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Simultaneous RP-HPLC determination of camylofin dihydrochloride 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 using methyl paraben as an internal standard. Chromatographic separation of these two drugs was performed on a waters symmetry C_{18} column (250mm × 4.6 mm, 5μm) as stationary phase with a mobile phase comprising of 0.05% trifluoro acetic acid in water and 0.05% trifluoro acetic acid in acetonitrile (40:60 *v/v)*, at a flow rate of 0.7mL min^{-1} and UV detection at 220 nm. The Retention time of Camylofin Dihydrochloride and Methyl paraben were 3.10 min and 4.77 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 camylofin Dihydrochloride. It can be conveniently adopted for routine quality control analysis.

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KEYWORDS

ICH guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Camylofin dihydrochloride.

INTRODUCTION

Camylofin Dihydrochloride 3-methylbutyl 2-(2-diethylaminoethylamino)-2-phenyl-acetate hydrochloride is a drug used an antispasmodic. The structure of the drug is shown in figure 1. One such combination contains 25 mg/mL of camylofin Dihydrochloride. The literature revealed no method was available for simultaneous determination of this drug in such pharmaceutical preparation by HPLC. Therefore an HPLC method was developed for determination of camylofin Dihydrochloride from their dosage form[1,2,3]. The method described is simple, fast, precise and accurate for simultaneous determination of camylofin dihydro chloride from pharmaceutical preparation.

Chemicals and reagents

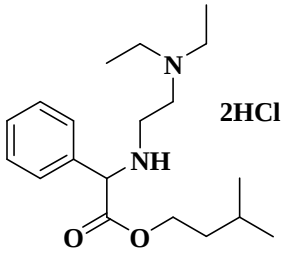
Standards were supplied from Accutest lab., Mumbai, India. Anafortan injections 25 mg/ml manufactured by Khandelwal lab, India was procured from the market. Acetonitrile and trifluroacetic acid were from Qualigens and across chemicals resp. Double distilled water was employed throughout the work. All 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 in their dosage form, dif-

ferent 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 was scanned on photo diode array detector for selecting the working wavelength. Peak purity of camylofin Dihydrochloride was checked using photo diode array detector. Chromatograms and data were recorded by means of chemstation software. Waters symmetry C_{18} column (250mm × 4.6 mm, 5µm particle) was used for the analysis. The mobile phase comprising of 0.05% trifluroacetic acid in water and 0.05% trifluroacetic acid in acetonitrile (40:60 v/v). The system was run at a flow rate of 0.7mL min^{-1}, 20µL of sample was injected in the chromatographic system and detection wavelength was set at 220 nm for simultaneous determination of camylofin Dihydrochloride. A typical HPLC chromatogram for simultaneous determination of camylofin Dihydrochloride from pharmaceutical formulation is shown in figures 2 and 3.



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

Figure 1: Structures of camylofin dihydrochloride

Preparation of standard stock solutions:

The stock solution of camylofin Dihydrochloride (2500µg mL^{-1}) was prepared by dissolving 125.2 mg of camylofin Dihydrochloride (99.9 %) in water: acetonitrile (1:1) in a standard 50mL volumetric flask

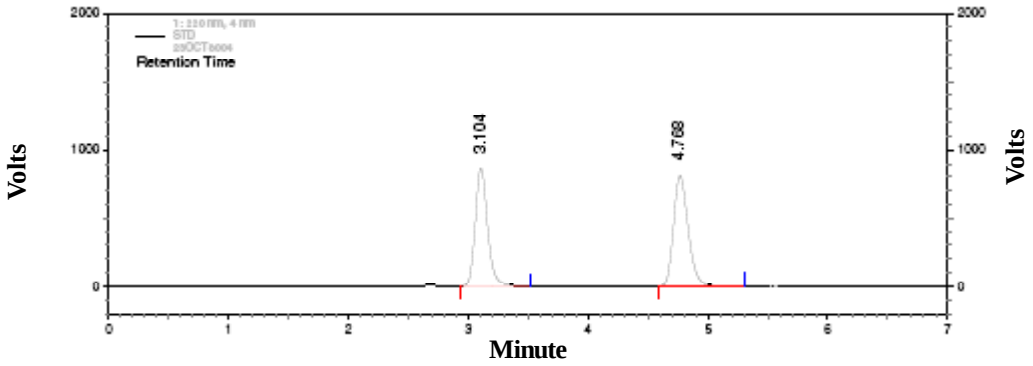


Figure 1: Chromatogram of camylofin Dihydrochloride with Methyl paraben (internal standard) in standard preparation

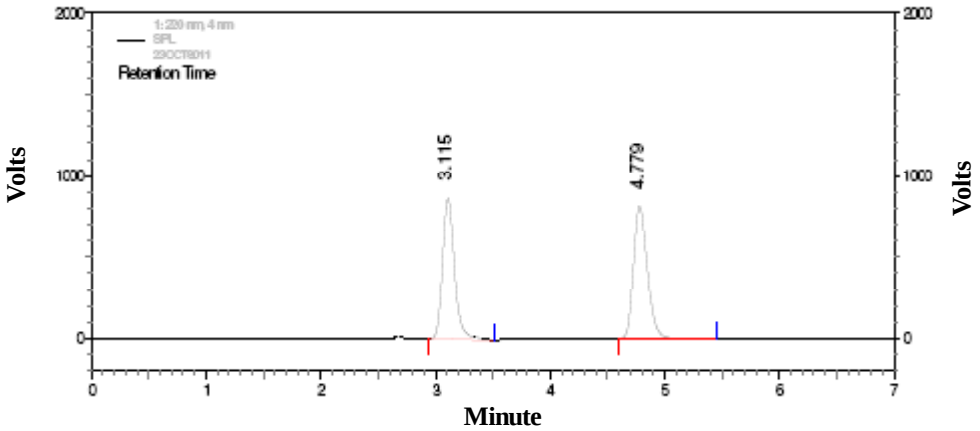


Figure 3: Chromatogram of camylofin dihydrochloride with methyl paraben (internal standard) in sample preparation

(solution A). Internal standard (methyl paraben) stock solution (750μg mL^{-1}) was prepared by dissolving 37.6 mg of methyl paraben in water:acetonitrile (1:1) in a 50mL standard volumetric flask (solution B).

Working standard solution

Transferred 5.0 mL of each stock solutions A and B to a 25 mL volumetric flask and diluted up to the mark with water:acetonitrile (1:1).

Sample preparation:

Ten ampules of injections were mixed homogeneously and 10 mL of solution were transferred in a 100mL volumetric flask, dissolved in water:acetonitrile (1:1), and filtered through Whatman no. 41 filter paper. The filtrate (5mL) was quantitatively transferred to a 25 mL volumetric flask, 5.0 mL of internal standard solution was added to it, and solution was diluted up to the mark with water:acetonitrile (1:1).

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 20μL standard solutions of camylofin Dihydrochloride of strengths 500μg mL^{-1} using methyl paraben as an internal standard. This was repeated five times. The RSD values of camylofin Dihydrochloride was 0.32. The RSD values was found to be satisfactory and meeting the requirements of USP 31 (RSD less than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

Linearity

Linearity was evaluated by analysis of working standard solutions of camylofin Dihydrochloride of seven different concentrations[1-2]. The range of linearity was from 250 - 750μg mL^{-1} for camylofin Dihydrochloride. 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 Dihydro chloride 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 1: Result of system suitability

Parameters	Camylofin dihydrochloride	Methyl paraben (IS)
Resolution	-	8.32
Tailing factor	1.31	1.24
Theoretical plates	4661	7582

TABLE 2 : Results of linearity

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n=7)
Camylofin dihydrochloride	0.002	-0.007	0.9998

TABLE 3: Results of assay experiment

	Camylofin dihydrochloride
Drug found in mg/ml (mean)	24.94
Mean %	99.76
RSD	0.40

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[3,4]. The LOD and LOQ of camylofin Dihydrochloride was experimentally determined by six injections of each drug. The LOD of camylofin Dihydrochloride was found to be 0.25μg mL^{-1}. The LOQ of camylofin Dihydrochloride was found to be 0.7μg mL^{-1}.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[3-4]. The relative standard deviations was less than 2%. Method precision was determined from results from six independent determinations at 100% of the test concentrations of camylofin Dihydrochloride in the product. The RSD was found to be 0.49. Refer TABLE 3.

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 la-

TABLE 4 : Accuracy of the method

Analyte	Initial conc. (mg)	Conc. added (mg)	Total conc. (mg)	Conc. found (mg)	RSD (%) n= 3	Recovery (%)
Camylofin Dihydrochloride	25	0	25.0	25.01	0.18	100.04
	25	2.5	27.5	27.45	0.39	99.82
	25	5.0	30.0	30.08	0.64	100.27
	25	7.5	32.5	32.49	0.46	99.97

bel claim of drug was added, to determine if there are positive or negative interferences from excipients present in the formulation[2]. Each set of addition was 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.

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 and Methyl paraben was 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 Dihydro chloride from pharmaceutical preparations.

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