

**SIMULTANEOUS RP HPLC DETERMINATION OF
CAMYLOFIN DIHYDROCHLORIDE AND PARACETAMOL IN
PHARMACEUTICAL PREPARATIONS.**

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ABSTRACT :

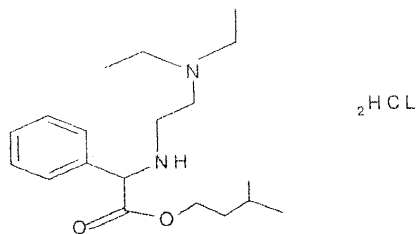
A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of camylofin dihydrochloride and paracetamol using methyl paraben as an internal standard. Chromatographic separation of these two drugs was performed on waters symmetry C18 column (250 mm x 4.6 mm, 5 mm) as stationary phase with a mobile phase comprising of 0.05% trifluoro acetic acid in water and 0.05% trifluoro acetic acid in acetonitrile (50 : 50 v/v), at a flow rate of 1.0 mL min⁻¹ and UV detection at 220 nm. The Retention time of Camylofin dihydrochloride and paracetamol and Methyl paraben were 3.10 min, 3.42 min and 4.70 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 25-75 mg mL⁻¹ for camylofin dihydrochloride and 250-750 - mg mL⁻¹ for paracetamol. It can be conveniently adopted for routine quality control analysis.

Key Words :

ICH Guidelines, Validation, Column liquid chromatography, Pharmaceutical preparations, Camylofin dihydrochloride, Paracetamol

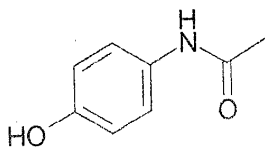
Introduction :

Camylofin dihydrochloride 3-methylbutyl 2 - (2- diethylaminoethylamino) - 2-phenyl-acetate hydrochloride is a drug used as an antispasmodic. Paracetamol is chemically 4 - hydroxy acetanilide, a centrally and peripherally acting analgesic and antipyretic agent [2] the structure of the drug is shown in Fig I and Fig. II. One such combination contains 11.5 mg of camylofin dihydrochloride and 125 mg of Paracetamol per 5 mL. 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 and paracetamol from their dosage form [1, 2, 3, 4]. The method described is simple, fast, precise and accurate for simultaneous determination of camylofin dihydrochloride and paracetamol from pharmaceutical preparation.



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

Fig. I – Structures of camylofin dihydrochloride



Paracetamol ($C_8H_9NO_2$)

Fig. II – Structure of Paracetamol

Chemicals and Reagents :

Anafortan syrup manufactured by Khandelwal lab, India was procured from the market. Acetonitrile and trifluoroacetic acid were from Qualigens & 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 and paracetamol 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 performed 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 paracetamol were scanned on photo diode array detector for selecting the working wavelength. Peak purity of camylofin dihydrochloride and paracetamol were checked using photo diode array detector. Chromatograms and data were recorded by means of chemstation software. Waters symmetry C18 column (250 mm x 4.6 mm, 5 mm particle) was used for the analysis. The mobile phase comprising of 0.05% trifluoroacetic acid in water and 0.05% trifluoroacetic acid in acetonitrile (50 : 50 v/v). The system was run at a flow rate of 1.0 mL min⁻¹, 5mL of sample was injected in the chromatographic system and detection wavelength was set at 220 nm for simultaneous determination of camylofin dihydrochloride and paracetamol. A typical HPLC chromatogram for simultaneous determination of camylofin dihydrochloride and paracetamol from pharmaceutical formulation is shown in Fig. III and Fig., IV

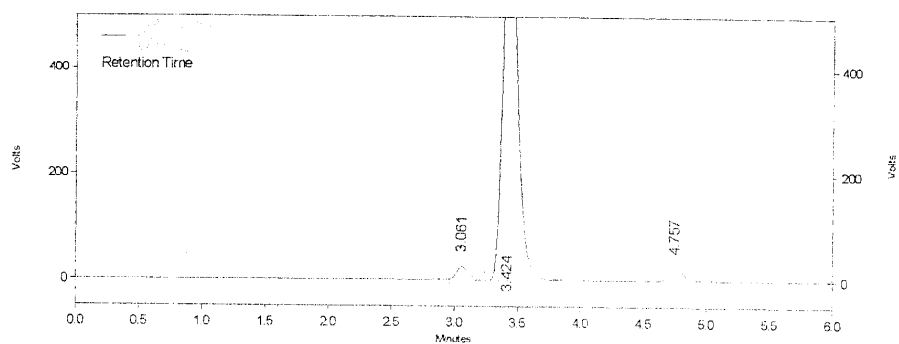


Fig. II chromatogram of Camylofin dihydrochloride and paracetamol with Methyl paraben (internal standard) in standard preparation

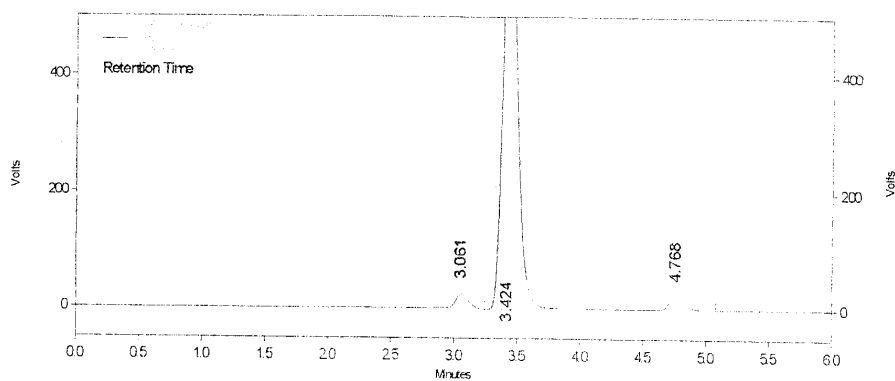


Fig. III chromatogram of Camylofin dihydrochloride and paracetamol with Methyl paraben (internal standard) in sample preparation

Preparation of Standard Stock Solutions :

The stock solution of camylofin dihydrochloride (250 mg mL⁻¹) was prepared by dissolving 25.2 mg of camylofin dihydrochloride (99.9%) in water acetonitrile (1:1) in a standard 100 mL volumetric flask (solution A). The stock solution of paracetamol (2500 mg mL⁻¹) was prepared by dissolving 250.4 mg of paracetamol (99.6%) in water acetonitrile (1:1) in a standard 100 mL volumetric flask (solution A). Internal standard (methyl paraben) stock solution (75 mg mL⁻¹) was prepared by dissolving 7.45 mg of methyl paraben in water : acetonitrile (1:1) in a 100mL standard volumetric flask (solution C).

Working Standard Solution :

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

Sample Preparation :

Transferred 5.0 mL of syrup in a 250 mL volumetric flask, dissolved in water:acetonitrile (1:1), and filtered through Whatman no. 41 filter paper. The filtrate (5 mL) 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 5-mL standard solutions of camylofin dihydrochloride, paracetamol of strengths 50 mg mL⁻¹, 500 mg mL⁻¹ using methyl paraben as an internal standard. This was repeated five times. The RSD values of camylofin dihydrochloride and paracetamol were 0.32 and 0.24 respectively. The RSD values were 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.

Parameters	Camylofin dihydrochloride	Paracetamol	Methyl paraben (IS)
Resolution	-	1.88	6.45
Tailing factor	1.33	1.33	1.35
Theoretical plates	4242	4745	7760

Table I - Result of System suitability.

Linearity

Linearity was evaluated by analysis of working standard solutions of camylofin dihydrochloride and paracetamol of seven different concentrations [1-2]. The range of linearity was from 25 - 75 mg mL⁻¹ for camylofin dihydrochloride and 250 - 750 mg mL⁻¹ for paracetamol. 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 paracetamol is represented in Table II. The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

Analyte	Slope	Intercept	Correlation coefficient (r ²) (n = 7)
Camylofin dihydrochloride	0.004	0.011	0.9993
Paracetamol	0.066	0.102	0.9992

Table II - Results of Linearity

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 and paracetamol was experimentally determined by six injections of each drug. The LOD of camylofin dihydrochloride and paracetamol was found to be 0.25 mg mL⁻¹ & 0.3 mg 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 [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 and paracetamol in the product. The RSD was found to be 0.49.

Refer Table III

	Camylofin dihydrochloride	Paracetamol
Drug found in mg/5ml (mean)	12.54	124.90
Mean %	100.29	99.92
RSD	0.80	0.80

Table III - Results of Assay experiment

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 [2]. Each set of addition were repeated three times. The accuracy was expressed as the percentage of analytes recovered by the assay. Table IV 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 paracetamol.

Analyte	Initial conc. (ppm)	Conc. added (ppm)	Total conc. (ppm)	Conc. found (ppm)	RSD (%) n = 3	Recovery (%)
Camylofin	50	0	50.0	50.16	0.59	100.32
	50	0.05	55.0	54.84	0.36	99.73
	50	10.0	60.0	60.22	0.23	100.37
	50	15.0	65.0	65.02	0.24	100.03
dihydrochloride	500	0	500.0	500.12	0.05	100.02
	500	50.0	550.0	550.16	0.12	100.03
	500	100.0	600.0	600.47	0.16	100.08
	500	150.0	650.0	649.95	0.12	99.99

Total IV - Accuracy of the method

Discussion and conclusion :

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried out good peak shape and good resolution between Camylofin dihydrochloride, paracetamol and Methyl paraben were observed using the mobile phase mentioned in chromatographic conditions. 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 paracetamol from pharmaceutical preparations.

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Development and Validation of HPLC Determination Of Camylofin Dihydrochloride And Paracetamol In Pharmaceutical Preparations

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A reversed phase high performance liquid chromatography (HPLC) method was developed for the simultaneous determination of camylofin dihydrochloride and paracetamol. Methyl paraben was used as an internal standard. The separation of these two drugs was achieved on a reversed phase C₁₈ column (250mm x 4.6 mm, 5 μm) using a mobile phase comprising of acetonitrile, water and 0.05% trifluoro acetic acid (15:85:0.1) at a flow rate of 1.0 mL min⁻¹ at 30°C. The retention time of Camylofin dihydrochloride, paracetamol and Methyl paraben were 1.2, 1.8 and 2.5 min respectively. The proposed method was validated for accuracy, precision, LOD and LOQ. The LOD and LOQ were found to be 0.05 μg mL⁻¹ and 0.25 μg mL⁻¹ for camylofin dihydrochloride and paracetamol respectively. It can be used for the routine quality control analysis.