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Research paper

Development and validation of HPTLC method for the estimation of cefadroxil in bulk and tablet dosage form

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Abstract

A novel and simple HPTLC method has been developed for the estimation of Cefadroxil (CFD) in bulk and pharmaceutical dosage form on silica gel precoated aluminum 60F254 plates, (10 cm x10 cm) with 0.25 mm thickness. The separation was carried out using ethyl acetate: chloroform (8:2v/v) as mobile phase. The Chromatographic scanning was carried out at 254 nm. The Rf (± SD) values were found to be 0.38 ± 0.05 for CFD. The linearity for analyte was in the range 100-500 ng/band for CFD with correlation coefficients, r2 = 0.9996. The percentage recovery obtained for CFD was in the range of 99.28-100.13% respectively. The proposed method was optimized and validated as per the ICH guidelines17.

Keywords: *Cefadroxil; HPTLC; Method Validation; Tablet Dosage Form.*

1. Introduction

Cefadroxil is a semi-synthetic first generation oral cephalosporin drug effective against gram-positive and gram-negative bacterial infections and similar to cephalexin and cephradine in structure and spectrum of antibacterial activity[1]. It is used in the treatment of mild to moderate infections of the respiratory and urinary tracts, skin and soft tissue infections. It has been used in the prophylaxis of recurrent urinary tract infections in children. The chemical name of Cefadroxil is (6R, 7R)-7-[(2R)-2-(4-hydrophenyl) acetamido]-3-methyl-8-OXO-5-thia-1- azabicycio [4.2.0] oct-2-ene-2-carboxylic acid hydrate. It has a molecular formula of $C_{16}H_{17}N_3O_5S$ and molecular weight of 363.38g/mol. Cefadroxil is freely soluble in water and methanol[2].

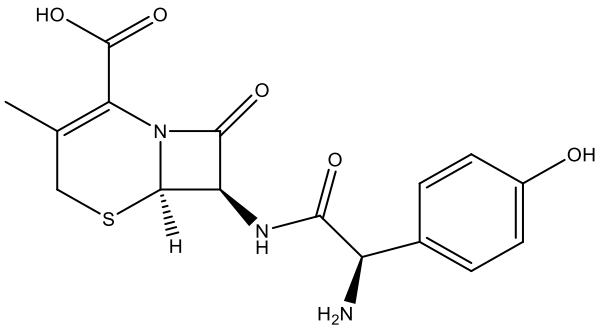

Fig. 1: Chemical Structure of Cefadroxil.

Literature survey reveals that there were some analytical methods have been reported for the estimation of Cefadroxil in pure drug and pharmaceutical dosage forms by using UV[3-8] spectrophotometric, HPLC[9-14] and HPTLC[15-16] so far.
The aim of present work is to develop and validate a novel, simple, accurate and specific HPTLC method for estimation of Cefadroxil in bulk and tablet dosage form.

2. Materials and methods

2.1. Chemicals and reagents

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- Cefadroxil pure form was obtained as a gifted sample from Microlabs pharma industry located at Bommasandra, jigani link road, Bangalore and its pharmaceutical dosage form Cefadrox 10 Tablets labelled claim 500mg. Manufactured by Aristo pharmaceuticals Pvt ltd, Baddi at Mumbai. Batch no: B712K117 procure from local pharmacy Mandya.
- Ethyl acetate and chloroform are of analytical grade were available in the laboratory of Bharathi College of pharmacy, bharathi-nagara.
- Silica gel 60 F_{254} 20×10 cm, precoated plates, layer thickness 0.25 mm sup- plied by Anchrom Technologists, Mumbai (Merck, Darmstadt, Germany).

2.2. Instrumentation and chromatographic conditions

The standard solution ranging from 100-500ng/band for CFD was applied as bands using a Camag 100 µl sample syringe (Hamilton, Bonaduz, Switzerland) with an automatic TLC Sampler Linomat V (Camag, Muttenz, Switzerland). (Number of tracks 5, track distance from the left plate edges 15 mm, distance from plate bottom 10 mm, band length 6 mm, distance between bands 4.5 mm.).Before analysis the layers were pre- developed with methanol to clean them and activated at 110^0C for 5 min. Analysis was performed on silica gel precoated aluminum plate 60 F_{254}, 10 × 10 cm, 0.25 mm layer thickness. The plates were developed in a twin trough glass chamber with saturation of 30 min with the mobile phase. The mobile phase consists of ethyl acetate: chloroform (8:2v/v). After development, plates were dried and were observed under Camag TLC Visualizer. Chromatographic scanning was per- formed using a Camag TLC scanner III in the reflectance absorbance mode at 254 nm for all measurements and operated by the WINCAT'S software version 2.0. The source of radiation utilized was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression.

2.3 Preparation of Standard Stock Solution and Working Standard Solution

The standard stock solution of CFD containing 1000µg/ml was prepared by dissolving accurately weighed quantity of 50mg of Cefadroxil into a 50ml volumetric flask and dissolved in a minimum volume of methanol and diluted to the required volume with methanol. The working standard solution was prepared in the concentration range 100-500 ng/band for CFD by appropriate dilutions of the above standard stock solution with methanol.

3. Results and discussion

3.1. Optimization of solvent system and chromatographic conditions

Chromatographic separation was carried out on the stock solution of CFD. Initially the plates were spotted with 10 µl of stock solution and developed by linear ascending development method using neat solvents like toluene, hexane, methanol, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, etc. with or without chamber saturation. Based on the results of these initial chromatograms, binary and ternary mixtures of solvents were tried to achieve optimum peak parameter. The final mobile phase consisting of Ethyl acetate: chloroform (8:2v/v).was optimized since good R_f value of 0.38 for CFD was obtained as shown in Fig. 1. The samples were applied in form of bands on precoated aluminum sheets of silica gel 60 F_{254}. Linear ascending development was carried out in a twin trough glass chamber (20 cm ×10 cm, 10 × 10 cm), without saturation. The length of chromatogram run was 85 mm. The developed plates were dried in the current of dry air. Chromatographic scanning was performed in the absorbance mode at 254 nm.

3.2. Method validation

As per the ICH guidelines[17], the method validation parameters checked were linearity, accuracy and precision, limit of detection (LOD), limit of quantitation (LOQ), robustness and specificity.

3.3. Calibration curve

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 100-500 ng/band. Linear regression equation was found to be Y=6.5528X + 283.94 and R^2=0.9996. Data of linearity study are listed in Table 1.

Table 1: Linearity Study of Cefadroxil

SL No	Concentration ng/band	Peak Area	Statistical analysis
1	100	933.8	
2	200	1590.5	Slope=6.5528
3	300	2280.7	Intercept=283.94
4	400	2876.9	Correlation coefficient=0.9996
5	500	3567	

*Mean of six estimations.

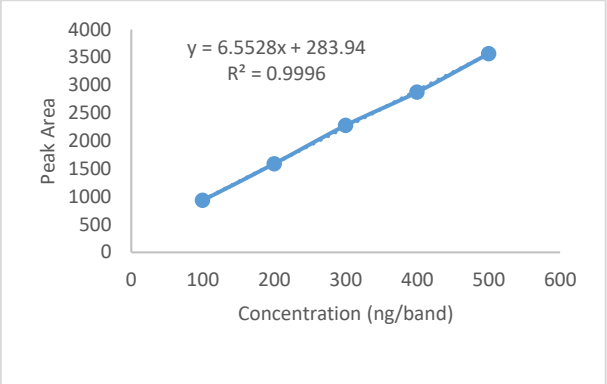

Fig. 2: Calibration Curve of Cefadroxil.

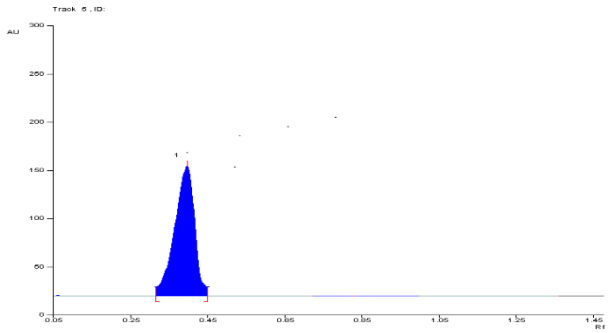

Fig. 3: HPTLC Chromatogram of Cefadroxil.

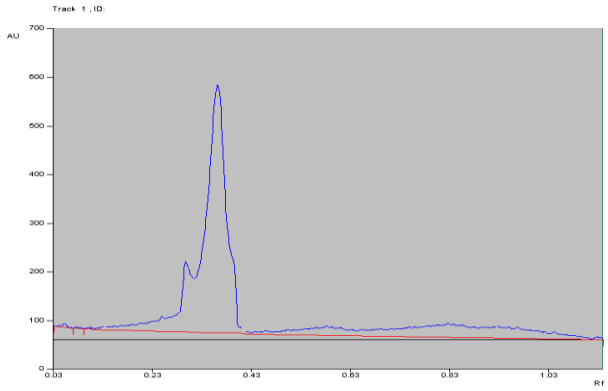

Fig. 4: Overlain Spectrum of Cefadroxil.

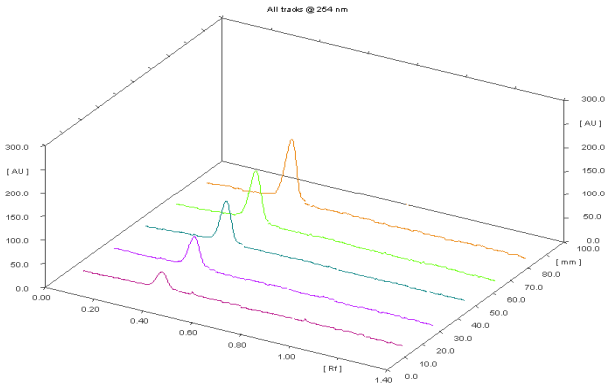

Fig. 5: 3D Spectra of All 5 Tracks.

3.4. Precision

The precision of the method is determined by intra-day and inter-day variation studies by using three concentrations over the range of the method. In the intra-day studies, the method was repeated 3 times a day for standard and sample solutions and % RSD was calculated. For inter day variation studies, 3 repeated measurements of standard and sample solutions were made on 3 consecutive days and % RSD was calculated. Results are listed in Table 2.

Table 2: Precision Study of Cefadroxil

SL No	Concentration ng/band	INTRADAY		INTERDAY	
		Mean of area	%RSD	Mean of area	%RSD
1	100	923.2	0.190	932.2	0.303
2	200	1587.9	0.127	1579.8	0.204
3	300	2275.7	0.086	2289.7	0.109
4	400	2887.4	0.076	2879.1	0.068
5	500	3575.2	0.080	3568.9	0.064

*Mean of three estimations at each level.

3.5. Method sensitivity (LOD and LOQ)

LOD and LOQ were calculated using S.D (standard deviation of the response) and b (slope of the calibration curve) using the relationship LOD = (3.3 x s.d)/ b and LOQ = (10 x s.d)/b. The LOD and LOQ were found to be 7.52 and 22.58 ng/spot, respectively. This indicates the sensitivity of the method.

3.6. Specificity

The method is found to be specific since no interfering spots were seen in Chromatogram of placebo and tablet triturate and R_f values of standard and sample were compared. Peak purity for both the analyte was assessed by comparing spectra at three different regions of the spot i.e. acquired at the peak start(S), peak apex(M) and end of the peak(E) obtained from the band (Table 3). As the correlation values are greater than 0.999 which indicates that the peaks are pure.

3.7. Ruggedness

When the method is performed under the variant conditions. This includes different analysts, laboratories, instruments, temperature etc. Ruggedness was determined between two different analysts. It was found to be rugged (Table 3).

Table 3: Summary of Validation Parameters of Cefadroxil

Parameters	Cefadroxil
Detection Wavelength (nm)	254
Beer's Law Limit (ng/band)	100-500
Regression Equation(y=mx+c)	Y=6.5528x +283.94
Slope(m)	6.5528
Intercept(c)	283.94
Correlation coefficient (r^2)	0.9996
Limit of detection (ng/spot)	7.52
Limit of quantitation (ng/spot)	22.58
Ruggedness	0.154
Robustness	Robust
Specificity	Specific

3.8. Accuracy

Accuracy of the analytical method is the closeness of test results obtained by the method to the true value. To assess the accuracy of the proposed method, recovery studies were carried out at 3 different levels i.e. 50%, 100% and 150%. The formulation concentration was kept constant and varied pure drug concentration. Accuracy results were shown in table.4.

Table 4: Accuracy Study of Cefadroxil

Drug/Label claim	Initial amount of drug (ng/band)	Amount of Standard drug (ng/band)	Drug Recovered	%RSD
Cefadroxil 500mg	300	150	100.13	0.733
		300	99.28	0.134
		450	99.81	0.077

*Mean of three estimations at each level.

- Robustness

Robustness study is done by changing the volume (± 2 ml) and composition (± 5%) of mobile phase and chromatograms were run. Robustness of the method was studied at 300 ng/band of CFD, respectively. During robustness study R_f values, standard deviation of the peak areas and% RSD was calculated for each parameter. % RSD values were found to be less than 2% which indicates robustness of the method (Table 5).

Table 5: Robustness of the Method of Cefadroxil

Parameters	%RSD
Mobile phase volume (± 2ml)	0.92
Development distance (±5cm)	0.75
Duration of saturation (±2)	0.78
Time from spotting to chromatography (±10 min)	0.39
Time from chromatography to scanning (±10 min)	0.81

*Mean of three estimations at each level.

- Analysis of Tablet Formulations

Twenty tablets were weighed accurately and a quantity of tablet triturate equivalent to 50 mg of CFD was weighed and dissolved in the 40 ml of methanol with the aid of ultra-sonication for 10 min and solution was filtered through Whatman filter paper No. 41 into a 50 ml volumetric flask. Filter paper was washed with the methanol, adding washings to the volumetric flask and volume was made up to mark. The solution was suitably diluted with methanol to get of 300 µg/ml of CFD was used for calibration curve. The amount of drug present per

tablet was estimated from the respective calibration curves. The method was evaluated in terms of assay of commercially available tablets containing CFD. Three replicate determinations were performed on the accurately weighed amounts of tablet triturate. After carrying assay, percentage found was 99.39 for CFD. The %RSD was found to be 0.95 for CFD

4. Conclusion

The present analytical method was validated as per the ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, specific, economical and sensitive and can be used for the routine analysis of Cefadroxil in bulk drug and its pharmaceutical dosage forms.

Acknowledgement

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