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Development and validation of a HPTLC method for simultaneous estimation of Drotaverine Hydrochloride and Diclofenac Potassium in combined dosage form

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ABSTRACT

A simple, precise, rapid and accurate HPTLC method has been developed for the simultaneous estimation of Drotaverine Hydrochloride (DRO) and Diclofenac Potassium (DFK) in bulk and pharmaceutical dosage form on silica gel precoated aluminum 60F₂₅₄ plates, (20 cm x10 cm) with 250 μ m thickness. The separation was carried out using Toluene: Ethyl acetate: Methanol (2:8:2 v/v/v) as mobile phase. The densitometric scanning was carried out at 298 nm. The R_f values were found to be 0.28 ± 0.05 for DRO and 0.51 ± 0.05 for DFK. The linearity was obtained in the range 160-1280 ng/band and 100-800ng/band with correlation coefficients ($r^2 = 0.99958$) and ($r^2 = 0.99959$) for DRO and DFK, respectively. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The percentage recovery obtained for DRO and DFK were in the range of 99.87-101.20% and 98.39-101.42%, respectively. The proposed method was optimized and validated as per the ICH guidelines.

Keywords: Drotaverine Hydrochloride, Diclofenac Potassium, HPTLC, Method Validation.

INTRODUCTION

Drotaverine Hydrochloride, 1-[(3, 4-diethoxy phenyl) methylene]-6, 7-diethoxy-1, 2, 3, 4-tetra hydro isoquinoline is an analogue of papaverine [1]. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain [2]. Drotaverine hydrochloride is official in Polish Pharmacopoeia [3]. Diclofenac Potassium is chemically potassium (o-(2, 6-dichloroanilino) phenyl) acetate, a non steroidal anti-inflammatory drug (NSAID) exhibits anti-inflammatory and analgesic properties [4]. Diclofenac Potassium is official in United States Pharmacopoeia[5], European Pharmacopoeia [6] and Martindale, The Extra Pharmacopoeia [7]. The exact mechanism of action of DFK is not entirely known, but it is thought that the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX) and it appears to inhibit DNA synthesis [8].

Literature survey reveals that various methods have been reported for estimation of DRO such as spectrophotometry [9], HPLC [10], HPTLC [11] and voltammetry [12] individually and in dosage form in combination with other drugs. For DFK various analytical methods have been reported for its individual estimation and in dosage form in combination with other drugs and in human plasma which includes spectrophotometry [13], liquid chromatography with UV detection [4, 14], HPLC with electrochemical detection [15].

Since no HPTLC method is reported for simultaneous estimation of DRO and DFK in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously. The present work describes a new method for simultaneous estimation of DRO and DFK in tablets using HPTLC-densitometry. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16].

MATERIALS AND METHODS

Pure drug sample of DRO, % purity 99.86 and DFK, % purity 99.91 was kindly supplied as a gift sample by Alkem Pharmaceutical Pvt. Ltd. Mumbai and Aarti Drugs Pvt Ltd., Mumbai, respectively. These samples were used without further purification. Two tablet formulations (Lot 304F-1 and 308F-2) were supplied by JCPL Pharma Ltd., Jalgaon were used for analysis containing DRO 80 mg and DFK 50 mg per tablet. HPTLC precoated plates silica gel 60 F₂₅₄ 20×10 cm, layer thickness 250 μm (Merck, Germany). Analytical grade methanol, toluene and ethyl acetate were procured from Merck Chemicals (Mumbai, India).

Instrumentation and chromatographic conditions

The standard solution ranging from 160-1280 ng/band of DRO and 100-800 ng/band of DFK were applied on precoated silica gel 60 F₂₅₄ plate in the form of bands with 100 μl sample syringe using Camag Linomat 5 (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate of 150 nL s⁻¹ was used and the space between two bands was 11.2 mm. The slit dimension was kept at 6 mm x 0.45 mm and the scanning speed was 10 mm s⁻¹. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. It was developed in a twin trough glass chamber (20 cm x 10 cm) without saturation. The mobile phase consisted of Toluene: Ethyl acetate: Methanol (2:8:2 v/v/v) and development distance was 85 mm. After development, plate was immediately dried with the help of dryer and was observed under CAMAG TLC Visualizer. The flow rate in the laboratory was maintained unidirectional. The well resolved bands of drugs were scanned at 298 nm with CAMAG TLC scanner III densitometer controlled by WINCAT's software version 4. The source of radiation used 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.

Preparation of standard solutions and calibration curve

The standard stock solutions of DRO and DFK of 1000 μg/ml were prepared separately in methanol. Mixed standard stock solution of DRO and DFK (in ratio 1.6:1) was prepared in methanol, suitably diluted to have solutions of concentration ranging from 160-1280 ng/band and 100-800 ng/band of DRO and DFK respectively. The solution was spotted and the plate was developed on previously described mobile phase and well resolved band of drug were scanned at 298 nm with scanner. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve.

Analysis of tablet formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg of DFK (80 mg of DRO) was weighed and dissolved in the 40 ml of methanol with the aid of ultrasonication 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 stock solution was suitably diluted with methanol to get of 80 µg/ml of DRO & 50 µg/ml of DFK. Solution was spotted on the plate to have 600 and 400 ng/band of DRO and DFK, respectively. The amount of each drug present per tablet was estimated from the respective calibration curves. A typical densitogram obtained from a sample solution along with structures of analytes is shown in Fig. 1.

Method Validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, and limit of detection, limit of quantitation and robustness and specificity.

Linearity:

Linearity of the method was studied by spotting eight concentrations of the drug prepared in the mobile phase in the range of 160-1280 ng/spot and 100-800 ng/spot of DRO and DFK, respectively and noting the peak areas.

Accuracy:

For accuracy of method, recovery study was carried out by applying the method to drug samples to which known amount of DRO and DFK was added at level of 80, 100 and 120% of label claim (standard addition method). At each level of the amount, three determinations were performed and the results obtained were compared with expected results.

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies by using three concentrations over the range of the method. In the intra day studies, 3 repeated measurements of standard and sample solutions were made in a day and % RSD were calculated. For inter day variation studies, 3 repeated measurements of standard and sample solutions were made on 3 consecutive days and percentage RSD was calculated.

Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response and Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOD and LOQ were calculated using σ (standard deviation of the response) and b (slope of the calibration curve) using the following formulae: $LOD = (3.3 \times \sigma) / b$ and $LOQ = (10 \times \sigma) / b$

Robustness:

Robustness is checked by making slight deliberate change in the experimental procedures. Mobile phases having different composition like ethyl acetate: toluene: methanol (1.9: 8.2: 1.9 v/v) and ethyl acetate: toluene: methanol (2.1: 7.8: 2.1 v/v) were tried and chromatograms were run. Robustness of the method was done at three different concentration levels 160, 600, 1280 ng / spot & 100, 400, 800 ng / spot for DRO and DFK, respectively.

Specificity:

The specificity of the method was determined by analyzing standard drug and test samples. The spot for DRO and DFK in the samples was confirmed by comparing the RF and spectrum of the

spot to that of a standard. The peak purity was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

RESULT AND DISCUSSION

Optimization of Solvent System and Chromatographic Conditions:

Chromatographic separation studies were carried out on the stock solution of DRO and DFK. Initially the plates were spotted with 10 μ L of stock solution and developed by linear ascending development method using neat solvents like toluene, hexane, methanol, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, etc. without chamber saturation. Based on the results of these initial chromatograms, binary and ternary mixtures of solvents were tried to achieve optimum peak parameter. Ultimately mobile phase consisting of ethyl acetate: toluene: methanol (2: 8: 2 v/v) was selected as acceptable R_f value of 0.28 ± 0.05 for DRO and 0.51 ± 0.05 for DFK was obtained as shown in Fig. 1. The samples were applied in form of bands on precoated aluminum sheets of silica gel 60 F₂₅₄ (20 cm x 10 cm) with 250 μ m thickness. Linear ascending development was carried out in a twin trough glass chamber without saturation. The length of chromatogram run was 85 mm. The developed plates were dried in the current of dry air. Densitometric scanning was performed in the absorbance mode at 298 nm.

Linearity:

When peak area was plotted Vs Concentration (ng/spot) DRO and DFK showed good correlation coefficient in concentration range of 160 - 1280 ng/spot and 100 - 800 ng/spot. Linearity was evaluated by determining eight standard working solutions. Table.1 summarizes Beer's law limit, linear regression equation and correlation coefficient for the method.

Analysis of tablet formulation:

The proposed method was also evaluated in terms of assay of commercially available tablets containing DRO and DFK. Three replicate determinations were performed on the accurately weighed amounts of tablets. The results obtained are shown in Table. 2

Precision and Accuracy:

The method was found to be precise as indicated by % RSD (Relative Standard Deviation) not more than 2. Intra day and inter day studies supports the precision of the method (Table 1). The proposed method when used for estimation of DRO and DFK from pharmaceutical dosage form after spiking with working standard afforded recovery of 98-102% (Table. 2).

Sensitivity of the methods (LOD and LOQ):

The limit of detection was found to be 53.33 ng/spot and 33.33 ng/spot, while the limit of quantitation was found to be 160 ng/spot and 100 ng/spot for DFK and DRO, respectively. The low value of LOD and LOQ indicates that the method is sensitive.

Robustness:

The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2%. The low values of the % RSD (Table 3) indicated robustness of the method.

Specificity:

The method was found to be specific since no interfering spots were seen when R_f values of standard and sample were compared. There is no difference in the spectra of sample and standard solution which indicate the specificity of the method (Fig. 2).

Table 1: Regression analysis of calibration curves and results of precision and sensitivity of the method

Parameters		DRO	DFK
Detection Wavelength (nm)		298	298
Beer's Law Limit (ng/band)		160 – 1280	100 – 800
Regression equation		4.6016x + 1619.07	6.8970x + 506.21
Slope (m)		4.6016	6.8970
Intercept (c)		1619.07	506.21
Limit of detection (ng/spot)		53.33	33.33
Limit of quantitation (ng/spot)		160	100
Precision (% RSD)	Actual Conc. ($\mu\text{g mL}^{-1}$)	160	100
	Intra-day	160.01, 0.47	100.19, 0.36
	Inter-day	160.09, 0.29	100.41, 0.45

Table 2: Results of Tablet analysis and accuracy study

Compound (Label Claim)	Formulation Study (n=6)		Recovery (accuracy) study	
	Tablet Lot	% Assay Found, % RSD	Recovery Level	% Recovery, % RSD (n=3)
Drotaverine Hydrochloride (80 mg)	Lot 304	100.4, 1.05	80	101.20, 0.93
	Lot 308	100.01, 1.26	100	99.37, 0.62
			120	100.16, 0.97
Diclofenac Potassium (50 mg)	Lot 304	99.80, 0.97	80	100.19, 0.99
	Lot 308	100.2, 1.32	100	98.39, 1.03
			120	101.42, 0.27

Table 3: Robustness Study of DRO and DFK (n = 3)

Parameter		SD of peak area		% RSD	
		DRO	DIC	DRO	DIC
Mobile phase composition		8.04	4.97	0.44	0.43
Amount of mobile phase		15.46	18.44	1.28	1.89
Development Distance		8.14	6.16	0.41	0.57
Plate pretreatment		11.82	7.66	0.59	0.71
Time from	application to chromatography	10.72	6.76	0.99	0.68
	chromatography to scanning	11.85	11.54	1.37	0.85
Plate from different lot	Lot304(F- 1)	9.32	7.54	0.79	0.75
	Lot 308(F-2)	10.82	7.06	0.39	0.43

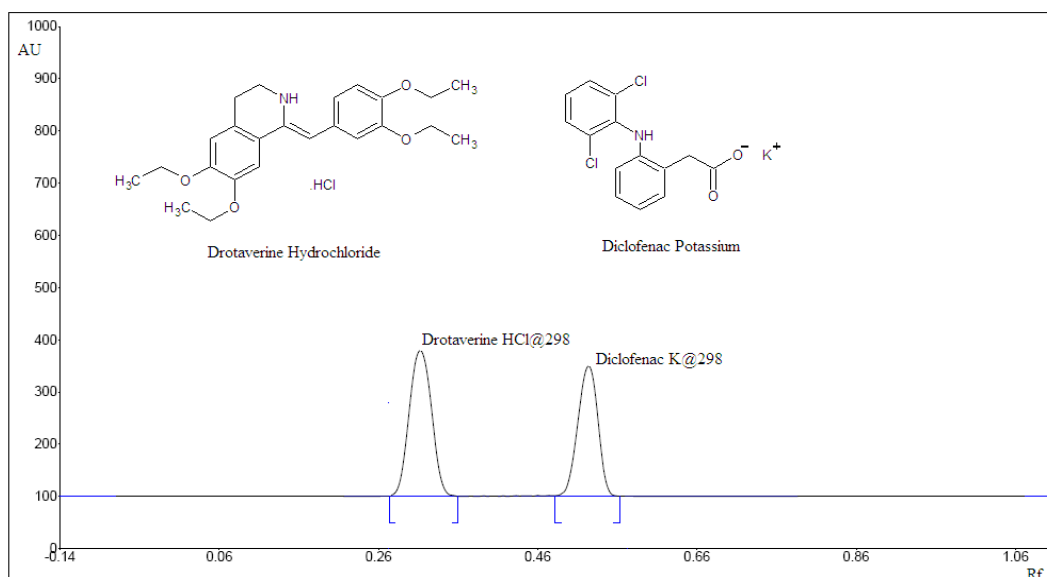


Fig.1: Chromatogram of working standard mixture of DRO (80µg/ml) and DFK (50 µg/ml) with the structures of the analytes

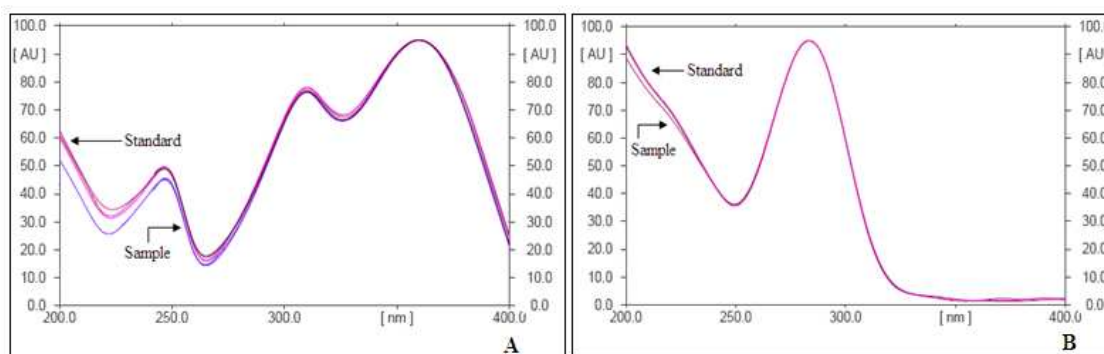


Fig.2: Specificity spectra of sample and standard of A) DRO (80 µg/ml) and B) DFK (50 µg/ml)

CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and sensitive, thus can be used for routine analysis of Drotaverine hydrochloride and Diclofenac potassium in tablet dosage form.

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