Development and Validation of Bioanalytical Spectrophotometric Method for Pharmacokinetic Study of Cefpodoxime Proxetil Microemulsion in Rats

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ABSTRACT

For the pharmacokinetic analysis of cefpodoxime proxetil SMEDDS in rats, a sensitive and accurate bioanalytical spectrophotometric technique was developed and validated in the visible range. The method was developed using oxidative chromogenic reagents such as potassium ferricyanide and ferric chloride to produce colour and methanol as a solvent which produced into green coloured chromogen. Rat plasma was used to validate the described procedure. It was found that plasma has a maximum absorbance at λmax 738 nm. According to USFDA guidelines, the method was validated over the concentration range of 100-8000ng/mL. The method's precision and accuracy were found to be within accepted limits. For the pharmacokinetic analysis of cefpodoxime proxetil, the established method can be used successfully.

Key words: *Cefpodoxime proxetil, SMEDDS, method validation, USFDA guidelines.*

INTRODUCTION

Cefpodoxime proxetil is a broad-spectrum, third-generation cephalosporin that is taken orally. It is a pro-drug that *in vivo* de-esterifies to cefpodoxime, a compound with strong antibacterial properties. It has a good therapeutic potential and is typically well tolerated by patients with a variety of common bacterial illnesses. Strong antibacterial action against both gram-positive and gram-negative bacteria is shown by cefpodoxime proxetil, along with good stability in the presence of beta-lactamases. Most respiratory pathogens are inhibited by cefpodoxime at low dosages [1-3].

The chemical name [4] of Cefpodoxime proxetil is 1-(isopropoxy carbonyloxy) ethyl (6R,7R)-7-[2-(2-amino-4-thiazolyl) -(z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate and its Empirical formula $C_{21}H_{27}N_5O_9S_2$. Structure of cefpodoxime proxetil is given in (Figure 1). The literature survey reveals [5-14] that there are UV-Visible spectrophotometric and HPLC methods reported for the estimation of cefpodoxime proxetil in dosage forms but there were are no bioanalytical methods for the analysis of cefpodoxime proxetil using Visible spectrophotometry. In view of this we planned to develop novel spectrophotometric method using chromogenic reagents and validate the method by using USFDA guidelines.

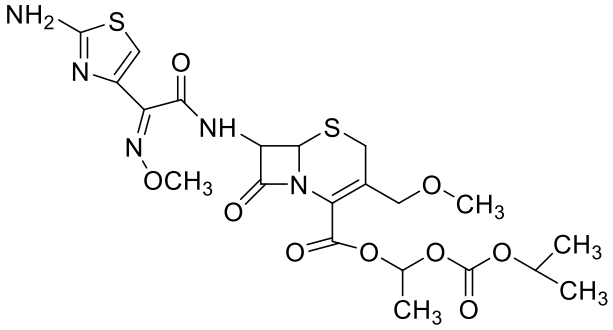

Figure1.Structure of cefpodoxime proxetil

MATERIALS AND METHODS

The development of the method used a visible spectrophotometer Shimadzu, UV-1800 with UVProbe 2.48 software; the instrument includes 10 mm matched quartz cells. All of the solvents and chemicals used in the experiment were of the AR grade. Our lab created the formulation as Cefpodoxime Proxetil - SMEDDS. I received a free sample of Cefpodoxime proxetil's pure API from Micro labs in Bangalore. From the National Institute of Nutrition in Hyderabad, albino Wistar rats (180-200g) were purchased. Animals were kept in standard cages in a carefully regulated laboratory environment. During the quarantine period, all animals had unlimited access to food (provided by the National Institute of Nutrition, Hyderabad) and water. The animal experimentation methodology has been authorised by the Institute of Animal Ethics Committee at G. Pulla Reddy College of Pharmacy in Hyderabad, Telangana, India.

Preparation of standard stock solutions

Preparation of ferric chloride (1.62% w/v)

Dissolve 1.62 gm. of ferric chloride in 100 mL of distilled water.

Preparation of potassium ferricyanide (0.1%w/v)
Dissolve 100 mg of potassium ferricyanide in 100 mL of distilled water.

Preparation of working stock solution for Calibration curve standards
100 mg equivalent weight of cefpodoxime proxetil was weighed and transferred into 100mL volumetric flask having 3-4mL of methanol and sonicated for 5 minutes and made the volume with methanol, which gives 1mg/mL stock solution. 0.1 mL was pipette out from the stock solution into 10 mL volumetric flask sonicated for 5 minutes, then the volume was made with distilled water, which gives 10µg/mL stock.

Selection of solvent
Various solvents such as water, methanol, 0.1N HCl, 0.2NHCl, 0.5N HCl, phosphate buffer of pH 3.6 and chloroform were tried to dissolve cefpodoxime proxetil the results are as follows like the drug was sparingly soluble in water, it was not completely soluble in different normality HCl and Phosphate buffer used, whereas the drug dissolved but there was no absorbance when treated with chromogenic agents when chloroform used as solvent. The drug was completely soluble in methanol.

Selection of reagent
Numerous reagents were employed, including 3-aminophenol, naphthol, the Bratton-Marshall reagent, ferric chloride, and potassium ferric cyanide. When 3-amino phenol was used, red chromogen was produced, however the colour was not stable. While red chromogen was formed using naphthol, its absorbance values were negative when it was measured at its maximum wavelength of 554 nm. The coloured compound was examined for absorbance at a maximum wavelength of 554 nm using the diazotization coupling technique, and it was found that there was no exponential increase in the absorbance as the drug concentration was increased. Finally, as an oxidising agent, ferric chloride and potassium ferricyanide produced a persistent green chromogen with a maximum wavelength of 738 nm.

Validation of the method
The method validation was carried out in accordance with USFDA recommendations. The method's linearity, accuracy, precision, selectivity, recovery, and stability of analyte solutions were all validated [15].

Formulation details
Phase Titration Method
Pseudo ternary phase diagram was constructed to find out different zones including micro emulsion zone, in which each corner of the diagram represents 100% of particular component. Phase diagrams are obtained by mixing of the ingredients which shall be pre weighed into glass vials and titrated with water and stirred well at room temperature. A clear transparent mixtures were visualized after stirring, the samples shall be marked as the points in the phase diagram. The area covered by these points is considered as the region of existence of micro emulsion.

Preparation of test formulation SMEDDS (Self micro emulsifying drug delivery system)
With stirring and heating to 60°C, 12g of castor oil was added to the 0.394 g of drug and 4.5 g of PEG 500 (co-surfactant). After a while, allow the temperature to drop until it reaches room temperature. Self-Micro Emulsifying Drug Delivery System is developed by adding Surfactant (13.5 g of tween 80) gradually drop by drop while stirring. The stability of the SMEEDS can be evaluated by keeping it at room temperature for 24 to 48 h without disturbing, there should be no phase separation in the emulsion. Then it is stable enough to administer.

Preparation of standard formulation
Preparation of 0.5% SCMC suspension
0.25 gms of sodium carboxy methyl cellulose was weighed and transferred into a mortar. Water was added slowly with trituration until it was mixed without any lumps and water was added up to 5 mL and mixed well. Then accurately weighed 0.0394 gms of pure cefpodoxime proxetil dug and mixed well in prepared SCMC suspension until dissolved. cefpodoxime proxetil suspension (pure formulation) was prepared. Check for the stability and administer required quantity to the rats.

Preparation of standard and sample solutions for the analysis
The processed plasma was collected into fresh Eppendorf tube and spiked the required quantity of drug from 10µg/mL Stock solution. 0.15mL of 1.62% w/v of ferric chloride solution and 0.1 mL of potassium ferricyanide solution were added. Kept the mixture of solutions aside for 20 minutes and made the volume to 1 mL with distilled water. The green-coloured standard solutions were scanned against λmax in visible range (400-1100) to obtain respective absorbance. Maximum absorbance of cefpodoxime with $FeCl_3$ in plasma was found to be at (λmax) 738nm with 10µg standard drug solution.

Application of the method for pharmacokinetic study
Male Wistar rats were used in pharmacokinetic experiments. Rat plasma samples were analysed using the established visible spectrophotometric method to determine the amounts of cefpodoxime proxetil. The institutional ethical committee at G. Pulla Reddy College of Pharmacy in Telangana, India, gave its approval to the protocol. Observations were made in accordance with CPCSEA recommendations. Except for the final 12 hours prior to the experiment, the 180–200 g rats were housed with unlimited access to food and drink. The dose for rats was estimated using the adult human dose and their respective body weights. Samples from the control and test groups were taken at intervals of 0 min, 10 min, 20 min, 1 h, 1.5 h, 2 h, 3 h, and 24 h. All of the rats were fed and given water after three hours of study, and the subsequent sampling was then conducted. Prior to analysis, all samples were prepared and kept at -20^0C. As the control or blank sample, samples from all the test and standard groups that were obtained at the beginning of time were used [16-18]. The amounts of cefpodoxime proxetil in the plasma samples were determined as previously mentioned. Using the Excel add-in programme pksolver, various pharmacokinetic parameters, including rate constant, elimination rate constant, absorption half-life, elimination half-life, Cmax, Tmax, and AUC_{0-t}, and $AUC_{0-\infty}$, were calculated. The findings are shown in (Table 5).

Plasma sample preparation

The blood samples were collected from retro-orbital puncture into disodium EDTA vials (20 mg disodium EDTA in 1 mL water, 1mL of blood requires 50µL of disodium EDTA). Plasma was separated from blood samples by centrifugation at 7000 rpm for 15 minutes. After centrifugation, plasma layer gets separated and it is collected and stored at -20^{0}C for further use [19-22].

Blank Plasma and standard preparation

The separated organic layer was taken from processed blank plasma and after filtration its absorbance was measured directly without spiking it with either sample or solvent. Standard solution of cefpodoxime proxetil 10µg/mL spiked in plasma was prepared and absorbance was measured. Blank plasma absorbance was also recorded.

RESULTS AND DISCUSSION

For the purpose of estimating cefpodoxime proxetil levels in rat plasma, a novel visual spectrophotometric was developed. Among the trials, trial-4 with 1.62 % w/v ferric chloride and potassium ferricyanide was successful.10 µg/mL of the 100 µg/mL solution was made. The absorbance of the coloured solution that resulted was measured in the visible range of 400 to 1100 nm, with a maximum absorption at 762 nm so this wavelength was selected as λmax (Figure 2) and the optimized conditions of the method are given in (Table 1).

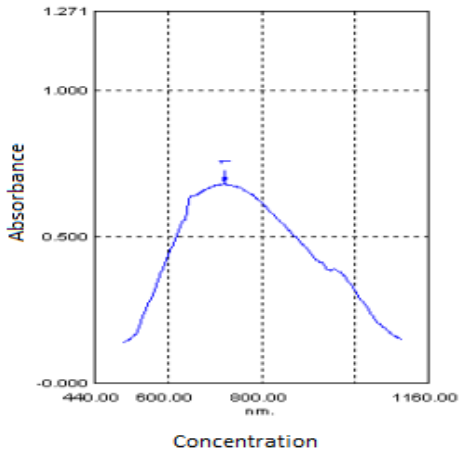

Figure2. Absorption spectrum of cefpodoxime proxetil

Table1. Optimized conditions of the developed method

λmax	**738nm**
Beer's law range (ng/mL)	100-8000
Sandell's sensitivity (µg/cm^{-2})	0.0452
Limit of detection (ng/mL)	32.885
Limit of quantification (ng/mL)	99.65
Regression equation	Y = 0.1144x+0.247

Slope (a)	0.1144
Intercept (c)	0.247
Correlation coefficient (r)	0.9941

Sensitivity of the developed method was checked by preparing 1 µg/mL solution of cefpodoxime proxetil and its absorbance was found to be 0.221 at 738nm. (Figure 3).

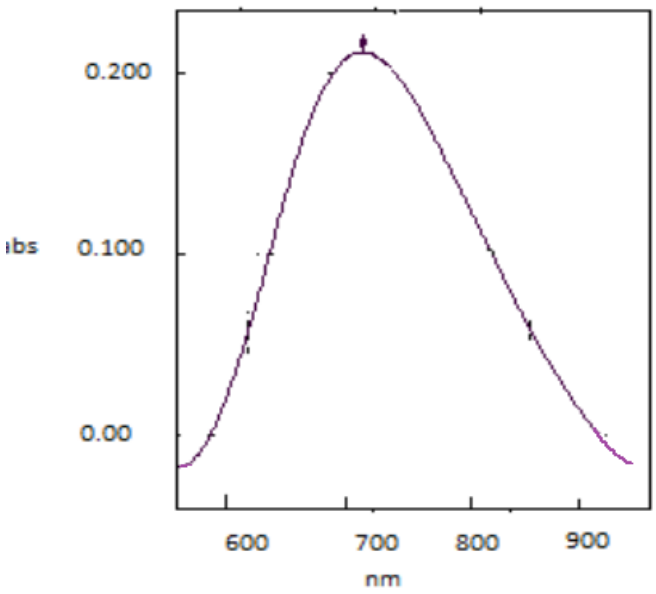

Figure 3. Absorbance spectrum for cefpodoxime proxetil at 1 µg/mL solution

Linear regression analysis was performed for checking the linearity of the data obtained. The response of the drug was found to be linear in the concentration range 100 – 8000 ng/mL. The linear regression equation for cefpodoxime proxetil was Y =0.114x + 0.247 with r2 0. 994.The calibration curve data is shown in (Table 2) and calibration curve is shown in (Figure 4&5).

Table 2. Calibration curve data of the developed method

S. No	Concentration (ng/mL)	Absorbance*
1	100	0.221
2	500	0.299
3	1000	0.396
4	2000	0.507
5	4000	0.679
6	6000	0.936
7	8000	1.161

*Average of three determinations.

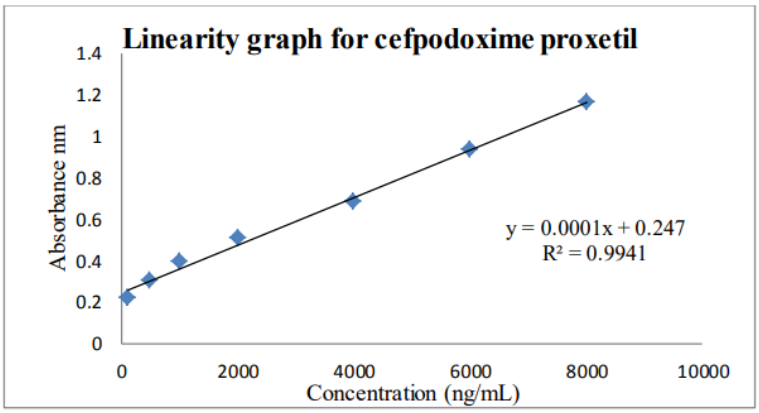

Figure 4. Calibration graph of cefpodoxime proxetil in rat plasma

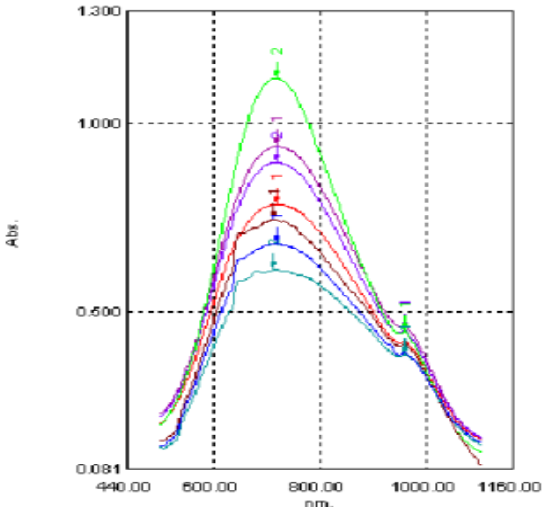

Figure 5. Overlay spectrum of linearity data.

Accuracy of the method was determined at three different concentration levels. Mean and % RSD values were calculated and are shown in (Table 3). The results indicate that the method was accurate as all the %RSD of different concentrations are within the limits.

Table 3. Accuracy data of cefpodoxime proxetil in spiked rat plasma

S. No	Accuracy level	Concentration (ng/mL)	Absorbance	Mean*±SD (n=3)	%RSD
1	80	1600	0.397	0.405 ± 0.0075	1.86
			0.409		
			0.411		
2	100	2000	0.497	0.4966 ± 0.0045	0.907
			0.492		
			0.501		
3	120	2400	0.529	0.532 ± 0.0036	0.677
			0.531		
			0.536		

*Average of three determinations

Precision of the data is reported in terms of repeatability and it is found to be within the limits as % RSD for analyte concentration in standard samples should not be more than 2.0 (Table 4).

Table 4. Repeatability data

S. No	Concentration (µg/mL)	Absorbance	Mean*±SD	%RSD
1	2	0.507	0.501± 0.0034	0.695
2	2	0.501		
3	2	0.500		
4	2	0.497		
5	2	0.503		
6	2	0.499		

*Average of six determinations

Pharmacokinetic parameters

Different pharmacokinetic parameters like rate constant, elimination rate constant, absorption half-life, elimination half-life, maximum plasma concentration (C_{max}), time taken to reach the same (t_{max}), AUC_{0-t}, and $AUC_{0-\infty,}$ were determined using pksolver, excel add-in software and results are tabulated in (Table 5). It was determined that the procedure was appropriate for analysing the plasma samples from the rat pharmacokinetic investigation.

Table 5. Pharmacokinetic parameters of cefpodoxime proxetil

Parameter	Result
K_a	3.8697616/1h
K_{10}	0.2292638/1h
$t1/2K_a$	0.1791188/h
$t1/2K_{10}$	3.0233607/h
t_{max}	0.7762880/h
C_{max}	4.8760225µg/mL
AUC_{0-t}	25.300979 µg.h/mL
$AUC_{0-\infty}$	25.411111 µg.h/mL

Note. K_a = rate constant, K_{10} = elimination rate constant, $t1/2K_a$ = absorption half-life, $t1/2K_{10}$ = elimination half-life, t_{max} = time taken to reach the plasma concentration, C_{max} = maximum plasma concentration, AUC_{0-t} = area under the curve from zero to t, $AUC_{0-\infty}$= area under the curve from zero to infinite.

CONCLUSION

In order to carry out a pharmacokinetic study of the cefpodoxime proxetil self-emulsifying drug delivery system in rats, a sensitive and selective bioanalytical spectrophotometric approach was devised. This method made use of ferric chloride and potassium ferricyanide as oxidising agents. agents. The approach had sufficient sensitivity to allow for the detection of drug concentrations to the nanogram level. The method is valid according to all of the validation parameters that were established in accordance with USFDA criteria. It is routine

practise to analyse drugs in their pure state and in their formulations using visible spectrophotometric methods. Comparing visible spectrophotometry to UV and chromatographic techniques, it has a high sensitivity. The approach in the current investigation was developed employing the inexpensive oxidizers potassium ferric cyanide and ferric chloride in spiked rat plasma. Since the method's sensitivity was high, it can be used for pharmacokinetic studies of newly created cefpodoxime proxetil formulations.

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CONFLICT OF INTEREST: None

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ETHICS STATEMENT: The work on animals has been approved from Institutional ethical committee at G. Pulla Reddy College of Pharmacy in Telangana, India dated on 20-02-2016, with student Id as follows GPRCP/IAEC/20/16/02/PCA/AE-11-RATS-M/F-20.

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