor for LLOQ and all quality control samples at LQC, MQC and HQC concentration levels are ranged from 1.60 to 6.33 %, which is within acceptance limit.

For Interday precision, the % CV of calculated concentrations for LLOQ and all quality control samples of LQC, MQC and HQC are 2.43 to 5.02% which is within the acceptance limit of 15.00 % and for LLOQ which is within 20%.

The results were shown in Table1.

Accuracy

The %CV of calculated response factor for all quality control samples at LLOQ, LQC, MQC and HQC concentration levels are ranged from 4.62% to 11.32%, which is within acceptance limit.

Extraction recovery

The % mean recovery for Cefditoren pivoxil at HQC, MQC and LQC levels are found to be 97.99, 104.55, and 97.15 respectively. The recovery of internal standard was also been calculated for three times and overall recovery was found to be 90.81% which is given in table 2.

Stability

The % mean stability for freeze thaw stability, LQC (200ng/spot) and HQC (600ng/spot) are found to be 95.32 and 92.37 respectively. For bench top stability, LQC (200ng/spot) and HQC (600ng/spot) are found to be 96.93 and 101.03 respectively.

The stock solution stability and Post preparative stability was found to be 101.23% and 97.29% for MQC samples respectively.

The long term stability for LQC and HQC, was found to be 98.27% and 95.78%.

The observations are given in table 3.

CONCLUSION

A rapid, simple and sensitive method has been developed for quantitative analysis of cefditoren pivoxil in human plasma. The chromatographic conditions described showed well resolved peaks. As well a very easy and economic sample preparation method was developed. So, this method is suitable for routine analysis of cefditoren pivoxil in human plasma.

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REFERENCES

1. Cefditoren, http://en.wikipedia.org/wiki/Cefditoren (accessed on 12-8-2010)
2. Cefditoren pivoxil, http://www.drugs.com/ppa/cefditoren-pivoxil (accessed on 15-8-2010)
3. Spectracef: Cefditoren pivoxil Description and clinical pharmacology, http://www.drugs.com/pro/cefditoren-pivoxil (accessed on 1-9-2010)
4. www.uso.org, authorized USP pending monograph version 1 (accessed on 25-9-2010)
5. Foye WO, Lemke TL, Williams DA, Antibiotics and Antimicrobial agents, Foye's principles of medicinal chemistry, 1063
6. Sweetman SC , Martindale, The complete drug reference, 35th ed., The Pharmaceutical Press, London (U.K) 2007, 199.
7. O'Neil MJ , The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, 14th ed., Merck & Co. Inc. 2006, 1921.
8. Cefditoren pivoxil: pharmacoogy http://www.ingentaconnect.com/content/adis/dgs/2002/00000062/00000002/art00006
9. Raju SA, Karadi AB, Manjunath S, Visible Spectrophotometric Determination of Cefditoren Pivoxil in Pharmaceutical Formulations, J. Ind. Council Chem. 2009; 26:54-57
10. Narala SR, Saraswathi K, Application of Oxidants to the Spectrophotometric Determination of Cephalosporins (Cefditoren Pivoxil and Cefdinir) In Formulations, Asian journal of research in chemistry 2011; 4:270
11. Dewani AP, Kochar NI, Abooj HC, Bakal RL, Chandewar AV, Barik BB, Determination Of Cefditoren Pivoxil In Bulk By Rp-Hplc In Presence Of Its Degradation products, Journal of Pharmacy Research 2010; 3:2588-2591
12. Qi Liu, Ji-Hong Yao, Cheng-Ye Su, Guo-Zhu Han, Hui-Jun Sun, Kun Wang, Xu Han, Bin Qu and Ke-Xin Liu, Bioequivalence of Cefditoren in human and pharmacokinetics of absorption in rat, Asian Journal of Pharmacodynamics and Pharmacokinetics 2006;6:214-218
13. Sadaba B, Azanza JR, Quetglas EG, Campanero MA, Honorato J, Coronel P, Gimeno M, Pharmacokinetic/pharmacodynamic serum and urine profile of cefditoren following single-dose and multiple twice- and thrice-daily regimens in healthy volunteers: A phase I study, Rev Esp Quimioterap, Enero 2007;20:51-60
14. Committee for medicinal products for human use (CHMP), Guideline on validation of Bioanalytical methods, European Medicinal Agency (EMEA), London, 2009.

Table1: Reproducibility of the analysis of cefditoren pivoxil in human plasma

Levels	Concentration (ng/spot)	(%CV)		(SD)	
		Intra day	Inter day	Intra day	Inter day
LLOQ	100	1.60	5.02	0.007	0.02
LQC	200	6.33	2.43	0.04	0.01
MQC	400	2.60	4.43	0.02	0.03
HQC	600	5.06	2.46	0.06	0.02

Table2: Extraction recovery of cefditoren pivoxil in human plasma

Concentration (ng/spot)	Recovery	(%CV)
200	97.99	4.31
400	104.55	5.55
600	97.15	3.09

Table3: Stability of cefditoren pivoxil in human plasma

Parameters	Nominal concentration (ng/spot)	Recovery ±%CV
Freeze thaw stability	200	95.32±2.95
	600	92.32±5.97
Bench top stability	200	96.93±5.004
	600	101.03±5.93
Long term stability	200	98.87±0.16
	600	97.53±5.03
Stock solution stability	400	101.23±4.30
Post preparative stability	400	97.29±0.71

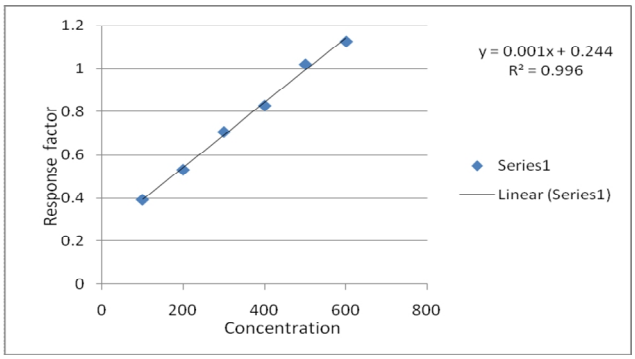

Figure1: Calibration curve for cefditoren pivoxil in human plasma

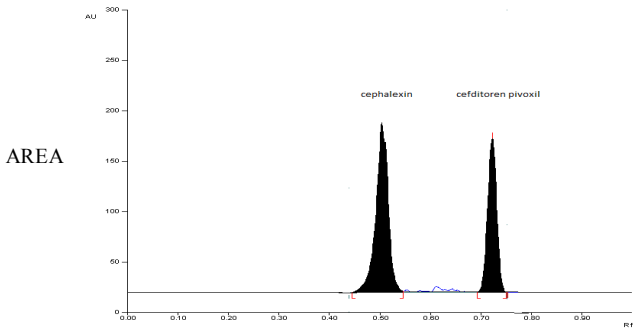

Figure 2: Chromatogram of cefditoren pivoxil (100ng ml^{-1}) and cephalexin (500ng ml^{-1}) internal standard

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