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## 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 C<sub>18</sub> 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 (50:50 v/v), at a flow rate of 1.0mL min<sup>-1</sup> 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 -μg mL<sup>-1</sup> for camylofin dihydrochloride and 250-750-μg mL<sup>-1</sup> for paracetamol. 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;  
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<sup>[1]</sup>. The structure of the drug is shown in figures 1 and 2. One such combination contains 12.5 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<sup>[4-7]</sup>. The method described is simple, fast, precise and accurate for simultaneous determination of camylofin dihydrochloride and paracetamol from pharmaceutical preparation.

### Chemicals and reagents

Anafortan syrup manufactured by Khandelwal lab, India was procured from the market. Acetonitrile and trifluoroacetic acid were from Qualigens and across chemicals resp. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

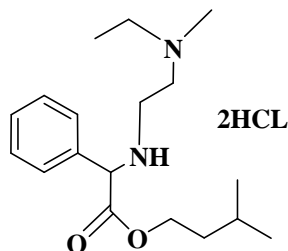
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### EXPERIMENTAL

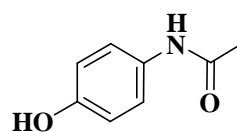
#### Method development and optimization of chromatographic conditions

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 C<sub>18</sub> column (250mm×4.6 mm, 5μm



Camylofin dihydrochloride (C<sub>19</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>·2HCl)  
Figure 1: Structures of camylofin dihydrochloride



Paracetamol (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>)  
Figure 2: Structure of paracetamol

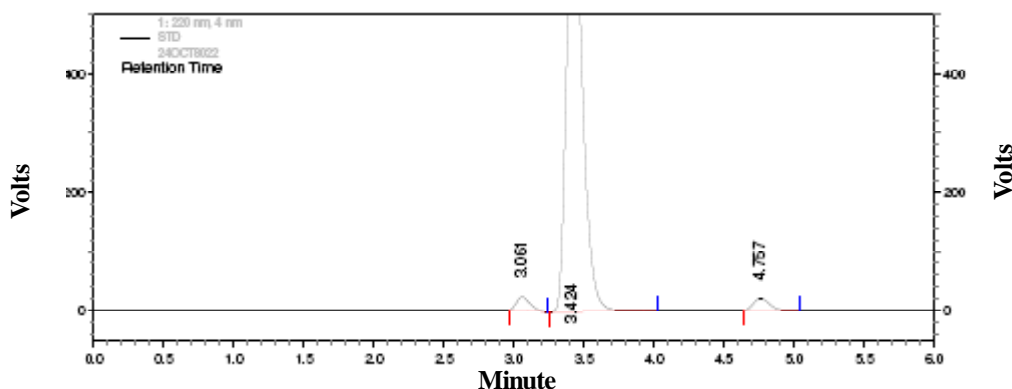


Figure 3 : Chromatogram of camylofin dihydrochloride and paracetamol with methyl paraben (internal standard) in standard preparation

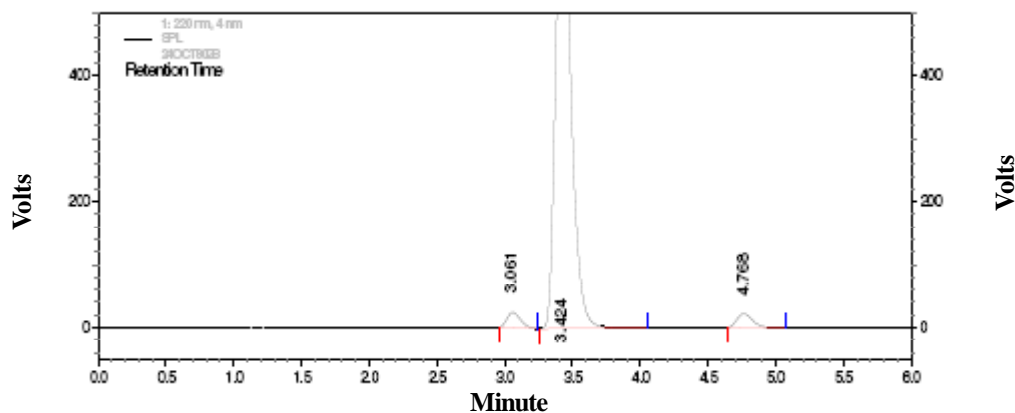


Figure 3 : Chromatogram of camylofin dihydrochloride and paracetamol with methyl paraben (internal standard) in standard preparation

TABLE 1 : Result of system suitability

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 2 : Results of linearity

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

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<sup>-1</sup>, 5  $\mu$ L 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 figures 3 and 4.

### Preparation of standard stock solutions

The stock solution of camylofin dihydrochloride (250  $\mu$ g mL<sup>-1</sup>) 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  $\mu$ g mL<sup>-1</sup>) 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  $\mu$ g mL<sup>-1</sup>) was prepared by dissolving 7.45 mg of methyl paraben in water:acetonitrile (1:1) in a 100 mL 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: acetonitrile (1:1).

### Sample preparation

Transferred 5.0 mL of syrup in a 250 mL volumetric flask, dissolved in water:acetonitrile (1:1), and fil-

tered 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<sup>[3,4]</sup>. 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- $\mu$ L standard solutions of camylofin dihydrochloride, paracetamol of strengths 50  $\mu$ g mL<sup>-1</sup>, 500  $\mu$ g mL<sup>-1</sup> 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.

### Linearity

Linearity was evaluated by analysis of working standard solutions of camylofin dihydrochloride and paracetamol of seven different concentrations<sup>[3,4]</sup>. The range of linearity was from 25 - 75  $\mu$ g mL<sup>-1</sup> for camylofin dihydrochloride and 250-750  $\mu$ g mL<sup>-1</sup> 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 2. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

### 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<sup>[3,4]</sup>. The LOD and LOQ of camylofin dihydrochloride and paracetamol was experimentally determined by six injections of each drug. The

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**TABLE 3 : Results of assay experiment**

	Camylofin dihydrochloride	Paracetamol
Drug found in mg/5ml (mean)	12.54	124.90
Mean %	100.29	99.92
RSD	0.80	0.08

**TABLE 4: Accuracy of the method**

Analyte	Initial Conc. (ppm)	Conc. added (ppm)	Total conc. (ppm)	Conc. found (ppm)	RSD (%) n= 3	Recovery (%)
Camylofin dihydrochloride	50	0	50.0	50.16	0.59	100.32
	50	5.0	55.0	54.85	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
	500	0	500.0	500.12	0.05	100.02
Paracetamol	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

LOD of camylofin dihydrochloride and paracetamol was found to be  $0.25\mu\text{g mL}^{-1}$  and  $0.3\mu\text{g mL}^{-1}$  respectively. The LOQ of camylofin dihydrochloride and paracetamol was found to be  $0.7\mu\text{g mL}^{-1}$  and  $0.8\mu\text{g mL}^{-1}$  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<sup>[3,4]</sup>. 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 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 label claim of drug were added, to determine if there are positive or negative interferences from excipients present in the formulation<sup>[3,4]</sup>. 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.

## 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, paracetamol 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 paracetamol from pharmaceutical preparations.

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