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EXTRACTION AND SEPARATION OF CIPROFLOXACIN BY HPLC FROM HUMAN PLASMA

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ABSTRACT: A selective, sensitive and accurate liquid chromatographic method with UV detection was developed, validated and applied for the determination of Ciprofloxacin in human plasma. The effects of mobile phase composition. buffers. pH, acetonitrile concentration and temperature were investigated on the separation/recovery of Ciprofloxacin from the standard preparations and spiked plasma. The standard samples concentration range 0.2ppm to 3ppm and then same concentration were spiked in human plasma. During process of extraction. acetonitrile was used as deproteinizing agent and separated through centrifuge. In this method UV detector and CIs column with temperature 50 degree centigrade were used. The recovery. selectivity. linearity. precision and accuracy of the method were evaluated from spiked human plasma samples. The method was successfully applied on plasma samples of healthy human volunteers during bioequivalence study. Ciprofloxacin tablets were prepared in lab and compared with market image preparation (Ciproxin).

KEYWORDS: Pharmacokinetics. Bioequivalence, Microbiological assay Ciprofloxacin & Ciproxin.

INTRODUCTION

Ciprofloxacin is a quinolone carboxylic acid derivative, which has gram negative and gram positive bacterial activity (Wise *et at.,* 1983; Roy *et at.,* 1983 and Chin & Neu, 1984), exhibits a rapid on set of action, and lacks cross-reactivity with penicillins, cephalosporins, and aminoglycosides (Zeeiler & Grohe, 1984). Although there are several published procedures for the analysis of ciprofloxacin and their metabolites Le., M1 (desethyleneciprofloxacin), M2 (sulphociprofloxacin), M3 (oxociprofloxacin) and M4 (formyl ciprofloxacin) in body fluids (Gau *et at.,*
1985), but still there is a need for a simple procedure that can be used for the simultaneous analysis of ciprofloxacin in serum and plasma as well as in urine. Such a procedure can save a considerable amount of time when concentration of ciprofloxacin have to be assayed in thousand of serum, plasma and urine samples collected during clinical, bioavailability, bioequivalence and pharmacokinetic studies.

The present procedure required modifications of the previously published procedures (Krol *et at.,* 1995; Gracia *et at.,* 1999; Hairrui *et at.,* 2002; Ulrike *et at.,* 2002; Rao *et at.,* 2002 and Samanidou *et at.,* 2003). The modification involved in the precipitation of plasma proteins with acetonitrile and using centrifuge for separation/extraction prior to chromatography, followed by direct injection on the waters micron bodapack CI8 reverse phase column at 50 degree centigrade and detection at max 285 nm using UV detector along with change in the composition of mobile phase and adjustment in pH.

EXPERIMENTAL

MATERIALS AND METHODS

Analytical grade potassium dihydrogen phosphate (Merck), acetonitrile HPLC grade (Merck), *N-N* dimethyl formamide (Reidel) and phosphoric acid (Merck) were purchased from local market. The de-ionized water was purified in the Lab. The pH of mobile phase was adjusted by adding phosphoric acid (85%). Dissolved gases were removed by 10 minutes sonication on an ultrasonic bath.

Sample Preparation:
One ml of plasma sample was taken in a test tube, added 0.5 ml different concentration of Ciprofloxacin standard preparations and to it 1 ml of acetonitrile and the mixture was vortexed for 5 minutes then centrifuged the sample and the supernated solution was transferred to a HPLC vial for injection and detection at 285 nm by using UV detector.

Chromatography:
Aliquots (20u1) of Ciprofloxaxcin reference standard and plasma spiked samples, were injected on to the water micro bodapack CI8 column at temperature 50 degree centigrade, with flow rate I ml/minute and running time 10 minutes. The mobile phase composition Acetonitrile: *N-N* Dimethyl formamide : 0.01 M Sodium Dihydrogen Phosphate Dihydrate (15 : 6 : 79) and pH 3.0 is adjusted by adding phosphoric acid (85%). The normal chromatograms of Ciprofloxacin standards with average retention time of different concentrations 6.33 minutes (Fig. 1) ciprofloxacin spiked plasma with average retention

time of different concentrations 6.354 minutes (Fig. 2). Ciprofloxacin in plasma samples were analyzed for study of bioavailability, bioequivalence and pharmacokinetic.

Measurement precision: In order to demonstrate acceptable measurement precision five runs of 0.5ppm solution were made and the relative standard deviations was not more than 2.0% was observed (Table-1).

Table 1
Concentration of sample Ciprofloxacin analyzed = 0.5ppm

Run	Average Area Response
1	1068854
2	1056648
3	106458
4	110254
5	108112
AVG.	107465.2
%RSD	1.67

Linearity: The area response of seven samples of different concentration between 0.2-3.0 ppm values was 0.999 (Table-4).

Limit of Detection: Limit of detection was established by stepwise dilution of a known standard of 0.2ppm until the point at which a real positive response was no longer detected and that the 0.02ppm (Table-2).

Table-2
Limit of Detection

Concentration (ppm)	Average Area Response
0.2	50318.5
0.15	38493
0.1	26416
0.05	13575
0.02	5631

Table-3
Limit of Quantitation
Concentration of sample (Ciprofloxacin) analyzed = 0.2ppm

Run	Area Response
1	51264
2	50647
3	54468
4	49658
5	53115

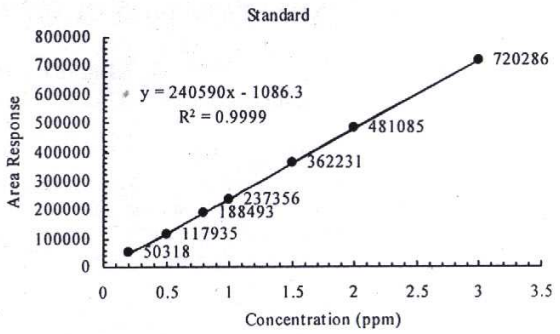

Fig. 1

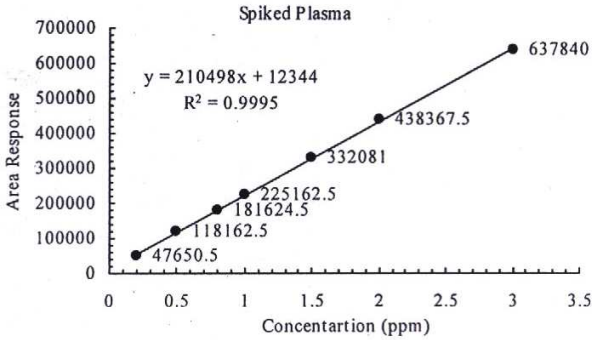

Fig. 2

Limit of Quantitation: Limit of detection is the lowest concentration point of the linear range i.e. 0.2ppm with relative standard deviation value of ≥10.0% (Table-3).

Spike and Recovery: To demonstrate acceptable recovery, known solutions of Ciprofloxacin were spiked and tested for recovery. Amount recovered versus amount spiked was plotted using each preparation as individual points (Table-4).

RESULTS AND DISCUSSION

Table-4 summarizes the observed recoveries of Ciprofloxacin standard preparation from concentration 0.2ppm to 3ppm with average area response from 50318.5 to 720286.5 with R2 = 0.9999 (Fig. 1 and Table-4) also summarizes the observed recoveries of Ciprofloxacin spiked plasma after the precipitation of plasma proteins with area response from 47650.5 to 637840 with R2 = 0.9995 (Fig. 2).

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Table-4
Linearity % RSD

Cone. (ppm)	A vg. area response standard	A vg. area response spiked plasma	% recovery	%RSD
0.2	50318.5	47650.5	94.70	2.88
0.5	117935	118612.5	100.57	4.37
0.8	188493	181624.5	96.36	0.91
1.0	2373356	225162.5	94.86	0.9
1.5	362232	332081	91.12	0.02
2.0	481085.5	438367.5	91.12	0.68
3.0	720286.5	637840	88.55	1.03

Table-5
Intra-day decision and accuracy observed with plasma calibration standards containing Ciprofloxacin.

Con. of Std Ciprofloxacin spiked in plasma	Theoretical area response	Mean Observed area response	RSD (%)	Variation (%)	Accuracy (%)
0.2 ppm	47650.5	46738.57	1.78	1.914	98.00
1.0ppm	225162.5	228529.43	2.105	1.500	101.5
2.0 ppm	438367.5	437777.13	2.395	0.135	99.87

(n =14 triplicate at three cone. levels w(:re analyzed)

Table-6
Inter-dav precision and accuracy observed with plasma calibration standards containing Ciprotloxacin.

Con. of Std Ciprotloxacin spiked in olasma	Theoretical area response	Mean Observed area response	RSD(%)	Variation (%)	Accuracy (%)
0.2 ppm	47650.5	46966.79	1.88	1.44	98.57
1.0 ppm	225162.5	1433387.154	2.58	1.14	198.86
2.0 ppm	438367.5	226449.93	2.04	0.57	100.57

(n =14 triplicate at three cone. levels were analyzed)

Table-4 further shows the % of recovery of each concentration varies from 88.55% to 1 00.57% and % RSD from 0.02 to 4.37. The described procedure is specific, accurate, precise and sufficiently sensitive for the analysis of Ciprofloxacin in plasma with limit of detection 0.2ppm and limit of quantitation 0.2ppm with RSD less than 10%. A relatively simple sample preparation step is involved and only one isocratic chromatographic elution that separates and quantitates. This procedure was used for analysis of about 500 blood samples collected during bioavailability, bioequivalence and pharmacokinetic study. The intra-day and inter-day relative standard deviation (n=14) of 0.2ppm 1.78% (accuracy 98.00%) and 1.88% (accuracy 98.57%) respectively, 1.0ppm 2.1055 (accuracy 101.5%) and 2.58% (accuracy 98.86%) respectively and for 2.0ppm 2.395% (accuracy 99.87%) and 2.04% (accuracy 100.57%) see Table-5 and 6. Calculated at triplicate samples of each concentration level of standard Ciprofloxacin solution spiked in plasma after precipitation of protein (Fig.3), were performed with high precision and accuracy.

This method can also be employed in the analysis of Ciprofloxacin in pharmaceutical dosage forms due to relative simple sample preparation step and only isocratic chromatographic elution that separate and quantitate in just 10 minutes of total run.

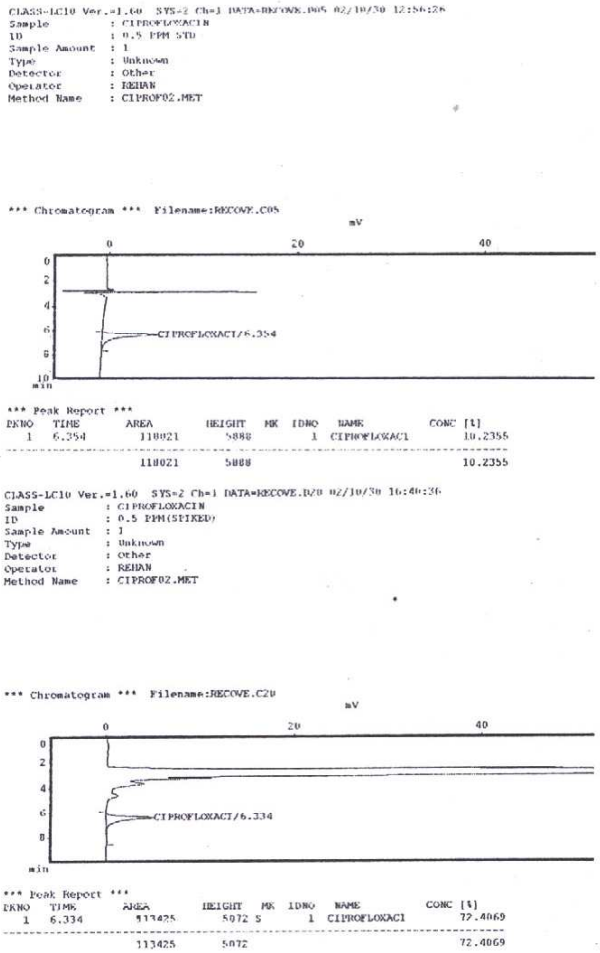

Fig. 3

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