ISSN : 0974-7419 Volume 10 Issue 3

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Review

ACAIJ, 10(3) 2011 [207-210]

Simultaneous HPTLC determination of Camylofin dihydrochloride in pharmaceutical preparations

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Received: 17th August, 2010 ; Accepted: 27th August, 2010

ABSTRACT

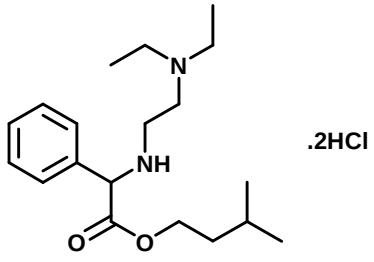
A simple, fast and precise high performance thin layer chromatographic method has been developed for the simultaneous determination of Camylofin dihydrochloride using Mebeverine as an internal standard. Chromatographic separation of these two drugs was performed using Silica gel 60F 254 as stationary phase with a mobile phase comprising of Benzene:Methanol and Ammonia (75:25:3 v/v). Under these conditions the Rf for Camylofin peak was 0.62. Quantitation was achieved by densitometric scanning at 254nm. The Rf values for Camylofin dihydrochloride and Mebeverine HCl peaks were 0.62 and 0.78 respectively. The proposed method was validated for linearity, accuracy, precision. The response to Camylofin dihydrochloride was a linear function of concentration over the range 125-1000 μg mL^{-1} i.e. 25% to 200% of the working assay concentration. The method permits reliable quantification of Camylofin dihydrochloride and good resolution and separation of Camylofin dihydrochloride from internal standard of Mebeverine HCl. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

ICH Guidelines;
Validation;
HPTLC;
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 Fig I. 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 HPTLC. Therefore an HPTLC method was developed for determination of Camylofin dihydrochloride from their dosage form[1-3]. The method described is simple, fast, precise and accurate for simultaneous determination of Camylofin dihydrochloride from pharmaceutical preparation.



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

Figure 1 : Structures of Camylofin dihydrochloride

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EXPERIMENTAL

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. Benzene, Methanol, used was from Rankem and ammonia used was from S.D Fine chemicals. All dilutions were performed in standard volumetric flasks.

Method development and optimization of chromatographic conditions

To develop a suitable HPTLC method for the analysis of Camylofin dihydrochloride 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.

Method development and optimization of high performance thin layer chromatographic conditions

To develop a suitable HPTLC method for the analysis of Camylofin dihydrochloride in their combined 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 using a CAMAG TLC system comprising of a Linomat-5 applicator and CAMG TLC scanner. Chromatography was performed on HPTLC silicagel 60F as stationary phase. The mobile phase comprising of Benzene: Methanol:Ammonia (75: 25: 3 v/v)., 5 µL of sample was spotted in the chromatographic system and detection wavelength was set at 254nm for simultaneous determination of Camylofin dihydrochloride. The plate was developed to a distance of 70 mm using Benzene: Methanol : Ammonia (75: 25 : 3 v/v), as a mobile phase with Camag twin trough chamber. The developed plates were dried with the help of a drier. Evaluation of the plates was performed at =254nm using tungsten lamp with the help of Camag TLC scanner. The wavelength used for evaluation was selected after acquiring spectra of the standard and the sample. A typical HPTLC chromatogram for simultaneous determination of Camylofin dihydrochloride from pharmaceutical formulation is shown in figure 2.

Preparation of standard stock solutions

The stock solution of Camylofin dihydrochloride (1000 µg mL^{-1}) was prepared by dissolving 25.1 mg of Camylofin dihydrochloride (99.9 %) in methanol in a standard 25mL volumetric flask (solution A). Internal standard (Mebeverine HCl) stock solution (1000 µg mL^{-1}) was prepared by dissolving 25.6 mg of Mebeverine HCl in methanol in a 25mL standard volumetric flask (solution B).

Working standard solution

Transferred 5.0 mL of each stock solutions A & B to a 10 mL volumetric flask and mix.

Sample preparation

Ten ampoules of injections were mixed homogeneously and 4 mL of solution were transferred in a 100mL volumetric flask, dissolved in methanol, and filtered through Whatman no. 41 filter paper. The filtrate (5mL) was quantitatively transferred to a 10 mL volumetric flask; 5.0 mL of internal standard solution was added to it, and mixed.

RESULTS AND DISCUSSION

Linearity

Standard Stock solution preparation

The stock solution of Camylofin dihydrochloride (1000 µg mL^{-1}) was prepared by dissolving 100.24 mg of Camylofin dihydrochloride (99.9 %) in methanol in a standard 100 mL volumetric flask (solution A).

The linearity of response was determined by preparing 5 different concentrations of standard solution of assay ranging from 25% to 200% of the working assay concentration (500 ppm)[2].

The test was carried out by injecting 5 µL standard solutions of Camylofin dihydrochloride of strengths 500µg mL^{-1} using Mebeverine HCl as an internal standard. Each of these solutions (5 µl) was applied to a plate, the plate was developed and detector response to the different concentrations was measured. A graph of peak area against concentration was plotted. The plot was linear in the range 125

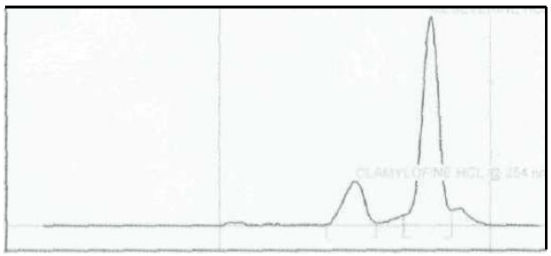


Figure 2 : A typical HPTLC chromatogram for simultaneous determination of Camylofin dihydrochloride from pharmaceutical formulation

TABLE 1 : Result of Linearity/ Range

Sr. No.	% Level	Conc. in ppm	Mean peak Area
1	25	125	695.31
2	50	250	1385.08
3	100	500	2835.40
4	150	750	4221.86
5	200	1000	5608.63
Slope			5.6275
Intercept			-5.1999
Correlation coeff. (r)			0.99996
r^2			0.9999

TABLE 2 : Results of assay experiment

	Camylofin dihydrochloride
Drug found in mg/mL (mean)	24.86
Mean %	99.44
RSD	0.48

TABLE 3 : Accuracy of the method

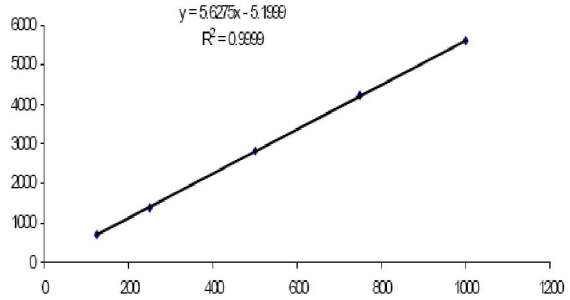
Analyte	Initial conc. (mg)	Conc. added (mg)	Total conc. (mg)	Conc. found (mg)	RSD (%) n= 3	Recovery (%)
Camylofin dihydrochloride	500	0	500	493.88	0.41	98.78
	500	50	550	542.81	0.34	98.69
	500	100	600	595.67	0.15	99.28

to 1000 μg mL^{-1}. The experiment was performed twice and mean was used for calculations. The data is summarized in TABLE 1.

The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration as evident from Graph 1 The correlation coefficient value found to be 0.99996.

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 were less than 2%. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of Camylofin dihydrochloride in the product. The RSD was found to be 0.48. Refer TABLE 2.



Graph 1 : Graph of Linearity/Range

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% and 120% of the label claim of drug was added, to determine if there are positive or negative interferences from excipients present in the formulation[4]. Each set of addition was repeated three times. The accuracy was expressed as the percentage of analytes recovered by the assay. TABLE 3 lists the recoveries of the drug from a series of spiked concentrations. The results indicate the method is accurate for simultaneous determination of Camylofin dihydrochloride.

DISCUSSION AND CONCLUSION

Several mobile phases such as Toluene, Ethyl acetate, ethanol, acetone, Glacial acetic acid in different ratios were tried but good peak shape and good resolution between Camylofin dihydrochloride and Mebeverine HCl was observed using the mobile phase mentioned in chromatographic conditions. The method after being 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

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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 from pharmaceutical preparations.

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