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SIMULTANEOUS HPTLC DETERMINATION OF CAMYLOFIN DIHYDROCHLROIDE AND DICLOFENAC POTASSIUM IN A PHARMACEUTICAL FORMULATION

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ABSTRACT

An HPTLC method for simultaneous determination of Camylofin Dihydrochloride and Diclofenac Potassium in a pharmaceutical formulation has been developed and validated. The analyte were separated on silica gel 60F254 HPTLC plates with Benzene: Methanol: Ammonia in the ratio of 8.0:2.0:0.2, as mobile phase, after chamber saturation for 15 min. The development distance was 9 cm. The plate was then dried in air and scanned and quantified at the wavelength at 220 nm. The limits of detection were 25 µg mL^{-1} and 30 µg mL^{-1} for Camylofin Dihydrochloride and Diclofenac Potassium respectively. The limits of quantification were 50 µg mL^{-1} and 60 µg mL^{-1} for Camylofin Dihydrochloride and Diclofenac Potassium respectively. The method enables accurate, precise, and rapid simultaneous analysis of Camylofin Dihydrochloride and Diclofenac Potassium. It can be conveniently adopted for routine quality control analysis.

Key words: HPTLC, Camylofin Dihydrochloride, Diclofenac Potassium.

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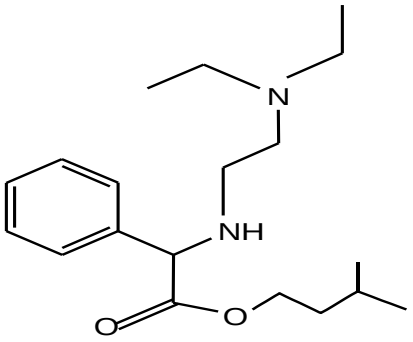
INTRODUCTION:

Camylofin dihydrochloride (CAM) is 3-methylbutyl 2-(2-diethylaminoethylamino)-2-phenyl-acetate hydrochloride is a drug used an antispasmodic[1]. Diclofenac potassium (DIC) is potassium-[(2, 6-dichlorophenyl) amino]-phenyl acetate[2]. It is a potassium salt of an aryl acetic acid derivative. It possesses analgesic, anti-inflammatory, and antipyretic activity. The structure of the drug is shown in Figure 1 and 2. One such combination contains 25 mg of Camylofin dihydrochloride and 25 mg of Diclofenac potassium. A literature survey indicated few methods for the determination of Camylofin dihydrochloride and Diclofenac Potassium individually or in combination with other drug preparations by HPLC.A simple uv spectrophotometric method for the estimation of Diclofenac Potassium in a formulation was reported[3]. An HPTLC method was reported for estimation of Diclofenac potassium in presence of another drug substance in a pharmaceutical preparation[4]. Other analytical method mentioned for assay of Diclofenac Potassium were HPLC method[5-7], UV-spectroscopy method[8-9]. An HPLC method was developed for the estimation of Diclofenac in complex matrix like gels and injections[10]. An HPTLC method was reported for the estimation Camylofin dihydrochloride in pharmaceutical preparation[11] .An HPLC method was also reported for the estimation of Camylofin dihydrochloride in pharmaceutical preparation[12]. HPLC method of Camylofin dihydrochloride in combination with other drug preparations were reported[13-16]. A GC method was reported for the determination of Camylofin dihydrochloride with other drug preparation[17].

The literature revealed that no HPTLC method was available for simultaneous determination of this drug in such pharmaceutical preparation. The method is very cost and time effective since it does not require any mobile phase preparation and can be easily adapted to Quality control testing laboratory.



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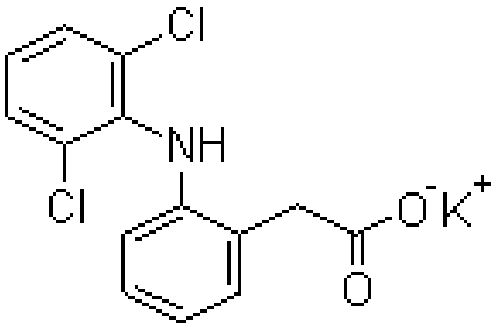

Figure 1: Camylofin dihydrochloride

Figure 2: Diclofenac Potassium

MATERIALS AND METHODS

Reagents and Materials

Active pharmaceutical ingredient of Camylofin dihydrochloride was procured from Sigma Aldrich and Diclofenac Potassium was procured from USP. Tablet dosage form developed by Khandelwal Laboratories, India. High purity Benzene, Methanol and Ammonia was purchased from S.D. Chemicals Limited, Mumbai.

Instrumentation

The HPTLC used is of Camag Linomat 5 sample applicator equipped with 100 µl syringe Camag TLC Scanner 3 was used for densitometric evaluation, equipped with mercury, tungsten and deuterium lamp for scanning of TLC plate.

The separation was achieved on thin layer plates of silica gel aluminum Plate 60 F-254 (20cm×10 cm) with 250 m thickness. Deuterium lamp was used as a source of radiation. Camag Twin Trough chamber was used as development chamber with saturation time of 15 minutes. The injector speed was set at 10 µL/mL. The monochromator bandwidth was set at 220 nm, each track was scanned thrice and baseline correction was used. Sartorius CP224S was used as an analytical balance.

Preparation of Mobile Phase

The mobile phase comprised of Benzene: Methanol: Ammonia (8.0:2.0:0.2 v/v). The chamber was saturated prior to use.

Preparation of Standard Solutions

The stock solution of Camylofin dihydrochloride (2500 µg mL^{-1}) was prepared by dissolving 125.6 mg of Camylofin dihydrochloride (99.9 %) in methanol in a standard 50 mL volumetric flask (stock solution A). The stock solution of Diclofenac potassium (2500 µg mL^{-1}) was prepared by dissolving 125.9 mg of Diclofenac Potassium (99.8 %) in methanol in a standard 50 mL volumetric flask (stock solution B).Transferred 10.0 mL of each stock solution A & B to a 50 mL volumetric flask and diluted up to the mark with methanol. This is working standard solution.

Preparation of Sample solution:

For analysis of the tablet dosage form, twenty tablets were weighed individually and their average weight was determined. The tablets were crushed to fine homogenous powder and quantity equivalent to ten tablets were transferred in a 200mL volumetric flask. Added about 100 mL of Methanol to the volumetric flask, shaken for 10 minutes and then sonicated for 15

minutes. The solution was allowed to stand at room temperature for 20-30 minutes and filtered through Whatman no. 41 filter paper. The residue was washed with Methanol and the combined filtrate was made up to the mark with the same solvent. 10.0 mL of filtrate was quantitatively transferred to a 50 mL volumetric flask was added to it, and solution was diluted up to the mark with methanol.

Validation of the proposed HPTLC method

The method validation was carried out as per ICH guidelines[18]. Various method validation parameters were performed.

Specificity

Specificity of the method was evaluated by injecting diluent, placebo, individual Camylofin dihydrochloride and Diclofenac Potassium and sample solution in to the HPTLC to check any interference of spot.

Linearity

Linearity was evaluated by analysis of working standard solutions of Camylofin dihydrochloride and Diclofenac Potassium of five different concentrations.

Linearity was evaluated by analysis of working standard solutions of Camylofin dihydrochloride and Diclofenac Potassium of seven different concentrations. The range of linearity for Camylofin dihydrochloride and Diclofenac Potassium were from 125 µg mL^{-1} to 375 µg mL^{-1} (250 µg/mL is 100% level).

LOD and LOQ / Sensitivity

Sensitivity was determined by establishing the limit of detection (LOD) and limit of quantification (LOQ). The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively.

Accuracy

Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of Camylofin dihydrochloride and Diclofenac Potassium from standard stock solution was added in placebo to attain 50%, 100% and 150% of sample concentration. Each sample was prepared in triplicate at each level. Blank and standard preparations were injected and chromatograms were recorded.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions. Method precision was

determined from results from six independent determinations at 100% of the test concentrations of Camylofin dihydrochloride and Diclofenac Potassium in the product.

Ruggedness (Intermediate Precision)

Ruggedness study was demonstrated by injecting five individual sample preparations at 100% of the test concentrations of Camylofin dihydrochloride and Diclofenac Potassium on different day using another column and system.

Ruggedness study was done by injecting six individual sample preparations at 100% of the test concentrations of Camylofin dihydrochloride and Diclofenac Potassium on different day and different HPTLC system.

Robustness

The robustness of the method was established by introducing small changes in various parameters like the mobile phase composition and wavelength. Mobile phases having different compositions like Benzene: Methanol: Ammonia (8.0: 2.0: 0.2 v/v), (7.8: 2.2: 0.2 v/v), (8.2: 1.8: 0.2 v/v), (8.1: 2.0: 0.1 v/v) and (7.9: 2.0: 0.3 v/v) were tried and chromatograms were run. The standard and sample were run at different wavelength of 218 nm, 220nm and 222nm.

Solution stability

The solution stability of Camylofin dihydrochloride and Diclofenac Potassium was carried out by leaving the test solutions of sample in a tightly capped volumetric flask at room temperature for 3 days. The same sample solutions were assayed for 24 hours interval up to the study period against freshly prepared standard solution.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions:

To optimize the HPTLC parameters, several mobile phase compositions were tried[19]. A satisfactory separation and good peak shape were obtained with the mobile phase ratio of Benzene: Methanol: Ammonia (8.0: 2.0: 0.2) v/v. A typical densitogram is shown in figure 3.A summary of method optimization is shown in table 1.

Table 1: **Summary of optimization of chromatographic conditions**

Solvent system	Ratio	Observation	Result
Toluene: Methanol	5:5	No spot observed	Method rejected
Toluene: Methanol: Chloroform	7:2:1	No spot observed	Method rejected
Benzene: Methanol	7:3	Spots observed but poor resolution	Method rejected
Benzene: Methanol: Ammonia	8.0:2.0:0.2	Good resolution and good peak shape	Method accepted

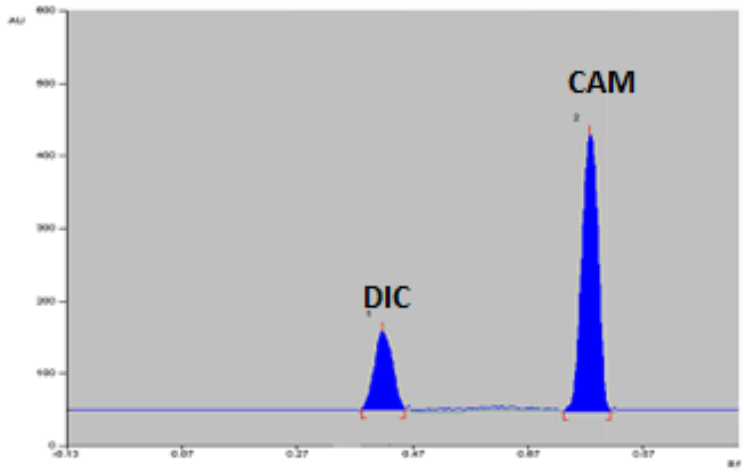

Figure 3: Densitogram of Diclofenac Potassium and Camylofin dihydrochloride at 220 nm.

Specificity

Diluent, placebo, Camylofin dihydrochloride and Diclofenac Potassium and sample solution were injected to check any interference of spot. No spot was observed at the retention time of Camylofin dihydrochloride and Diclofenac Potassium in diluents and Placebo chromatogram. Hence the method was specific.

Linearity

The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. Figure 4 and 5 represents the linearity plots of Camylofin dihydrochloride and Diclofenac Potassium respectively. The regression data obtained for the Camylofin dihydrochloride and Diclofenac Potassium is represented in Table 2. The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

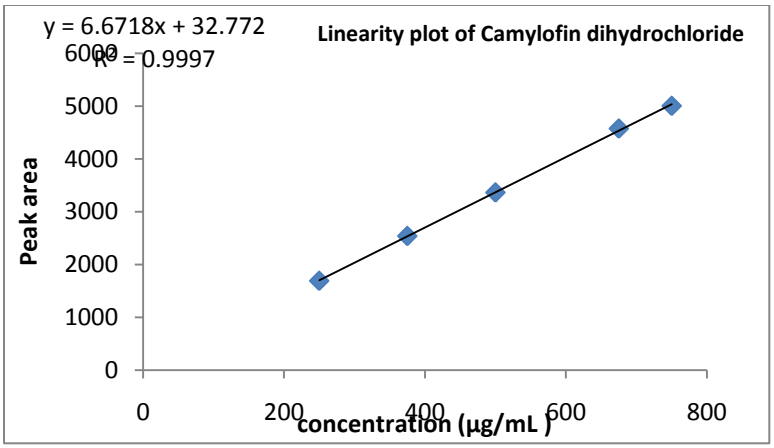

Figure 4: Linearity plot of Camylofin dihydrochloride

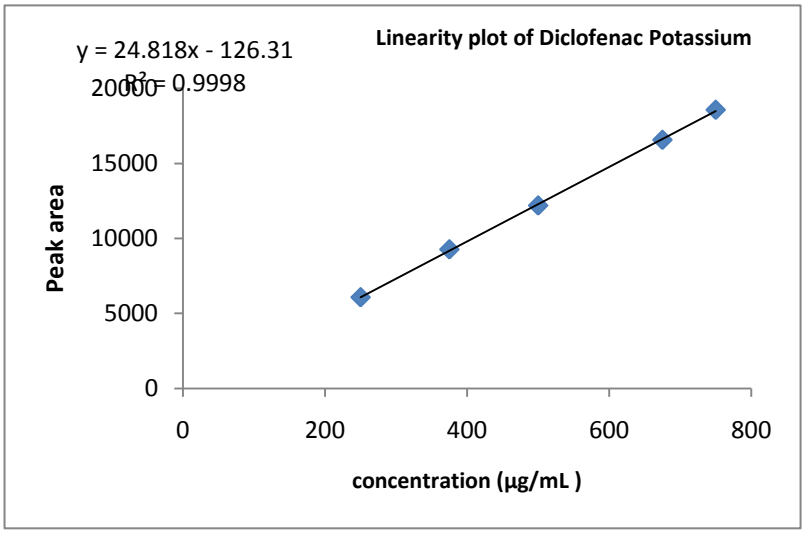

Figure 5: Linearity plot of Diclofenac Potassium

Table 2: Results of Linearity study

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n=5)
Camylofin dihydrochloride	6.67	32.77	0.9997
Diclofenac Potassium	24.82	-126.31	0.9998

LOD and LOQ / Sensitivity

The LOD and LOQ of Camylofin dihydrochloride and Diclofenac Potassium was experimentally determined by six injections of each drug. The LOD of Camylofin dihydrochloride and Diclofenac Potassium was found to be 38 µg mL^{-1} & 15 µg mL^{-1} respectively. The LOQ of Camylofin dihydrochloride and Diclofenac Potassium was found to be 68 µg mL^{-1} & 32 µg mL^{-1} respectively.

Accuracy

Accuracy was expressed as the percentage of analyte recovered by the assay. Table 3 lists the recoveries of the drug from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of Camylofin dihydrochloride and Diclofenac Potassium.

Table 3: Accuracy of the method

Analyte	Recovery Level (%)	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	RSD (%) n= 3	(%) Recovery
Camylofin dihydrochloride	50	250.3	253.1	0.81	101.1
	100	500.6	497.0	0.36	99.3
	150	750.9	746.5	0.52	99.4
Diclofenac Potassium	50	250.9	249.2	0.46	99.3
	100	501.8	500.3	0.35	99.7
	150	752.7	750.2	0.52	99.7

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions. Method precision was determined from results from six independent determinations at 100% of the test concentrations of Camylofin dihydrochloride and Diclofenac Potassium in the product. The result of Precision is given in table 4.

Table 4: Results of Precision experiment.

Results	Camylofin dihydrochloride	Diclofenac Potassium
Drug found in mg/tab (mean)	25.2	24.8
% Mean Assay	100.8	99.2
% RSD	0.35	0.26

Ruggedness (Intermediate Precision)

The mean % Assay obtained was compared with mean % Assay of precision study. The relative standard deviation (RSD) was less than 2%. Refer Table 5.

Table 5: Ruggedness of Assay experiment

Results	Camylofin dihydrochloride	Diclofenac Potassium
Drug found in mg/tab (mean)	24.8	25.1
% Mean Assay	99.2	100.4
% RSD	0.61	0.55
% Difference w.r.t. Precision	1.6	1.2

Solution stability

The % assay of Camylofin dihydrochloride and Diclofenac Potassium were checked in the test solutions. The % difference of assay of Camylofin dihydrochloride and Diclofenac Potassium with respect to initial assay during solution stability experiment was within 2.0. No significant changes were observed in the content of Camylofin dihydrochloride and Diclofenac Potassium during solution stability experiment. Sample solutions used during the experiment were stable upto the study period of 3 days. The results are reported in Table 6.

Table 6: Results of Solution stability

Condition	% Assay of Camylofin dihydrochloride	% Difference w.r.t. initial assay	% Assay of Diclofenac Potassium	% Difference w.r.t. initial assay
Initial	100.8	NA	99.2	NA
Day 1	100.1	0.7	99.5	0.3
Day 2	99.6	1.2	99.6	0.4
Day 3	99.5	1.3	99.3	0.1

Robustness

The robustness of the method was established by introducing small changes in various parameters like the mobile phase composition and wavelength. The % RSD for standard injections was less than 2.0% and the % Assay for Camylofin dihydrochloride and Diclofenac Potassium were within 98.0% to 102.0%.

CONCLUSION

The proposed HPTLC technique is simple, precise, specific, robust and accurate. Statistical analysis proves that the method is suitable for analysis of Camylofin dihydrochloride and Diclofenac Potassium in pharmaceutical tablet formulation.

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