



DEVELOPMENT AND VALIDATION OF A HPTLC METHOD FOR THE ESTIMATION OF CEFETAMET

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ABSTRACT

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of cefetamet in bulk and pharmaceutical dosage forms. The stationary phase used was precoated silica gel 60F₂₅₄. The mobile phase used was a mixture of chloroform: methanol: toluene (6:1:3v/v/v). The detection of spots was carried out at 236nm. The method was validated in terms of specificity, accuracy, linearity and precision. The calibration curve was found to be linear between 1-5µg/spot. The proposed method can be successfully used to determine the drug in bulk and marketed formulations.

Keywords: Cefetamet, Validation, Precision.

INTRODUCTION

Cefetamet is a third generation cephalosporin antibiotic. It is given orally as cefetamet pivoxil hydrochloride. Cefetamet was effective in the treatment of otitis media, pneumonia, uncomplicated gonorrhoea, pharyngotonsillitis, urinary tract, upper and lower respiratory tract infections¹⁻². Cefetamet is chemically 7-[2-(2-aminothiazol-4-yl)-2-methoxyimino acetamido]-3-methyl 3-cephem-4-carboxylic acid. It is not official in any pharmacopoeia. Literature survey reveals that few analytical methods were found for the quantitative estimation of cefetamet in bulk drug and pharmaceutical dosage forms³⁻¹⁰. So far no HPTLC method has been reported for the estimation of cefetamet in bulk and pharmaceutical dosage forms. In present investigation an attempt has been made to develop accurate and precise HPTLC method for the estimation of cefetamet in bulk and pharmaceutical dosage forms.

EXPERIMENTAL

Materials and Method

Cefetamet working standard drug was produced as a gift sample from Alembic Limited, Ahmedabad. Slica gel 60F₂₅₄ TLC plates (10x10cm, layer thickness 0.2mm, E.Merck, Mumbai) were used as a stationary phase. All chemicals and reagents used were of analytical grade. A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe (100µl), Camag TLC Scanner 3, Camag WinCATS software, Camag Twin-trough chamber (10x10cm) and ultra sonicator were used in the study.

Preparation of Standard and Sample Solutions

Working standard of Cefetamet (100mg) was weighed accurately and diluted with methanol to obtain the final concentration of 100µg/ml. 20 tablets were weighed and ground to fine powder. An accurately weighed quantity of powdered sample, equivalent to 100mg of cefetamet pivoxil hydrochloride was

transferred to a conical flask and dissolved in methanol. The solution was sonicated for 20 minutes. Then it was filtered through Whatmann filter paper No.42 into a calibrated 100ml volumetric flask and required dilutions were made to obtain a final concentration of 100µg/ml.

HPTLC Method and Chromatographic Conditions

TLC plates were prewashed with methanol. Activation of plates was done in an oven at 50⁰C for 5-10min. The chromatographic conditions maintained were precoated silica gel 60F₂₅₄ aluminum sheets (10x10cm) as stationary phase, chloroform: methanol: toluene (6:1:3v/v/v) as mobile phase, chamber and plate saturation time of 35min, migration distance allowed was 72mm, wave length scanning was done at 236nm keeping the slit dimension at 5x0.45mm. A deuterium lamp provided the source of radiation. Ten microliters of standard solution of cefetamet was spotted and developed at constant temperature. Wavelength was selected by scanning standard solution over 200-400nm wavelengths. Cefetamet show maximum absorbance at 236nm in reflectance mode with Camag TLC Scanner 3 using WinCATS software.

Calibration Curve

Aliquot of 10, 20, 30, 40, 50 µl of standard solution of cefetamet (100µg/ml) was applied on the TLC plate. TLC plate was dried, developed and analyzed photometrically as described earlier. The standard calibration curve was plotted using regression analysis with Microsoft excel.

Validation of the Method¹¹

The developed method was validated in terms of linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application.

Analysis of the marketed formulations

Ten µl of sample solution of the marketed formulation was spotted on to the plate followed by development scanning. The analysis was repeated in triplicate. The content of the drug was calculated from the peak area recorded.

RESULTS AND DISCUSSION

Different solvent systems were tried to get dense and compact spots with significant R_f value for the quantification of cefetamet in pharmaceutical formulations. The mobile phase consisting of chloroform: methanol: toluene (6:1:3v/v/v) gave R_f values of 0.35 ± 0.05 (Fig. 1). The linear regression data (n = 5, Table-1) showed a good linear relationship over a concentration range of 1-5µg/spot. The limit of detection and limit of quantification was found to be 2µg/spot and 8µg/spot.

The intra-day precision was determined by analyzing standard solutions in the concentration range of 2µg/spot to 4 µg/spot for 6 times on the same day while inter-day precision was determined by analyzing corresponding standards daily for 3 days over a period of one week. Repeatability of sample application was assessed by spotting 10µl of drug solution 6 times on a TLC plate followed by development of plate and recording the peak area for all spots. The % RSD for peak area value was found to be 0.53. Repeatability of measurement of peak area was determined by spotting 10µl on a TLC plate and developing the plate. The separated spot was scanned 6 times without changing the position of the plate and % RSD for measurement of peak area was found to be 0.19. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere.

Recovery studies of the drug (Table-2) were carried out for the accuracy parameter. The assay value for the marketed formulation was found to be within limits. The low RSD value indicated the suitability of the method for routine analysis of cefetamet in pharmaceutical dosage forms.

CONCLUSION

The developed HPTLC method for the determination of cefetamet is simple, precise, specific, accurate, selective, sensitive, and reproducible. The amounts found in formulations are well agreed with label claim. The proposed method can be applied to routine analysis in quality control laboratories.

ACKNOWLEDGEMENTS

The authors are grateful to Laila Impex, Vijayawada for providing the research facilities. The authors wish to express their gratitude to Alembic Limited, Ahmedabad for providing the gift sample of cefetamet.

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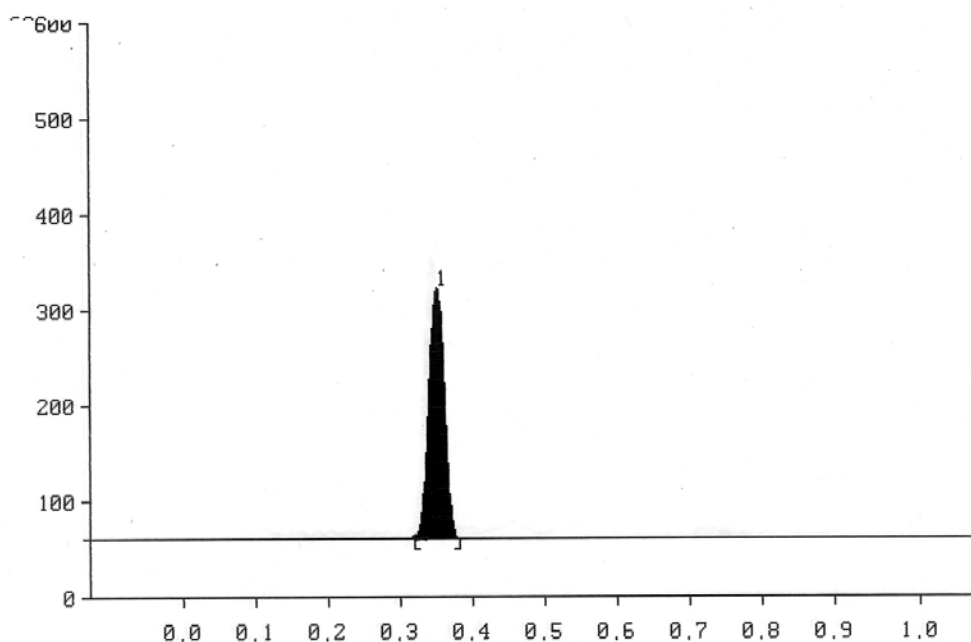


Fig.-1: Typical Chromatogram of Cefetamet

Table-1: Method Validation Parameters

Parameters	Values
Linearity range ($\mu\text{g}/\text{spot}$)	1-5
Correlation coefficient (r)	0.9996
Regression equation ($y = a+bx$)	
Slope (b)	2627.0
Intercept (a)	-51.8
Limit of detection (LOD)	$0.2\mu\text{g}/\text{spot}$
Limit of quantification (LOQ)	$0.8\mu\text{g}/\text{spot}$
Precision (% CV)	
Repeatability of application (n= 6)	0.53
Repeatability of measurement (n= 6)	0.19

Table-2: Recovery of Cefetamet using the proposed method

Dosage form	Labeled amount (mg/tablet)	Amount added (mg)	Amount obtained by Proposed method \pm SD	Percentage recovery* \pm SD
Brand-1	250	100	98.04 ± 0.98	99.72 ± 1.02
Brand-2	250	100	100.12 ± 0.66	101.49 ± 1.46

*Each value is a mean \pm standard deviation of three determinations.

(Received: 4 September 2009

Accepted: 13 September 2009

RJC-445)

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