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LC method for the analysis of cefetamet pivoxil hydrochloride in drug substance and powder for oral suspension

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Abstract

A high-performance liquid chromatography isocratic procedure was developed for the assay of cefetamet pivoxil hydrochloride in drug substance and powder for oral suspension. The method validation yielded good results and included the range, linearity, precision intra- inter-day, accuracy, specificity, LOD and LOQ values. The chromatographic system consisted of a C_{18} absorbosphere column (150 × 4.6 mm i.d., 5 μm particle size), a mobile phase composed of water–acetonitrile–methanol–phosphate buffer, pH 3.5 (50:35:10:5, v/v), flow rate of 1.5 ml min^{-1} and UV detection at 254 nm. The relative standard deviation varied between 0.03 and 1.76%, and accuracy of 100.09% was found. Calibration curve was linear from 30.0–80.0 μg ml^{-1}; its correlation coefficient was 0.99989. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

During the last few years, one of the major advances in the field of antimicrobial therapy has been in the area of oral derivatives of the aminocephalosporanic ring, the cephalosporins [1]. Cephalosporins are among the safest and the most effective broad spectrum bactericidal antimicrobial agents available to the clinician, and have therefore become the most widely prescribed of all antibiotics [2]. Cefetamet pivoxil hydrochloride (Fig. 1) is an orally absorbed prodrug ester of the microbiologically active cephalosporin, cefetamet

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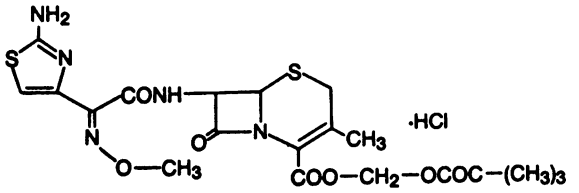

Fig. 1. Chemical structure of cefetamet pivoxil hydrochloride.

[3]. Cefetamet is classified as a third generation cephalosporin which has excellent in vitro activity against a wide range of Gram-negative and Gram-positive community acquired pathogens, and possess a very high intrinsic stability to the hydrolytic action of the most common β-lactamases and penicilinases [3,4]. Cefetamet pivoxil hydrochloride is available in tablet and powder for oral suspension dosage forms. Chemically it is [6R-[(6α,7β(Z)]] - 7 - [[(2 - Amino - 4 - thiazol)(methoxy-imino)acetyl]amino]-3methyl-8-oxo-5-thia-1-aza-byciclo [4.2.0] oct-2-ene-2carboxilic acid [5] and is not yet official in any pharmacopeia. Many methods have been reported in the literature for the determination of cefetamet in biological fluids [6] plasma [7] and tablets [8] using high-performance liquid chromatography; in drug substance using potentiometry, spectrofotometry [9] and polarographic methods [10]. However, there is no method reported for determination of cefetamet pivoxil hydrochloride in powder for oral suspension formulations. HPLC methods are very useful in the determination of drugs. During the last few years, many HPLC methods, using various stationary phases, mobile phases and sample preparation procedures, have been described for the determination of drugs in pharmaceutical preparations. We believe that the availability of this new method, with increased sensitivity, selectivity, will be very useful for the determination of cefetamet pivoxil hydrochloride in pharmaceutical preparations. The aim of this study was to develop and validate a specific, accurate, precise and reproducible quality control method of cefetamet pivoxil hydrochloride in drug substance and powder for oral suspension.

2. Experimental

2.1. Samples

The cefetamet pivoxil hydrochloride reference substance (assigned purity 99.57%) was generously supplied by Roche Laboratories, São Paulo, Brazil. It was tested for purity by controlling its melting point, UV and NMR spectrum. No impurities were found. The powder for oral suspension was obtained commercially and claimed to contain 3000 mg of cefetamet pivoxil hydrochloride.

2.2. Reagents and solvents

All chemicals used were of pharmaceutical or special analytical grade. Methanol and acetonitrile were HPLC grade (Merck, Darmstadt, Germany). Analytical reagent grade monobasic potassium phosphate, monobasic sodium phosphate and ethanol were purchased from Merck, Rio de Janeiro, Brazil. Orthophosphoric acid was purchased from Merck, Darmstadt, Germany. Water HPLC grade obtained from Milli-Q RO system, was used.

2.3. Apparatus and chromatographic conditions

A Shimadzu® HPLC SCL–10A–VP (Shimadzu Corporation, Kyoto, Japan) system was used for the analysis. The method was carried out on a Shim-pack® C_{18} (150×4.6 mm i.d., 5 μm particle size) column as a stationary phase and a mobile phase composed of water-acetonitrile–methanol–phosphate buffer (containing 5 mg ml^{-1} NaH_2PO_4 and 35 mg ml^{-1} KH_2PO_4) (50:35:10:5, v/v) and pH adjusted to 3.5 using orthophosphoric acid. The flow rate used was 1.5 ml min^{-1}. A Rheodyne injector with a 20 μl loop was used for the injection of samples. The SPD 10A-VP, variable-wavelength UV–Vis detector was used to detect the drug at 254 nm and CLASS-VP data station was used to process the chromatograms. The sensitivity was 1.0 AUFS. The HPLC system was operated isocratically at room temperature (20 ± 1 °C). The mobile phase was filtered through a 0.45 μ membrane filter and degassed with a helium sparge for 15 min.

2.4. Procedure

2.4.1. Cefetamet pivoxil hydrochloride reference standard

Stock solutions of cefetamet pivoxil hydrochloride were prepared at a concentration of 100 μg ml^{-1} in the mobile phase and kept at 4 °C. Stability of cefetamet pivoxil hydrochloride stock solutions were tested during 7 days. Working standard solutions were daily prepared by diluting stock solutions at the final concentration of 60 μg ml^{-1} in the mobile phase.

2.4.2. Assay of cefetamet pivoxil hydrochloride

The powder of cefetamet pivoxil hydrochloride for oral suspension was diluted with a mixture of ethanol–methanol–water (45:40:15, v/v) and transferred to 500 ml volumetric flask, followed by adding 400 ml of the same mixture of solvents. Then this solution was heated in a double-boiler at 35 °C temperature for 30 min. After cooling to room temperature and sonicated for 10 min, the volume was completed with ethanol:methanol:water (45:40:15, v/v). This sample solution had a final concentration of 6 mg ml^{-1} (Solution A). Aliquots (10 ml) of the solution A were centrifuged at 6000 rpm for 15 min; 5 ml of the supernatant was transferred to 50 ml volumetric flask, followed by adding the mobile phase to complete the volume (Solution B). Aliquots (5 ml) of the solution B were transferred to 50 ml volumetric flasks and diluted to volume with the mobile phase, to provide a final solution containing 60 μg ml^{-1}. All determinations were conducted in triplicate.

2.4.3. Calculations

With the above chromatographic conditions, the standard solution and sample solution were injected and the chromatograms were recorded (Fig. 2). The concentrations of the drugs were calculated using the following formula:

$$\text{concentrations of drugs} = \frac{\text{response factor of the sample}}{\text{response factor of the standard}} \times \text{concentration of standard}$$

Having been established the quantitative relationships between the parameters studied, and knowing the predictive performance of their association model, a simple linear regression analysis by the least squares method was applied.

3. Validation of the method

The evaluation of intra- inter-day precision, linearity and range, specificity, accuracy, ruggedness, limit of detection and quantification validated the method.

3.1. Linearity and range

Linearity was evaluated by preparing six standard concentrations of cefetamet pivoxil hydrochloride in the range of 30.0–80.0 μg ml^{-1}.

Triplicate 20 μl injections were made for standard solution to verify the reproducibility of the detector response at each concentration level. The linearity of the calibration curve was then determined for intra- and inter-day experiments on three different days. The peak area of cefetamet pivoxil hydrochloride was plotted versus the concentration to obtain the calibration graph. The six concentration of the standard solution were subjected to regression analysis to calculate calibration equation and correlation coefficients.

3.2. Precision

The method precision was demonstrated by repeatability studies. It was performed by assaying nine samples of powder for oral suspension, at same concentration, during the same day and under the same experimental conditions. The response factor of the drug peaks, mean and percentage RSD of the response factor of the peaks were calculated. These studies were also repeated on different days to determine inter-day precision.

3.3. Accuracy

To confirm the accuracy of the proposed method, the recovery test was performed, following the ICH guideline recommendations [11]. It

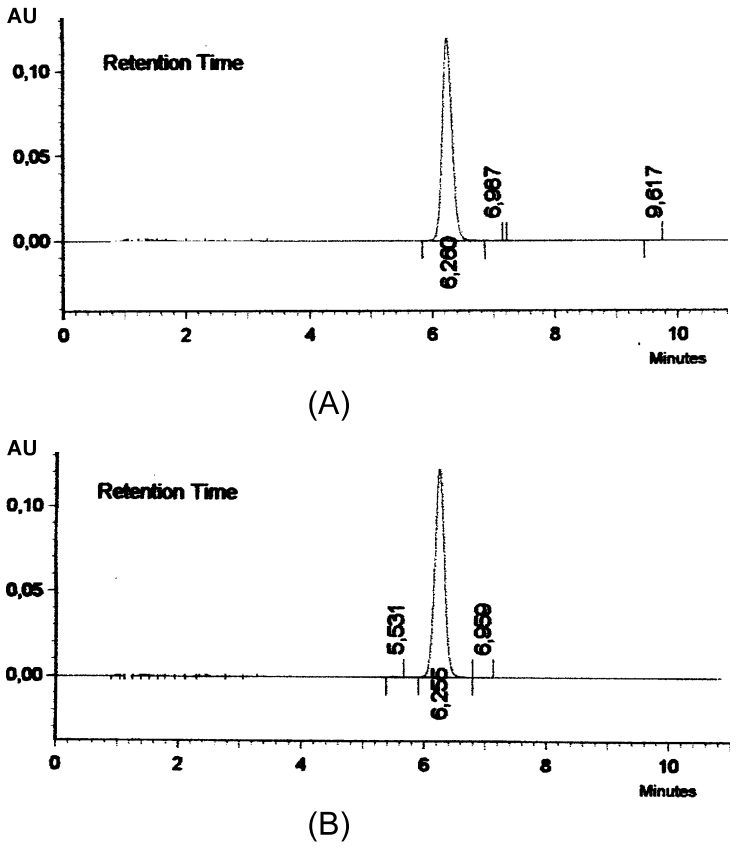

Fig. 2. Chromatograms of cefetamet pivoxil hydrochloride, at 60 μg ml^{-1}: (A) reference substance and (B) powder for oral suspension. Chromatographic conditions: column: Shim-pack® C_{18} (150 mm x 4.6 mm i.d.; 5 μm particle size); mobile phase: water-acetonitrile-methanol-phosphate buffer (pH adjusted to 3.5 using orthophosphoric acid) (50:35:10:5, v/v); flow rate: 1.5 ml min^{-1}; detection wavelength 254 nm (1.0 a.u.f.s.); retention time: 6.2 min.

was determined by adding known amounts of the cefetamet pivoxil hydrochloride drug substance (0.05, 0.10 and 0.15 mg ml^{-1}) from the solution B of the process.

These solutions were further diluted using the sample preparation procedure. The resulting solutions (65.0, 70.0 and 75.0 μg ml^{-1}) were analyzed and the recovered percentages were calculated.

3.4. Ruggedness

The ruggedness of the method was carrying out by changing the experimental conditions such as, changing the source of reagents and solvents (different manufacturers), changing to another stationary phase of similar type (Shim-pack C_{18}, Nova-pack C_{18}) and changing the flow rate to 1.0 ml min^{-1} and the chromatographic patterns was studied. It was also established through separation studies on the mixture of reaction solutions by different persons on the same chromatographic system.

The analytes, mobile phase, standard and sample solutions were subjected to long term (7 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for the change in the chromatographic pattern compared with freshly prepared solutions.

3.5. Specificity

The specificity test involved forced degradation of cefetamet pivoxil hydrochloride, to determine whether the degradation products are resolved from the cefetamet pivoxil hydrochloride peak. This was done by submitting cefetamet pivoxil hydrochloride drug substance to stress conditions that would cause up to 5% degradation products that would resemble those seen by the drug substance under longer term room temperature storage.

3.5.1. Degradation in acidic medium

Cefetamet pivoxil hydrochloride drug substance (20 mg) was transferred into 100 ml volumetric flask containing 0.1N hydrochloric acid. This solution were refluxed for 4h and diluted to nominal assay concentration with mobile phase.

3.5.2. Peroxide degradation

Cefetamet pivoxil hydrochloride drug substance (20 mg) was dissolved in 50 ml of 3% hydrogen peroxide and stored at room temperature. This solution was sampled after 4 h and diluted to nominal assay concentration with mobile phase.

3.6. Limit of detection/quantification

The limits of detection (LOD) and quantification (LOQ) were obtained by use of the slope and the standard deviation of the intercept from four calibrations graphs determined by linear regression line as defined by ICH [11]. The calibration graphs were constructed in the range of 30.0–80.0 μg ml^{-1}.

4. Results and discussion

Reversed phase HPLC is the separation method of choice for most pharmaceutical compounds, both hydrophilic and hydrophobic, due to the stable reproducible nature of the HPLC columns, the largely aqueous composition of the mobile phase, and the relative ease of reproducing the methods in a variety of laboratories.

In this study, the chromatographic conditions were influenced by the physico–chemical properties of cefetamt pivoxil hydrochloride, such as solubility, polarity and UV absorption.

The chromatographic conditions were used to separate the cefetamet pivoxil hydrochloride and its metabolites in a complex media. The chromatogram of the sample matched with the corresponding chromatogram of the standard drug, showing that the analyte was pure and also formulation excipients and impurities did not interfere with the analyte peak. The retention time repeatability during the precision studies was found to be excellent for all the solutions. The retention time (R_t) of cefetamet pivoxil hydrochloride was 6.2 min that provides adequate run time analysis. The HPLC chromatogram shows R_t (Fig. 2).

The calibration curves for cefetamet pivoxil hydrochloride were constructed by plotting the peak areas versus concentration. It was found to be linear in the 30.0–80.0 μg ml^{-1} range with a correlation coefficient of 0.99989; the representative linear regression equation $y = 7425.69 + 21358.11 \times (n = 6,\ r^2 = 0.9997)$.

The relative standard deviation (R.S.D.) of the intercept and the slope of the curves were 1.79 and 1.52%, respectively. Analysis of variance of the date indicate no significant differences in slopes of the three calibration curves ($P < 0.01$).

The intra- and inter-day precision were determined in the range of 30.0–80.0 μg ml^{-1} of standard solutions and the percentage RSD values were found to be between 0.03 and 0.51% and 0.36 and 1.76%, respectively.

The repeatability of the method was studied by assaying nine samples of powder for oral suspension, at the same concentration, during the same day under the same experimental conditions. The relative standard deviation was 0.33%. The results of cefetamet pivoxil hydrochloride determination in the pharmaceutical formulation are shown in Table 1.

The accuracy of the method was determined by recovery studies. These were carried out and the recovery percentage was calculated (Table 2). From the obtained data, recoveries of the standard drug were accurate. The mean absolute recovery was found to be 100.09%.

Table 1
Data obtained from pharmaceutical formulation analysis by HPLC

Theoretical amount (mg ml^{-1})	Experimental amount[a] (mg ml^{-})	Purity (%)	R.S.D. (%)
250	247.03	98.81	0.13
250	246.85	98.74	0.34
250	246.50	98.60	0.53

[a] Mean of three determination.

Table 2
Experimental values obtained in the recovery test for cefetamet pivoxil hydrochloride in powder for oral suspension by HPLC

	Amount of standard (μg ml^{-1})		Recovery[a] (%)	R.S.D. (%)
	Added	Recovered		
R1	5	5	100.00	0.72
R2	10	9.97	99.73	1.12
R3	15	15.08	100.55	0.29

[a] Mean of three replicate analysis.

The ruggedness of the method was studied and it was observed that there were no significant changes in the chromatographic pattern when slight changes were made in experimental conditions demonstrating that the method is rugged.

The stability of the standard and sample solutions was evaluated by analyzing these solutions after aging at room temperature and storage at 4 °C, comparing with freshly prepared standards. The results demonstrated that the working standard solutions, as well as the sample solutions are stable for at least 7 days, when stored at 4 °C.

Forced degradation studies were performed to evaluate the specificity and the percentage of the cefetamet pivoxil hydrochloride recovered in each case. The purpose of the specificity test was to determine whether new degradation products were produced from the forced degradation of cefetamet pivoxil hydrochloride drug substance, whether these degradation products are resolved from the cefetamet pivoxil hydrochloride chromatographic peak. Degradation of 57.8% was observed in cefetamet pivoxil hydrochloride drug substance when refluxed in 0.1 N hydrochloric acid for 4 h. The degradants products were observed at relative retention time 1.32 min and 1.80 min. The drug was also found to degrade in 3% hydrogen peroxide at room temperature. It was decomposed to an extent of 14.6% in 2 h. The retention time of the degradants products was 1.12 and 3.35 min. It is evident that the method was able to separate the peaks due to the degraded products from that of cefetamet pivoxil hydrochloride. It is of the rare studies where forced decomposition studies were done under al different suggested conditions and the products were resolved in a single isocratic run.

The LOD and LOQ were calculated to be 1.03 and 3.15 μg ml^{-1}, respectively.

5. Conclusion

HPLC methods are commonly used for determining drugs in final pharmaceutical products. The developed method is suitable for the identification and quantification of the cefetamet pivoxil hydrochloride as a drug substance and in powder for oral suspension.

In conclusion, the developed HPLC method has been successfully used on a routine basis and allows the quantification of the drug in pharma-

ceutical formulations in a short analytical time. This method is sensitive, simple, uses a fast, ease extraction procedure and possesses excellent linearity and precision characteristics. These observations made it possible to anticipate the use of this method as an official procedure.

The data validation also shows that the proposed method is selective, precise, accurate and adequate to determine the content of cefetamt pivoxil hydrochloride as pure drug substance and in formulated products.

This method has been applied in the analysis of tablets dosage form.

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