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Development and Validation of a HPTLC Method for the Simultaneous Estimation of Cefuroxime Axetil and Probenecid

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A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the determination of cefuroxime axetil and probenecid simultaneously in combined dosage form. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used was a mixture of chloroform: acetonitrile: toluene: acetate buffer of pH 6 (5:4:1:0.3 v/v). Detection of spots was carried out at 266 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The limit of detection and the limit of quantification for the drug combination were found to be 50 ng/spot and 100 ng/spot, respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

The combination of cefuroxime axetil and probenecid has recently been introduced in the market. Cefuroxime is a second-generation cephalosporin and probenecid is uricosuric agent[1,2]. This combination is based on the mechanism of inhibition of tubular secretion, of cefuroxime by probenecid and thus getting an increase in plasma concentration of cefuroxime by 2 to 4 times. Cefuroxime axetil is an ester prodrug of cefuroxime, which is rendered more lipophilic by esterification of carboxyl group of the molecule by the racemic 1-acetoxyethyl bromide, thus enhancing absorption. The absorbed ester is hydrolyzed in the intestinal mucosa and in portal circulation. Products of the de-esterification are active cefuroxime, acetaldehyde and acetic acid. Cefuroxime is chemically (1RS)-1-[(acetyl) oxy] ethyl- (6R,7R)-3-[(carbamoyloxy) methyl]-7-[(Z-2-furan-2yl)-2-(methoxy imino) acetyl) amino]-8-oxo-5-thia–1-aza bicyclo-(4,2,0)-oct–2-ene-2-carboxylate and probenecid is 4-(dipropyl sulphamoyl) benzoic acid. No analytical method has so far been reported for the simultaneous determination of cefuroxime and probenecid in pharmaceutical dosage forms.

Over the past decade HPTLC has been successfully used in the analysis of pharmaceuticals, plant constituents, and biomacromolecules. Several samples can be run simultaneously using a small quantity of mobile phase, thus lowering analysis time and cost per analysis. It also facilitates automatic application and scanning *in situ*. The objective of the present work was to develop an accurate, specific and reproducible method for the simultaneous determination of cefuroxime and probenecid in pharmaceutical formulations.

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MATERIALS AND METHODS

Cefuroxime axetil and probenecid working standards were procured as samples from Glenmark Pharmaceuticals Ltd., Nashik. Silica gel $60F_{254}$ TLC plates (E. Merck, Mumbai) were used as stationary phase. Chloroform:acetonitrile: toluene:acetate buffer of pH 6 (5:4:1:0.3 v/v) was used as mobile phase. Methanol was used as solvent. Capsules containing 250 mg cefuroxime axetil and 250 mg probenecid were purchased from local market (Altacef-LA, Glenmark). A Camag HPTLC system comprising of Camag linomat IV semiautomatic sample applicator, Hamilton syringe, Camag TLC scanner, Camag CATS IV software, Camag twin trough chamber and a sonicator were used during study.

Preparation of standard and sample drug solutions:

Working standards of cefuroxime axetil and probenecid (10 mg each) were weighed accurately and diluted with methanol to obtain the final concentration of 100 µg/ml of each drug. The contents of twenty capsules were ground to a fine powder. Weight equivalent to 100 mg each of cefuroxime axetil and probenecid were transferred to a conical flask and dissolved in methanol. The solution was sonicated for 15 min. The extracts were filtered through Whatmann filter paper No. 41 and residue was washed with methanol. The extracts and washings were pooled and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol. Required dilutions were made to get 100 µg/ml each of cefuroxime and probenecid.

HPTLC method and chromatographic conditions:

TLC plates were prewashed with methanol. Activation of plates was done in an oven at 50° for 5 min. The chromatographic conditions maintained were precoated silica gel $60F_{254}$ aluminum sheets as stationary phase, chloroform: acetonitrile:toluene:acetate buffer of pH 6 (5:4:1:0.3 v/v) as mobile phase, chamber and plate saturation time of 30 min, migration distance allowed was 75 mm, wavelength scanning was done at 266 nm keeping the slit dimension at 4x0.45 mm. A Deuterium lamp provided the source of radiation. Ten microlitres of standard solution (100 µg/ml of each drug) was applied and developed at a constant temperature. Photometric measurements were performed at 266 nm (as shown in fig. 1) in reflectance mode with a Camag TLC scanner 3, using CATS IV software.

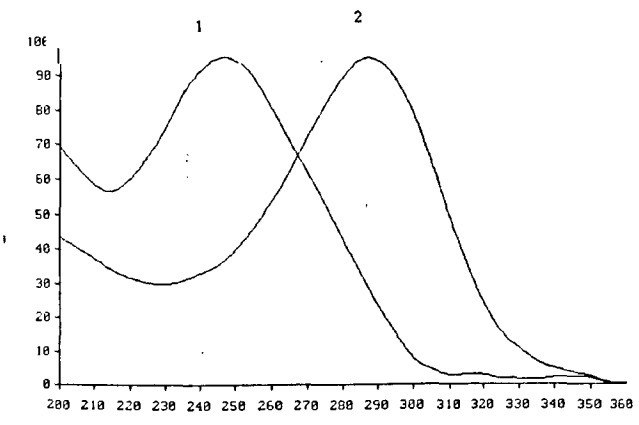

Fig. 1: Spectrum of probenecid and cefuroxime axetil. Peak 1 and 2 are spectrum of probenecid and cefuroxime axetil, respectively.

Calibration curve:

Aliquots of 1, 2, 3, 4, 5 and 6 µl of standard solution of cefuroxime axetil and probenecid were applied on the TLC plate (100 µg/ml of each drug). The TLC plate was dried, developed and analyzed photometrically as described earlier.

Validation of the method and detection of related impurities[5-9]:

The developed method was validated in terms of linearity, accuracy, specificity, and limit of detection, limit of quantification, inter-day and intra-day precision and repeatability of measurement as well as repeatability of sample application. The related impurities were determined by spotting higher concentration of the drugs so as to detect and quantify them.

Assay of the marketed formulation[10]:

Ten microlitres of the filtered solution of the marketed formulation was spotted on to the plate followed by development scanning. The analysis was repeated in triplicate. The spot was resolved into two peaks in the chromatogram of drug samples, extracted from the marketed formulation. The content of the drug was calculated from the peak areas recorded.

Stability studies[11,12]:

In planar chromatography, when the analytes are adsorbed on the highly active polar surface of silica and solvent layer in the presence of air, substances can decompose more easily, than for example in HPLC. Considering that the cephalosporins undergo degradation reactions and isomerisation on silica gel plate to Δ^2 cephalosporins, stability and related impurities were tested on chromatographic plate for different time periods prior to densitometry.

RESULTS AND DISCUSSION

A solvent system that would give dense and compact spots with appropriate and significantly different R_f values was desired for quantification of cefuroxime axetil and probenecid in pharmaceutical formulations. The mobile phase consisting of chloroform:acetonitrile:toluene: acetate buffer of pH 6 (5:4:1:0.3 v/v) gave R_f values of 0.28 (±0.04) and 0.58 (±0.04) for probenecid and cefuroxime, respectively (fig. 2). The linear regression data (n=6, Table 1) showed a good linear relationship over a concentration range of 100-500 ng for both probenecid and cefuroxime axetil. The limit of detection and limit of quantification were found to be 50 ng/spot and 100 ng/spot, respectively.

The intra-day precision was determined by analyzing standard solutions in the concentration range of 100 ng/µl to 500 ng/µl of each drug for 3 times on the same day while inter-day precision was determined by analyzing corresponding standards daily for 3 day over a period of one week. The intra-day and inter-day coefficients of variation are given in Table 2.

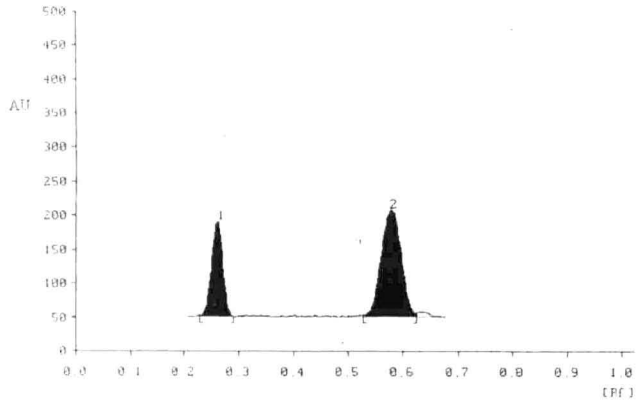

Fig. 2: Chromatogram of probenecid and cefuroxime axetil.

Peak 1 and 2 are probenecid and cefuroxime axetil, respectively.

Repeatability of sample application was assessed by spotting 10 µl of drug solution 7 times on a TLC plate followed by development of plate and recording the peak height and area for 7 spots. The % RSD for peak height and peak area values of probenecid were found to be 1.04 and 1.01 respectively and for cefuroxime axetil 1.58 and 0.77, respectively.

Repeatability of measurement of peak height and area were determined by spotting 10 µl of standard drug solution on TLC plate and developing the plate. The separated spot was scanned 7 times without changing position of the plate and % RSD for measurement of peak height and area of probenecid were 0.043 and 0.155, respectively and % RSD for measurement of peak height and area of cefuroxime were 0.073 and 0.073, respectively.

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 with the peaks

TABLE 1: CALIBRATION, LINEARITY AND RANGE OF PROBENECID AND CEFUROXIME AXETIL

Drug	Data	R^2	Slope	Constant
Probenecid	Peak height	0.998	0.337	9.71
	Peak area	0.999	6.40	152
Cefuroxime axetil	Peak height	0.998	0.447	10.2
	Peak area	0.998	10.8	418

R^2 = Correlation coefficient.

TABLE 2: PRECISION OF PROBENECID AND CEFUROXIME AXETIL

Drug	Concentration (ng/µl)	Intra-day precision % RSD	Inter-day precision % RSD
Probenecid	200	0.78	1.04
	300	0.72	0.93
	400	0.59	0.91
Cefuroxime axetil	200	0.64	1.00
	300	0.46	0.99
	400	0.36	0.88

RSD - Relative standard deviation.

TABLE 3: RECOVERY OF PROBENECID AND CEFUROXIME AXETIL

Label Claim mg/capsule	Amount added %	Total Amount Added (mg)	Amount Recovered* (mg) ± SD	% Recovery ±SD	% RSD
Probenecid	80	450	449±1.58	99.4±0.71	0.71
250	100	500	497±0.98	99.4±0.20	0.20
	120	550	550±1.20	99.9±0.20	0.21
Cefuroxime	80	450	448±2.51	99.5±0.51	0.51
axetil	100	500	499±1.28	99.7±0.25	0.25
250	120	550	546±0.94	99.2±0.17	0.17

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

of probenecid and cefuroxime axetil.

Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels i.e. multiple level recovery studies. Sample stock solution from capsule formulation of 250 μg/ml of each drug was prepared. To the above-prepared solutions, 80 %, 100 % and 120 % of the standard drug solutions were added. Dilutions were made and recovery studies were performed. % Recovery was found to be within the limits as listed in Table 3. For the detection of related impurities cefuroxime axetil and probenecid (0.2 g each) was dissolved separately in 10 ml of methanol and this solution was termed as sample solution (20 μg/ml). One millilitre of the sample solution was diluted to 100 ml with methanol and this solution was termed as standard solution (0.2 mg/ml). Aliquots of both the standard and sample solution (10 μl) were spotted on the plate and chromatograms run as described earlier. The spot other than the principle spot and the spot of the starting point from the sample solution were not intense than the spot from the standard solution. The sample solution of cefuroxime showed three additional spots at R_f of 0.14, 0.15 and 0.52. However, the area of these spots was found to be much less as compared to the spot of the standard solution as indicated in Table 4. But Probenecid showed no additional peaks.

The assay value for the marketed formulation was found to be within the limits as listed in Table 5. The low RSD value indicated the suitability of this method for routine analysis of probenecid and cefuroxime in pharmaceutical dosage

TABLE 4: RELATED IMPURITIES OF CEFUROXIME AXETIL

Concentration of drug (μg/μl)	R_f	Area (AU)
0.2	0.63	23545.6
Related impurities	0.14	1117.3
	0.15	1010.9
	0.52	712.2
		Total Area=2840.4

R_f -Retention factor

TABLE 5: ASSAY OF PROBENECID AND CEFUROXIME AXETIL

Label Claim mg/capsule	Amount found*	% of drug found*	% RSD
Probenecid 250	249.5	99.81	0.17
Cefuroxime axetil 250	246.7	98.43	0.078

*Each value is a mean of three determinations.

TABLE 6: STABILITY OF PROBENECID AND CEFUROXIME AXETIL ON TLC PLATES

Drug	% Drug loss ± SD after		
	3 h	24 h	48 h
Probenecid	No loss	3.61±0.8	4.08±0.56
Cefuroxime axetil	1.25±0.7	8.66±0.8	14.4±0.7

forms. To test the stability of drugs on the TLC plate's analyte was tested against freshly prepared standard solution and following results were obtained. No decomposition of drugs was observed during chromatogram development. No decrease in the concentration of drugs on plate was observed within 3 h. Decrease in concentration of cefuroxime was observed 3 h after the development as listed in Table 6. Therefore, chromatograms containing cefuroxime were scanned within 2 h after development.

The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that the method is reproducible and selective for the analysis of probenecid and cefuroxime simultaneously in bulk drug and in capsule formulations.

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