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Research Article

Simultaneous Estimation of Chlorzoxazone Paracetamol Famotidine and Diclofenac Potassium in Their Combined Dosage Form by Thin Layer Chromatography

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Abstract

A sensitive, selective and precise high performance thin layer chromatographic method has been developed for the estimation of chlorzoxazone, paracetamol, diclofenac potassium and famotidine in the pharmaceutical dosage form. TLC aluminum plates pre-coated with silica gel 60F254 used as the stationary phase, while chloroform: methanol: ethyl acetate: hexane: ammonia (10: 2.5: 1.5: 1: 0.1, v/v/v/v/v) used as mobile phase. The Rf values were observed 0.74 ± 0.01, 0.52 ± 0.01, 0.30 ± 0.01 and 0.14 ± 0.01 for chlorzoxazone, paracetamol, diclofenac potassium and famotidine, respectively. The densitometry analysis was carried out in absorbance mode at 282 nm. The method was linear in the range of 250-1500 ng/spot for chlorzoxazone, diclofenac potassium and famotidine and 500- 3000 ng/spot for paracetamol and method was validated as per ICH guideline. The limit of detection and limit of quantization were found to be 35.98 ng/spot and 109.05 ng/spot, respectively for chlorzoxazone, 99.74 ng/spot and 302.25 ng/spot, respectively for paracetamol, 58.63 ng/spot and 177.69 ng/spot, respectively for diclofenac, and 50.93 ng/spot and 154.35 ng/spot, respectively for famotidine. The proposed method was successfully applied to the estimation of chlorzoxazone, paracetamol, diclofenac potassium and famotidine in the pharmaceutical dosage form.

Keywords: Chlorzoxazone (CLZ); Paracetamol (PCM); Diclofenac potassium (DCL); Famotidine (FAM); HPTLC; Validation

Introduction

Chlorzoxazone (CLZ) is chemically 5-chloro-2, 3-dihydro-1, 3-benzoxazol-2-one. The empirical formula of CLZ is C7H4ClNO2 and a molecular weight is 169.56 g/mol. It is NSAID. It inhibits multisynaptic reflex a.c. involved in producing and maintaining skeletal muscle spasm. Paracetamol (PCM) is chemically N-(4-hydroxyphenyl) acetamide. The empirical formula for PCM is C8H9-NO2 and a molecular weight is 151.163 g/mol. It inhibiting both is forms of cyclooxygenase; COX-1, COX-2, and COX-3 enzymes involved in prostaglandin (PG) synthesis. Diclofenac potassium (DCL) is chemically 2-{2-[(2, 6-dichlorophenyl) amino] phenyl} acetic acid and empirical formula of DCL is C14H11Cl2NO2 and molecular weight is 318.13g/mol. It inhibition of leukocyte migration and the enzyme cylooxygenase (COX-1 and COX-2), leading to the peripheral inhibition of prostaglandin synthesis. Famotidine (FAM) is chemically 3-[({2-(diaminomethylidene) amino]-1, 3-thiazol-4-yl} methyl) sulfanyl]-N' sulfamoylpropanimidamide and empirical formula for FAM is C8H15N7O2S3 and molecular weight is 337.44 g/mol. It is competitive

Histamine H2-receptor antagonist and inhibits many of the isoenzymes of the hepatic CYP450 enzyme system.

The combined dosage form of CLZ, PCM, DCL and FAM is used as muscle relaxant. CLZ, PCM, DCL and FAM are official in United State Pharmacopoeia and British Pharmacopoeia. Official method has been reported for CLZ, PCM, DCL and FAM in United State Pharmacopoeia and British Pharmacopoeia. Some of UV, HPLC, TLC methods has been reported for the estimation of CLZ, PCM, DCL and FAM alone and with other drug combination. No method has been reported for the estimation of CLZ, PCM, DCL

and FAM in their combined dosage form [1-27]. In comparison to LC and LC-MS/MS methods, HPTLC method is considered to be a good alternative, and it should be widely explored as an important tool in routine drug analysis. A major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces the time and cost of analysis.

Experimental

HPTLC instrument

The samples were applied in the form of a bands of width 8 mm with a Camag 100 µl sample syringe (Hamilton, Switzerland) using Camag Linomat 5 (Switzerland) sample applicator on precoated silica gel aluminum plate 60 F254 (10 cm x 10 cm with 0.2 mm thickness, E. Merck, Germany). Camag TLC scanner 4 was used for the densitometric scanning.

Chemicals and reagents

Analytically pure CLZ, PCM, DCL and FAM from Sun pharmaceutical industry ltd. Vadodara, India were obtained as gift samples. Methanol (AR grade) of SRL Private Ltd. and chloroform of Chemdyes Corporation (AR grade) were used. Ammonia and hexane of Chiti-Chem Corporation (AR grade) were used. Ethyl acetate of Astron Chemicals (AR grade) Tablet formulation fast ran MR (Horizon biocauticals Pvt. Ltd.) containing 500 mg of PCM, 250 mg CLZ, 50 mg DCL and 10 mg FAM was procured from local pharmacy.

Chromatographic system

Sample application

Standards and formulation samples of CLZ, PCM, DCL and FAM were applied on the HPTLC plates in the form of narrow bands of 6 mm length, 10 mm from the bottom and left edge, and with 9 mm distance between two bands. Samples were applied under a continuous stream of nitrogen gas.

Mobile phase and development

Plates were developed using a mobile phase consisting of chloroform: methanol: ethyl acetate: hexane: ammonia (10: 2.5: 1.5: 1: 0.1, v/v/v/v/v). Linear ascending development was carried out in a twin-trough glass chamber equilibrated with the mobile phase vapors for 30 min at 25 ± 20C. Ten milliliters of the mobile phase (5 ml in the trough containing the plate and 5 ml in the other trough) was used for each development and was allowed to migrate a distance of 80 mm, sample application rate is 200nl/sec. After development, the HPTLC plates were dried completely using continuous stream of nitrogen.

Densitometric analysis

Densitometric scanning was performed in the absorbance mode under control by win CATS planar chromatography software. The source of radiation was the deuterium lamp and bands were scanned at 282 nm. The slit dimensions were 6 mm length and 0.45 mm width, with a scanning rate of 20 mm/s. Concentrations of the compound were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using a linear regression equation.

Preparation of standard stock solution

PCM (10 mg), CLZ (5 mg), DCL (5mg) and FAM (5mg) were accurately weighed and transferred to 10 ml volumetric flasks and dissolved in few ml of methanol. Volumes were made up to the mark with methanol to yield a solution containing 1000µg/ml of PCM and 500 µ/ml of CLZ, DCL and FAM. Aliquot from the stock solutions of PCM, CLZ, DCL and FAM were appropriately diluted with mobile phase to obtain working standard of 100 µg/ml of PCM and 50 µg/ml of CLZ, DCL and FAM respectively.

Validation

Validation of the developed HPTLC method was carried out according to International Conference on Harmonization (ICH) guidelines Q2 (R1) for specificity, sensitivity, accuracy, precision, repeatability, and robustness [28].

Linearity of calibration curves

Linearity of the method was evaluated by constructing calibration curves at six concentration levels over a range of 500–3000 ng/band for PCM and 250-1500 ng/band for CLZ, DCL and, FAM by applying 5µl to 30µl from stock solution has been applied on HPTLC plate using sample applicator. The calibration curves were developed by plotting peak area versus concentration (n = 6) with the help of the win CATS software.

Accuracy

Accuracy is closeness of the test results obtained by the method to the true value and should be established across specified range of analytical Procedure. The accuracy of the method was determined by calculating recoveries of PCM, CLZ, DCL and FAM by method of standard additions. Known amount of PCM (0, 500, 1000, 1500 ng/spot) and CLZ, DCL and FAM (0, 250, 500, 750 ng/spot) were taken from the working standard solutions (1000 ng/spot of PCM and 500 ng/spot of CLZ, DCL and FAM respec-

tively). It was added to a pre quantified sample and the amount of PCM, CLZ, DCL and FAM were estimated by measuring the peak area and by fitting these values to the straight-line equation of calibration curve. The proposed acceptance criteria for the accuracy studies are ranges from 95-105 %.

Precision

Precision is closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample. Precision was evaluated in terms of intraday and interday precisions. Standard solutions of 100 µg/ml of PCM and 50 µg/ml of CLZ, DCL, and FAM, were prepared and used for the precision study. Intraday precision was determined by analyzing sample solutions of PCM (500 ng/spot, 1000 ng/spot, and 3000 ng/spot), CLZ, DCL and FAM (250 ng/spot, 750 ng/spot, 1500 ng/spot) at three levels covering low, medium, and high concentrations of the calibration curve three times on the same day. Interday precision was determined by analyzing sample solutions of PCM, CLZ, DCL and FAM at three levels covering low, medium, and high concentrations over a period of 3 days. The peak areas obtained were used to calculate mean and RSD values. Less than 5 % RSD values indicate that the method is precise.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the method was ascertained by analyzing PCM, CLZ, DCL and FAM in presence of excipients commonly used for tablet formulations. The bands of PCM, CLZ, DCL and FAM were confirmed by comparing Rf values and respective spectra of sample with those of standards. The peak purity of PCM, CLZ, DCL and FAM was assured by comparing the spectra at three different levels, that is, peak start, peak apex and peak end positions. Selectivity describes the ability of an analytical method to differentiate various substances in a sample. The proposed method shows well resolution of all four molecules from their pharmaceutical dosage form.

Sensitivity

Sensitivity of the method was determined with respect to LOD and LOQ. Noise was determined by scanning a blank band (methanol) six times. LOD was calculated as 3 times the noise level, and LOQ was calculated as 10 times the noise level.

Robustness

Small changes in the chamber saturation time, solvent migration distance and mobile phase composition were introduced and the effects on the results were examined. Robustness of the method was determined in triplicate at a concentration level of 2000 ng/band for PCM and 1000 ng/spot for DCL and 750 ng/spot for CLZ and FAM. The mean and RSD of peak areas were calculated.

Analysis of marketed formulations

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 500 mg of PCM, 250 mg of CLZ, 50 mg of DCL and 10 mg of FAM was accurately weighed and transferred to a 100 ml volumetric flask. A few ml (40 ml) of methanol was added to the above flask and flask was sonicated for 15 min. The solution was filtered using what man filter paper No. 41 in another 100 ml volumetric flask and make up the volume up to the mark with the methanol.

A solution containing 300 ng/band FAM and 1500 ng/band DCL were injected as per the above chromatographic conditions and peak areas were recorded. Appropriate volume of the aliquot was transferred to a 10 ml volumetric flask and the volume was made up to the mark with the mobile phase to obtain a solution containing 750 ng/band CLZ and 1500 ng/band PCM. The quantifications were carried out by keeping these values to the straight line equation of calibration curve.

Results and Discussion

Optimization of the Mobile Phase

To develop the HPTLC method for analysis of PCM, CLZ, DCL and FAM in the pharmaceutical dosage form for routine analysis, selection of the mobile phase was carried out on the basis of polarity. A mobile phase that would give a dense and compact band with an appropriate Rf value for PCM, CLZ, DCL and FAM was desired. Various mobile phases such as acetone-methanol, methanol-chloroform acetic acid, methanol-toluene-ammonia, methanol-toluene-glacial acetic acid, toluene-ethyl acetate-methanol, methanol-acetonitrile-glacial acetic acid were evaluated in different proportions. A mobile consisting of chloroform: methanol: ethyl acetate: hexane: ammonia (10: 2.5: 1.5: 1: 0.1, v/v/v/v/v) gave good separation of PCM, CLZ, DCL and FAM from its matrix. It was also observed that chamber saturation time and solvent migration distance were crucial in the chromatographic separation. Therefore, chloroform: methanol: ethyl acetate: hexane : ammonia (10: 2.5: 1.5: 1: 0.1, v/v/v/v/v) mobile phase with a chamber saturation time of 30 min at 25 0C and solvent migration distance of 80 mm was used. Densitogram of PCM, CLZ, DCL and FAM,

photograph of TLC plate and three dimensional overlays of HPTLC densitograms of calibration bands of PCM, CLZ, DCL and FAM are depicted in figures (Figure 1-3).

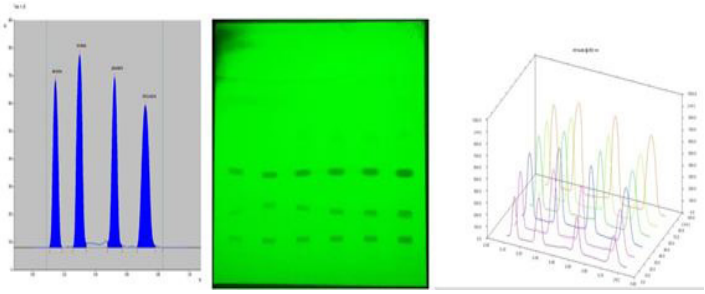

Figure 1-3: Densitogram of PCM, CLZ, DCL and FAM, photograph of TLC plate and three dimensional overlays of HPTLC densitograms of calibration bands of PCM, CLZ, DCL and FAM are depicted in figures (Figure 1-3).

Validation

Linearity and calibration curves.

The method was found to be linear for PCM in concentration range of 500-3000 ng/band (n = 6) and for CLZ, DCL and FAM 250-1500 ng/band (n = 6), respectively. Figure-3 displays a three-dimensional overlay of HPTLC densitograms of the calibration bands of PCM, CLZ, DCL and FAM at 282 nm. The regression data shown in (Table 1) reveal a good linear relationship over the concentration range studied, demonstrating the suitability of the method for analysis.

PARAMETERS	CLZ	PCM	DCL	FAM
Linearity range (ng/spot)	250-1500	500-3000	250-1500	250-1500
Slope	14.278	5.682	10.902	10.808
Standard deviation of slope	0.2094	0.03033	0.2148	0.294737
Intercept	4619.6	4953.4	9033.6	4310.6
Standard deviation of intercept	155.7122	171.73	193.72	166.82
Correlation coefficient	0.995	0.997	0.995	0.996

Table 1: The regression data shown

Accuracy

Accuracy was determined by the application of analytical procedure to recovery studies, where a known amount of standard is spiked into preanalyzed samples solutions. Results of the accuracy studies from

Amount of Sample (ng/spot)			Amount drug Spiked (ng/spot)		Average amount recovered (ng/spot)		% Recovery	
CLZ	PCM	Set	CLZ	PCM	CLZ	PCM	CLZ	PCM
		1	0	0				
		2	0	0				
500	1000	3	0	0	495.47	994.47	99.09	99.44
		1	250	500				
		2	250	500				
500	1000	3	250	500	747.89	1498.8	99.58	99.88
		1	500	1000				
		2	500	1000				
500	1000	3	500	1000	997.13	1985.6	99.42	98.55
		1	750	1500				
		2	750	1500				
500	1000	3	750	1500	1244.1	2489.8	98.81	98.93

Table 4: Recovery values demonstrated the accuracy of the method in the desired range.

Amount of Sample (ng/spot)			Amount drug Spiked (ng/spot)		Average amount recovered (ng/spot)		% Recovery	
FAM	DCL	Set	FAM	DCL	FAM	DCL	FAM	DCL
		1	0	0				
		2	0	0				
500	500	3	0	0	496.73	504.38	99.01	100.3

		1	250	250				
		2	250	250				
500	500	3	250	250	744.73	749.28	999.6	99.85
		1	500	500				
		2	500	500				
500	500	3	500	500	997.75	998.99	98.94	99.79
		1	750	750				
		2	750	750				
500	500	3	750	750	1245.07	1246.1	99.34	99.22

Table 4: Recovery values demonstrated the accuracy of the method in the desired range

Precision

In all instances, RSD values were less than 2%, confirming the precision of the method. Repeatability of the scanning device was studied by applying and analyzing sample seven times. RSD was less than 2%, which was well below the instrumental specifications. Summary of validation parameters are shown in (table 2).

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PARAMETERS	CLZ	PCM	DCL	FAM
Rf	0.74	0.52	0.29	0.14
Detection limit (ng/spot)	35.98	99.74	58.63	50.93
Quantization limit (ng/spot)	109.05	302.25	177.69	154.35
Accuracy (%)	98.81-99.57	98.55-99.88	99.22-100.27	98.94-99.55
Intra-day (n=3) (% RSD)	1.18-1.249	0.94-1.31	1.33-1.60	0.82-1.06
Inter-day (n=3) (% RSD)	1.71-1.89	1.45-1.68	1.44-1.83	1.57-1.93
Repeatability study (n=6) (% RSD)	1.77-1.96	1.77-1.86	1.71-1.85	1.58-1.83

Table 2: Summary of validation parameters

The RSD values obtained were less than 2%, which was under the acceptance criteria of ICH method validation guideline (<2%). The results indicated that the method is repeatable and reproducible.

Limit of detection and limit of quantification

Under the experimental conditions used, the lowest amount of drug that could be detected LOD was found to be 35.98 ng/band, 99.74 ng/band, 58.63 ng/band and 50.93 ng/band for PCM, CLZ, DCL and FAM, respectively and LOQ was found to be 109.05 ng/band 302.25 ng/band, 177.69 ng/band and 154.35 ng/band for PCM, CLZ, DCL and FAM, respectively. It indicate that the nanogram quantity of all the drugs can be estimated accurately and precisely which means that the method is sensitive.

Specificity

There was no interfering peak at the Rf value of PCM, CLZ, DCL and FAM from excipients added in the synthetic formulation. In addition, there was no interference from excipients present in the commercial formulation, thereby confirming the specificity of the method.

Robustness

The low values of RSD obtained after introducing small, deliberate changes in parameters of the developed HPTLC method confirmed its robustness. The robustness data of the proposed method are shown in (table 3).

Parameters	Amt of CLZ	Amt of ClZ Recovered ± SD	Amt of PCM	Amt of PCM Recovered ± SD	Amt of DCL	Amt of DCL Recovered ± SD	Amt of FAM	Amt of FAM Recovered ± SD
Chamber saturation time : 20 min	750	741±22.9	2000	1960.3±38.07	1000	962.7±31.89	750	766.8±21.65
Chamber saturation time : 40 min	750	753.6±29.50	2000	1941.3±48.22	1000	1014.1±36.90	750	774.9±6.24
Wave length 280	750	762.6±22.85	2000	1943.4±32.71	1000	953.5±28.53	750	780.3±17.92
Wave length 280	750	768.6±17.38	2000	1977.6±12.01	1000	949.6±22.03	750	757.5±10.40
chloroform:methanol:ethayl acetate:hexane:ammonia (9: 3.5: 1.5: 1: 0.1v/v/v/v/v)	750	732.6±10.40	2000	1978.4±34.58	1000	940.2±30.55	750	771.6±23.62
chloroform:methanol:ethayl acetate:hexane:ammonia (11: 2: 1: 1: 0.1 v/v/v/v/v)	750	771.2±24.57	2000	1952.6±36.115	1000	956.7±19.73	750	748.7±14.9

Table 3: The robustness data of the proposed method

Analysis of marketed formulation

Marketed formulation was analyzed using proposed method which gave percentage recovery of 98.46%, 98.26%, 98.09% and 99.01% for PCM, CLZ, DCL and FAM, respectively. No interference from the excipients present in the marketed tablet formulation was observed.

Conclusions

A selective, sensitive, accurate and precise high performance thin layer chromatography method has been developed for the simultaneous identification and quantification of chlorzoxazone, paracetamol, and famotidine and diclofenac potassium in their combined pharmaceutical dosage form. The method was successfully validated in accordance with ICH guidelines. It can be conveniently used for routine quality control analysis of chlorzoxazone, paracetamol, and famotidine and diclofenac potassium in marketed tablet without any interference from excipients.

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