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Simultaneous RP HPLC determination of camylofin dihydrochloride and diclofenac potassium in pharmaceutical preparations

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ABSTRACT

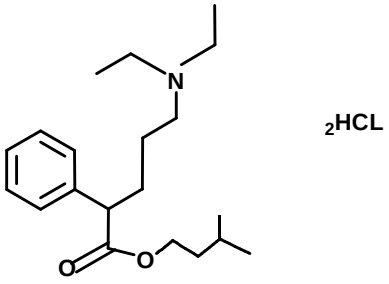
A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of camylofin dihydrochloride and diclofenac potassium using methyl paraben as an internal standard. Chromatographic separation of these two drugs was performed on inertsil C_{18} column (250mm × 4.6 mm, 5 µm) as stationary phase with a mobile phase comprising of 0.05 M KH2PO4 **:** methanol (35:65) at a flow rate of 1.5mL min^{-1} and UV detection at 220 nm. The Retention time of Methyl paraben, Camylofin dihydrochloride and diclofenac potassium were 3.60 min, 4.85 min and 13.10 min respectively. The proposed method was validated for linearity, accuracy, precision, LOD, LOQ. Linearity, accuracy and precision were found to be acceptable over the ranges of 250-750 - µg mL^{-1} for both camylofin dihydrochloride and diclofenac potassium. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

ICH Guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Camylofin dihydrochloride;
Diclofenac potassium.

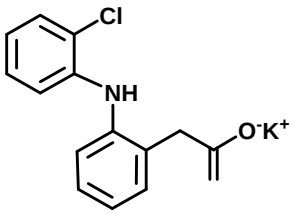
INTRODUCTION

Camylofin dihydrochloride 3-methylbutyl 2-(2-diethylaminoethylamino)-2-phenyl-acetate hydrochloride is a drug used an antispasmodic. Diclofenac potassium is potassium-[(2,6-dichlorophenyl)amino]-phenyl acetate. 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 & Figure 2. One such combination contains 25 mg of camylofin dihydrochloride and 25 mg of Diclofenac potassium. The literature revealed no method was available for simultaneous determination of this drug in such pharmaceutical preparation by HPLC[3-8]. Therefore an HPLC method was developed for determination of camylofin dihydrochloride and diclofenac potassium from their dosage form. The method described is simple, fast, precise and accurate for simultaneous determination of camylofin dihydrochloride and diclofenac potassium from pharmaceutical preparation.



Camylofin dihydrochloride ($C_{19}H_{32}N_2O_2$, 2HCl)

Figure 1 : Structures of camylofin dihydrochloride



Diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$)

Figure 2 : Structure of diclofenac potassium

Chemicals and reagents

Anaspas tablets manufactured by Khandelwal lab, India were procured from the market. Potassium dihydrogen orthophosphate and methanol were from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

EXPERIMENTAL

Method development and optimization of chromatographic conditions

To develop a suitable LC method for the analysis of camylofin dihydrochloride and diclofenac potassium in their dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involve, time required for the analysis, better separation of drugs. Chromatographic separation was preformed with Shimadzu LC 2010 High performance liquid chromatography having HPLC isocratic pump, equipped with auto sampler and a photo-diode array detector. The uv spectrum of camylofin dihydrochloride and diclofenac potassium were scanned on photo diode array detector for selecting the working wavelength. Peak purity of camylofin dihydrochloride and diclofenac potassium were checked using photo diode array detector. Chromatograms and data were recorded by means of Class VP software. Inertsil C_{18} column (250mm x 4.6 mm, 5 µm particle) was used for the analysis. The mobile phase comprising of 0.05 M KH_2PO_4 in water **:** Methanol (35:65 *v/v)*. The system was run at a flow rate of 1.5mL min^{-1}, 20 µL of sample was injected in the chromatographic system and detection wavelength was set at 220 nm for simultaneous determination of camylofin dihydrochloride and diclofenac potassium. A typical HPLC chromatogram for simultaneous determination of camylofin dihydrochloride and diclofenac potassium from pharmaceutical formulation is shown in Figure 3 and Figure 4.

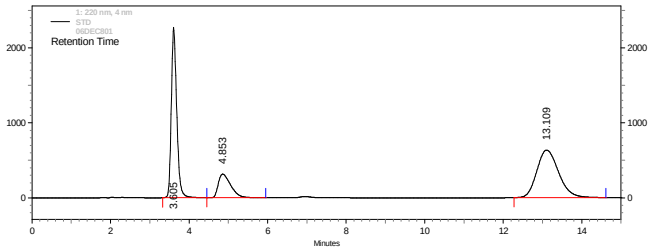


Figure 3 : Chromatogram of camylofin dihydrochloride and diclofenac potassium with methyl paraben (Internal standard) in standard preparation

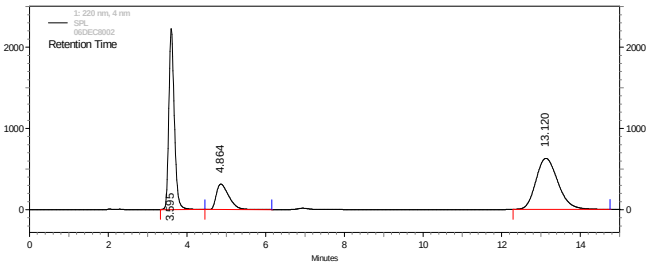


Figure 4 : Chromatogram of camylofin dihydrochloride and diclofenac potassium with methyl paraben (Internal standard) in sample preparation

Preparation of standard stock solutions

The stock solution of camylofin dihydrochloride (2500 µg mL^{-1}) was prepared by dissolving 250.2 mg of camylofin dihydrochloride (99.9 %) in methanol in a standard 100mL volumetric flask (solution A). The stock solution of diclofenac potassium (2500 µg mL^{-1}) was prepared by dissolving 250.4 mg of diclofenac potassium (99.6 %) in methanol in a standard 100mL volumetric flask (solution A). Internal standard (methyl paraben) stock solution (5000 µg mL^{-1}) was prepared by dissolving 499.8 mg of methyl paraben in methanol in a 100mL standard volumetric flask (solution C).

Working standard solution

Transferred 10.0 mL of each stock solutions A, B & C to a 50 mL volumetric flask and diluted up to the mark with methanol.

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed into a homogeneous powder and quantity equivalents to ten tablets were transferred in a 100mL volumetric flask,

dissolved in methanol and filtered through Whatman no. 41 filter paper. The 10.0 mL of filtrate was quantitatively transferred to a 50 mL volumetric flask, 10.0 mL of internal standard solution was added to it, and solution was diluted up to the mark with methanol.

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out[1,2]. System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 20-µL standard solutions of camylofin dihydrochloride, diclofenac potassium of strengths 500 µg mL^{-1} using methyl paraben as an internal standard. This was repeated five times. The RSD values of camylofin dihydrochloride and diclofenac potassium were 0.31 and 0.18 respectively. The RSD values were found to be satisfactory and meeting the requirements of USP 32 (RSD less than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

TABLE 1 : Result of system suitability

Parameters	Methyl paraben (IS)	Camylofin dihydrochloride	Diclofenac potassium
Resolution	-	2.94	10.32
Tailing factor	1.29	1.69	1.20
Theoretical plates	3034	1097	2676

Linearity

Linearity was evaluated by analysis of working standard solutions of camylofin dihydrochloride and diclofenac potassium of seven different concentrations[1,2]. The range of linearity was from 250 - 750 µg mL^{-1} for both camylofin dihydrochloride and diclofenac potassium. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the camylofin dihydrochloride and diclofenac potassium is represented in TABLE 2. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

TABLE 2 : Results of linearity

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n=7)
Camylofin dihydrochloride	0.001	-0.006	0.9993
Diclofenac potassium	0.002	0.002	0.9999

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively[1,2]. The LOD and LOQ of camylofin dihydrochloride and diclofenac potassium were experimentally determined by six injections of each drug. The LOD of camylofin dihydrochloride and diclofenac potassium was found to be 0.05µg mL^{-1} & 0.08 µg mL^{-1} respectively. The LOQ of camylofin dihydrochloride and diclofenac potassium was found to be 0.2 µg mL^{-1} & 0.3 µg mL^{-1} respectively.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[1,2]. The relative standard deviation was less than 2%. 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 RSD was found to be 0.49. Refer TABLE 3.

TABLE 3 : Results of assay experiment

	Camylofin dihydrochloride	Diclofenac potassium
Drug found in mg/tab (mean)	25.03	25.10
Mean %	100.11	100.40
RSD	0.40	0.40

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of drug were added, to determine if there are positive or negative interferences from excipients present in the formulation[1,2]. Each set of addition were repeated three times .The accuracy was expressed as the percentage of analytes recovered by the assay. TABLE 4 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 4

Analyte	Initial conc. (ppm)	Conc. added (ppm)	Total conc. (ppm)	Conc. found (ppm)	RSD (%) n= 3	Recovery (%)
Camylofin dihydrochloride	500	0	500.0	501.57	0.59	100.31
	500	50.0	550.0	548.50	0.36	99.73
	500	100.0	600.0	602.20	0.23	100.37
	500	150.0	650.0	650.23	0.23	100.04
Diclofenac potassium	500	0	500.0	500.12	0.05	99.89
	500	50.0	550.0	550.16	0.12	100.01
	500	100.0	600.0	600.47	0.16	100.35
	500	150.0	650.0	649.95	0.12	100.32

DISCUSSION AND CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried but good peak shape and good resolution between Camylofin dihydrochloride, diclofenac potassium and Methyl paraben were observed using the mobile phase mentioned in chromatographic conditions. The method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of %RSD for Method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate, easily reproducible and can be used for simultaneous determination of camylofin dihydrochloride and diclofenac potassium from pharmaceutical preparations.

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