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Research Article

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VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF DICLOFENAC POTASSIUM AND METAXALONE IN BULK DRUG AND FORMULATION

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ABSTRACT

This work is concerned with the simultaneous determination of diclofenac potassium and metaxalone in a bulk drug and pharmaceutical formulation by high performance thin layer chtromagraphic method (HPTLC). The precoated silica gel 60 F_{254} aluminum plate was selected as the stationary phase and solvent system consisted of chloroform: methanol (8:1 v/v) used as developing solvents. The detection of diclofenac potassium and metaxalone was carried out at 281 nm. The developed method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness parameters. The correlation coefficient of diclofenac potassium and metaxalone were 0.996 and 0.998 observed respectively. The average percentage recovery of diclofenac potassium and metaxalone were found to be 99.39 % and 99.85 % respectively. Intra and inter day precision measured as coefficient of variation were less than 2% for both analytes.The proposed HPTLC method has potential applications for determination of diclofenac potassium and metaxalone in combined tablet dosage form.

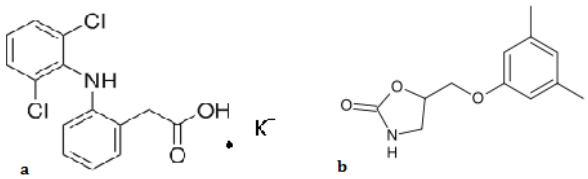
Keywords: Thin layer Chromatography, densitometry, validation, quantification, diclofenac potassium, metaxalone.

INTRODUCTION

Diclofenac potassium is chemically known as 2-(2-(2, 6-dichlorophenylamino) phenyl) acetic acid (Figure **1a**). Diclofenac potassium (DICLO) is responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis.[1]

Metaxalone (META) chemically, 5-[(3, 5-dimethyl phenoxy) methyl]-1,3-oxazolidin-2-one (Figure **1b**) is a muscle relaxant used to relax muscles and relieve pain caused by strains, sprains, and other musculoskeletal conditions.[2] Literature review reveals that methods have been reported for analysis of diclofenac potassium by high performance liquid chromatography (HPLC)[3-7] and high performance thin layer chromatography (HPTLC)[8-10] and for estimation of metaxalone by HPLC[11,12] either alone or in combination with other drugs.

Figure 1. Chemical structure of diclofenac potassium and metaxalone



Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC - thus reducing the analysis time and cost per analysis.

To date, there have been no published reports about the simultaneous estimation of diclofenac potassium and metaxalone by HPTLC in bulk drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous estimation of Diclofenac Potassium and Metaxalone by HPTLC in bulk drug and in pharmaceutical dosage form. The proposed method is validated as per ICH guidelines.[13-15]

MATERIALS AND METHODS

Materials

Working standards of pharmaceutical grade diclofenac potassium (batch no. AF01/10/034) and metaxalone (batch no. 290682) were obtained as generous gifts from Aarti Pharmaceutical pvt. Ltd, (Maharashtra, India). It was used without further purification and certified to contain 99.09 % and 99.10 % (w/w) on dry weight basis, diclofenac potassium and metaxalone respectively. Fixed dose combination tablet (Flexura-D) containing 50 mg Diclofenac Potassium and 400 mg Metaxalone were procured from local market. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton,

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Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60_{F-254} plates, [20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (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 0.1µL/s was used and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of chloroform: methanol (8:1 v/v).Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C ± 2). The length of each chromatogram run was 8 cm. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 281 nm and operated by CATS software (V 3.15, Camag).

Preparation of standard and construction of calibration curve

The standard stock solutions of Diclofenac Potassium and Metaxalone were prepared by dissolving 10 mg of each drug in 10 ml of methanol. From this solution, 1 ml of solution were taken and diluted to 10 ml with the same to get a solution containing 100 µg/ml of each drug. A calibration curve was plotted between concentration against their respective area for Diclofenac Potassium and Metaxalone separately. From the calibration curve, it was found that Diclofenac Potassium had linearity range between 100-180 ng/spot, whereas Metaxalone had a range between 800-1600 ng/spot.

Assay of marketed formulation

For the analysis of pharmaceutical formulation (Brand name: Flexura-D, Label claim: 50 mg diclofenac potassium and 400 mg metaxalone per tablet), twenty tablet of each drug were weighed and powdered individually. The mixture of formulation was prepared by weighing amount equivalent to labeled claim from the powdered formulation. To this, a suitable amount of methanol was added. The mixture was subjected to sonication for 30 min for a complete extraction of the drugs, and then filtered and diluted with methanol at a suitable concentration range and the samples were spotted on the HPTLC for the analysis. The amounts of Diclofenac Potassium and Metaxalone per tablet were calculated by extrapolating the value of area from the calibration curve. Procedure was repeated six times with tablet formulation. The result of analysis of tablet formulation is reported in Table **1**.

Table 1. Analysis of commercial formulation (n=6)

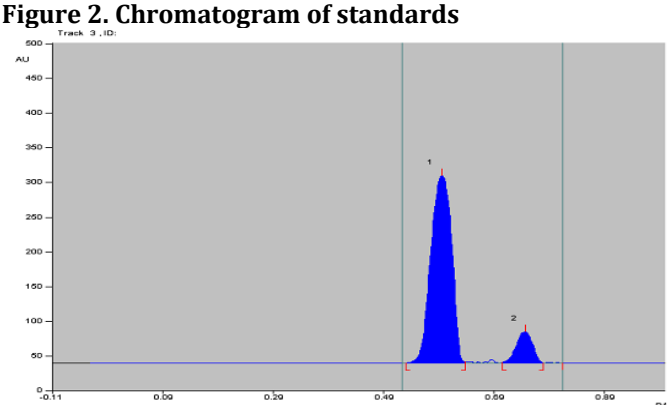
Drug	Label claim (mg/tablet)	Amount found (mg/tablet ± SD)
Diclofenac Potassium	50 mg	99.39 ± 0.31
Metaxalone	400 mg	99.85 ± 0.36

RESULTS AND DISCUSSION

Method development

The HPTLC method was optimized for simultaneous determination of DICLO and META. The mobile phase chloroform: methanol (8:1 v/v) resulted in good resolution and sharp and symmetrical peaks of R_F 0.68 for DICLO and 0.72 for META. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured good repeatability and peak shape of both drugs. (Figure **2**)

Figure 2. Chromatogram of standards



Diclofenac Potassium Rf (0.68) and Metaxalone R_f (0.72)

Method validation

The method was validated for linearity, accuracy, precision, LOD and LOQ, robustness and specificity study. All the validation study was carried out by replicate injection of the sample and standard solutions.

Linearity: The linearity was determined for two drugs diclofenac potassium and metaxalone, separately by plotting a calibration graph of peak area against their respective concentration. From the calibration curve, it was clear that diclofenac potassium had linearity between 100 to 180 ng/spot, whereas metaxalone had a range between 800 to 1600 ng/spot. The linear regression equation for two drugs:

Diclofenac Potassium

$$y = 8.034x + 3266 \ (r^2 = 0.996)$$

Metaxalone

$$y = 0.680x - 610.0 \ (r^2 = 0.998)$$

Where y is peak area and x is concentration.

Accuracy: Accuracy of the developed method was confirmed by doing a recovery study as per ICH guidelines at three different concentration levels (80%, 100% and 120%) by replicate analysis (n=3). Standard drug solutions were added to a preanalyzed sample solution, and then percentage of drug content was calculated. The results of the accuracy study are reported in Table **2**. From the recovery study, it was clear that the method is very accurate for quantitative estimation diclofenac potassium and metaxalone in tablet dosage form because all the statistical results were within the acceptance range (i.e., % RSD <2.0).

Table 2. Recovery studies (n =3)

Label claim	Amount of drug added (%)	Total amount of drug present (mg/mL)	Amount found (mg/mL)	% Recovery
DICLO 50 mg	80	90.0	89.7	99.67
	100	100.0	99.3	99.3
	120	110.0	109.58	99.62
Metaxalone 400 mg	80	720.0	720.62	100.60
	100	800.0	799.83	99.33
	120	880.0	880.67	100.67

Precision: Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and interassay

precision. The standard deviation and relative standard deviation were calculated for two drugs. Intermediate precision was carried out by doing intra- and interday precision studies. In the intraday study, the concentrations of two drugs were calculated on the same day at an interval of 1 h. In the interday study, the concentrations of drug contents were calculated on three different days, and the study expresses within-laboratory variation in different days (Table **3**). The developed method was precise for quantitative study because the precision study was found statistically significant (% RSD <2.0 for intra- and interday studies).

Table 3 Precision studies

Concentration (ng/spot)	Repeatability precision (n=6)			Intermediate precision (n=6)		
	Measured conc.	(%) RSD	Recovery (%)	Measured conc.	(%) RSD	Recovery (%)
DICLO						
100	99.32	0.82	99.32	99.23	0.76	99.23
120	119.8	0.94	99.83	119.25	0.68	99.37
140	139.52	0.89	99.65	140.20	0.71	100.14
META						
800	799.40	1.21	99.92	799.38	1.08	99.92
1000	998.88	1.07	99.88	998.68	1.01	99.86
1200	1198.20	1.12	99.85	1191.32	1.15	99.27

Limits of Detection and Quantitation: The LOD and LOQ were found to be 40 and 60 ng/spot for Diclofenac Potassium and 40 and 60 ng/spot for Metaxalone, respectively.

Robustness: Robustness is checked by making slight deliberate change in the experimental procedures. The result obtained is shown in Table **4**.

Table 4. Robustness testing (n = 3)

Parameter	SD of peak area		% RSD	
	DICLO	META	DICLO	META
Mobile phase composition (± 0.1 ml)	5.35	5.56	0.65	0.29
Time from spotting to chromatography (+20 min)	3.31	3.61	0.49	0.10
Time from chromatography to scanning (+20 min)	3.97	3.14	0.59	0.09

Specificity

Specificity of the method was assessed by comparing the chromatograms obtained from standard drugs with the chromatogram obtained from tablet solutions. Because the retention factor of standard drugs and the retention factor of two drugs in sample solutions were the same, the method was specific. The developed method was specific as no interference of excipients was found (Figure **2**).

CONCLUSION

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of diclofenac potassium and metaxalone in tablets. The method was validated as per ICH guidelines. The proposed HPTLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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