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Research Article

A STABILITY-INDICATING RP-HPLC DETERMINATION OF CAMYLOFIN DIHYDROCHLORIDE IN DRUG SUBSTANCE

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ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the determination of Camylofin Dihydrochloride in drug substance, using Zorbax eclipse-XDB C_{18} column (150mm x 4.6 mm, 5 µm) and a mobile phase composed of 0.05% trifluoro acetic acid in water (Mobile phase A) and 0.05% trifluoro acetic acid in acetonitrile (Mobile phase B*)*, at a flow rate of 1.0 mL min^{-1} using gradient program and UV detection at 220 nm. The Retention time of Phenyl glycine, Phenyl glycine isoamyl ester and Camylofin Dihydrochloride were found to be 1.841 min, 17.524 min and 18.663 min respectively. The proposed method was validated as per ICH guidelines. The Linearity for Camylofin Dihydrochloride was in the range of 250-750 µg mL^{-1}. The recovery was found to be in the range of 99.7-100.2%. Camylofin Dihydrochloride was subjected to acid, alkali and neutral hydrolysis, chemical oxidation and dry environmental condition. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. This method can be successfully employed for quantitative analysis of Camylofin Dihydrochloride in drug substance.

Keywords: ICH Guidelines, Validation, Column liquid chromatography, Drug substance, Camylofin dihydrochloride

INTRODUCTION

Camylofin Dihydrochloride 3-methylbutyl 2-(2-diethyl-aminoethylamino)-2-phenyl-acetate hydrochloride ($C_{19}H_{32}N_2O_2$,2HCl) Fig 1 is a drug used as an antispasmodic[1]. The literature survey reveals method for determination of Camylofin Dihydrochloride in drug product by HPLC[2-5] but no method is reported for Camylofin Dihyrochloride in drug substance using its probable impurities. A new stability indicating RP- HPLC method was thus developed for the determination of Camylofin Dihydrochloride from drug substance in presence of their degradation products. The method described is specific, precise and accurate for determination of Camylofin Dihydrochloride in drug substance.

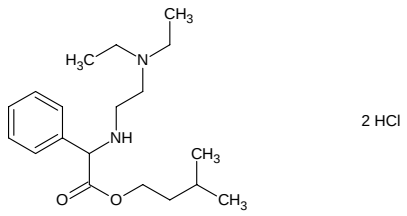

Fig. 1: Structure of Camylofin dihydrochloride

MATERIALS AND METHODS

Chemicals and reagents

Standard and Sample were supplied from Khandelwal labs., Mumbai, India. Acetonitrile and trifluroacetic 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

The aim was to develop a suitable stability indicating RP-LC method for the analysis of Camylofin Dihydrochloride in presence of their degradation products, accordingly different mobile phases were tried. The criteria employed for selecting the mobile phase for the analysis of the drug were better separation of drug with its probable impurities, time required for the analysis and the cost involved. Chromatographic separation was preformed with Agilent 1100 series High performance liquid chromatography having quaternary gradient pump, equipped with auto sampler and a photo-diode array detector. The UV spectrums of Camylofin Dihydrochloride and its impurities were scanned on photo diode array detector for selecting the working wavelength. Peak purity of Camylofin Dihydrochloride and its impurities were checked using photo diode array detector. Chromatograms and data were recorded by means of Waters millennium software. Zorbax Eclipse-XDB C-18, (150 x 4.6mm, 5um particle) was used for the analysis. The mobile phase comprising of 0.05% trifluoro acetic acid in water (Mobile phase A) and 0.05% trifluoro acetic acid in acetonitrile (Mobile phase B*)* was used. The system was run at a flow rate of 1.0 mL min^{-1} and 10µL of sample was injected in the chromatographic system. The column temperature was maintained at 25°C and detection wavelength was set at 220 nm for determination of Camylofin Dihydrochloride.

Chromatographic conditions

Column: Zorbax Eclipse-XDB C-18, (150 x 4.6mm, 5um)
Make- Agilent Part No: (P/N 993967-902)
Mobile Phase: Mobile phase A: 0.05% Trifluoro acetic acid in water
Mobile phase B: 0.05% Trifluoro acetic acid in Acetonitrile

Table 1: Gradient composition program

Time	% Mobile phase (A)	% Mobile phase (B)
0	95	5
5.0	95	5
30.0	20	80
32.0	20	80
35.0	95	5
45.0	95	5

λ_{max}: 220nm; Flow: 1.0ml/min; Temperature: Ambient; Run Time: 45mins; Injection Volume: 10 Ul; Diluent: Acetonitrile : water (50 :50)v/v

Resolution mixture solution

Resolution mixture solution was prepared by dissolving 5 mg of each Phenyl glycine, Phenyl glycine isoamyl ester and Camylofin Dihydrochloride in diluent in a standard 10 mL volumetric flask.

Standard solution

The standard solution of Camylofin Dihydrochloride (500µg mL^{-1}) was prepared by dissolving 25.05 mg of Camylofin Dihydrochloride (99.9 %) in diluent in a standard 50 mL volumetric flask.

Preparation of sample solution

The sample solution was prepared by dissolving about 25.00 mg of sample in diluent in a standard 50 mL volumetric flask.

Linearity stock solution

The Linearity stock solution of Camylofin Dihydrochloride (5000 µg mL^{-1}) was prepared by dissolving 124.98 mg of Camylofin Dihydrochloride (99.9 %) in diluent in a standard 25mL volumetric flask.

RESULTS AND DISCUSSION

Method development

Several mobile phases using different organic solvents as part of mobile phase were tried. Water and acetonitrile in the ratio of 750:250 v/v was chosen for initial trial with a 25 cm length, 4.6 mm ID and 5 micron particle size C-18 stationary phase. Flow rate was 1.5 mL min^{-1}. When test solution was injected the resolution between Phenyl glycine isoamyl ester and Camylofin Dihydrochloride was less than (<1.2). The peak shape of Camylofin was not symmetrical. To improve the peak shape of Camylofin Dihydrochloride, 0.05% Trifluoro acetic acid in water (mobile phase A) and 0.05% Trifluoro acetic acid in acetonitrile (mobile phase B) in 60:40 v/v was used as a mobile phase. When system suitability solution was injected in the above conditions the resolution between Phenyl glycine isoamyl ester and Camylofin Dihydrochloride was less than 1.5, the peak shape of Camylofin Dihydrochloride was found symmetric with tailing factor less than 1.5, but the retention time of Phenyl glycine was found to be ~ 0.2 min. To further improve the resolution between Phenyl glycine isoamyl ester and Camylofin Dihydrochloride, to retain the phenyl glycine, time gradient program was introduced. In the optimized conditions Phenyl glycine, Phenyl glycine isoamyl ester and Camylofin Dihydrochloride were well separated with a resolution greater than 2.0 and the typical retention times of Phenyl glycine, Phenyl glycine isoamyl ester and Camylofin Dihydrochloride were 1.841 min, 17.524 min and 18.663 min respectively.

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out[6]. System suitability tests were performed as per the general chapter <621> in USP 34 NF 29 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 10-µL resolution mixture solution and standard solution of Camylofin Dihydrochloride. In resolution mixture solution, the resolution between Phenyl glycine isoamyl ester and Camylofin Dihydrochloride is found to be 2.28. Six replicate injections of standard solution were made. The %RSD value of Camylofin Dihydrochloride was 0.55. The %RSD value was found to be satisfactory and meeting the requirements of the general chapter <621> in USP 34 NF 29 (%RSD not more than 2.0). Theoretical plates, resolution, tailing factor were determined and are presented in Table 2.

Table 2: Result of system suitability

Component	Retention Time	Resolution	Tailing Factor	Theoretical Plates
Phenyl glycine	1.841 min	----	1.34	2396
Phenyl glycine isoamyl ester	17.524 min	14.21	1.19	2254
Camylofin Dihydrochloride	18.663 min	2.28	1.23	6932

Method validation

Method validation was done as per ICH guidelines[7].

Specificity (Forced degradation study)

Forced degradation study is carried out to know in advance likely degradation products that may be generated during stability study or shelf life. In forced degradation study, it is observed that under dry environmental conditions, i. e. UV radiation and thermal exposure, no major degradation of sample was observed. In aqueous condition sample shows no significant degradation. Alkaline condition causes significant degradation of sample with assay value of Camylofin Dihydrochloride reduced to 85.3%. The degradation profile shows major peaks of 12.23% at RRT 0.10 and 2.89% at RRT 0.20, which corresponds to Phenyl glycine and 2-(2-diethylamino ethylamino)-2-phenyl acetic acid (identified by LC-MS) respectively. Phenyl glycine is a raw material used in the synthesis of Camylofin Dihydrochloride and is formed due to degradation of aliphatic side chain of Camylofin Dihydrochloride. 2-(2-diethylamino ethylamino)-2-phenyl acetic acid is possibly formed due to hydrolysis of Camylofin Dihydrochloride, which is an ester. Acidic condition shows significant degradation with formation of impurities at RRT – about 0.10, 0.20, 0.97 and 1.15 to the extent of 11.09% Phenyl glycine, 1.84% 2-(2-diethylamino ethylamino)-2-phenyl acetic acid, 0.38% and 0.23% respectively. The assay value of Camylofin reduced to 86.5%. In Oxidation condition, sample shows significant degradation with formation of impurities at RRT – 0.10, 0.73, 0.79, 0.80, 0.85, 0.87, 0.90, 0.97, 1.10, 1.13, 1.34, 1.56 and 1.58 to the extent of 2.99% (Phenyl glycine), 0.74%, 1.06%, 0.99%, 2.17%, 0.26%, 0.53%, 2.19%, 1.37%, 1.92%, 4.49%, 3.57% and 6.99% (Di-isopentyl 3-3 diamino-N-oxide di-benzoate identified by LC-MS) respectively. The assay value of the sample is reduced to 70.2%. Di-isopentyl 3,3 diamino-N-oxide di-benzoate is formed due to dimer formation of Camylofin Dihydrochloride by self-condensation and further formation of corresponding N-oxide of dimer base. In all above conditions, where the degradation is observed, main peak of Camylofin Dihydrochloride is found to be pure and no any other peak is merged in it (as indicated by PDA detector). All degradants are well separated from Camylofin peak indicating specificity and stability indicating nature of the method. Refer figure 2 to 10 for chromatograms of forced degradation study.

Linearity

Linearity was evaluated by analysis of working standard solution of Camylofin Dihydrochloride at seven different concentrations. The range of linearity was from 250 – 750 µg mL^{-1}. The peak area and concentration of Camylofin Dihydrochloride was subjected to regression analysis to calculate the calibration equations and correlation coefficient. The regression data obtained for the Camylofin Dihydrochloride is represented in Table 3. The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration.

Table 3: Results of linearity experiment

Analyte	Slope	Intercept	Correlation coefficient
Camylofin Dihydrochloride	5467.9	-53705	0.9996

Sensitivity

Sensitivity was determined by establishing the limit of detection (LOD) and limit of quantification (LOQ). The limit of detection (LOD) and limit of quantification (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of Camylofin Dihydrochloride was experimentally determined by six replicate injections. The LOD of Camylofin Dihydrochloride was found to be 0.05µg mL^{-1}. The LOQ of Camylofin Dihydrochloride was found to be 0.125µg mL^{-1}.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the standard solution. The relative standard deviation (RSD) was less than 2.0%. Method precision was determined from results of six independent determinations at 100% of the test concentrations of Camylofin Dihydrochloride. The % RSD for Camylofin Dihydrochloride was found to be 0.34.

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 50%, 100% and 150% of the working concentration of Camylofin Dihydrochloride was added. Each set of addition were repeated three times .The accuracy was expressed as the percentage of analyte recovered by the assay. Table 5 lists the recoveries of the drug from a series of spiked concentrations. The results indicate the method is highly accurate for determination of Camylofin Dihydrochloride.

Table 4: Results of precision experiment

Parameter	Mean assay (%)	Standard deviation (%)	Relative standard deviation
Method Precision	99.9	0.34	0.34

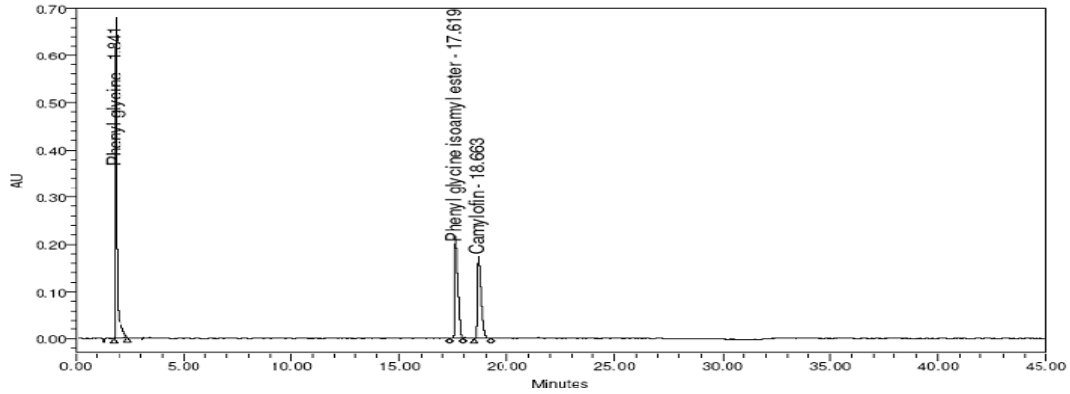

Fig. 2: Chromatogram of resolution mixture solution

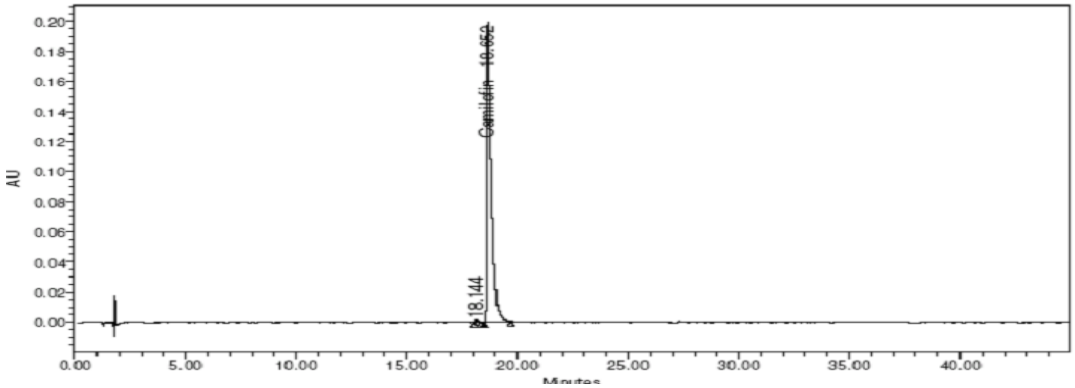

Fig. 3: Chromatogram of standard solution

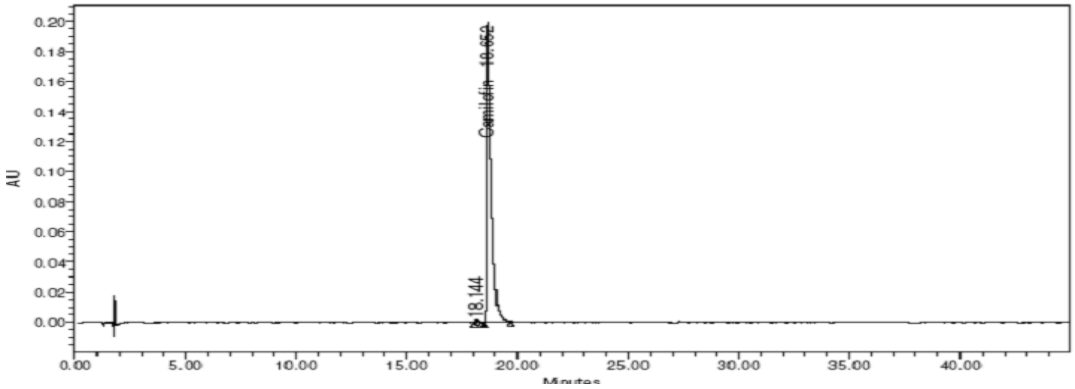

Fig. 4: Chromatogram of sample solution

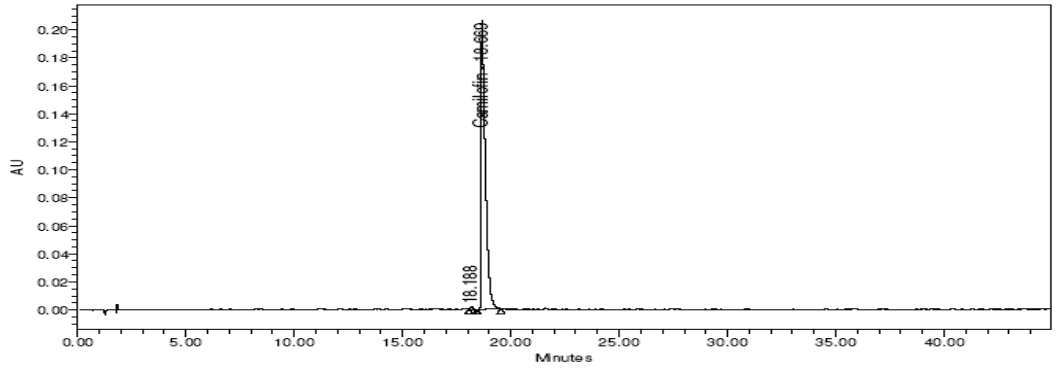

Fig. 5: Chromatogram of sample exposed to UV condition

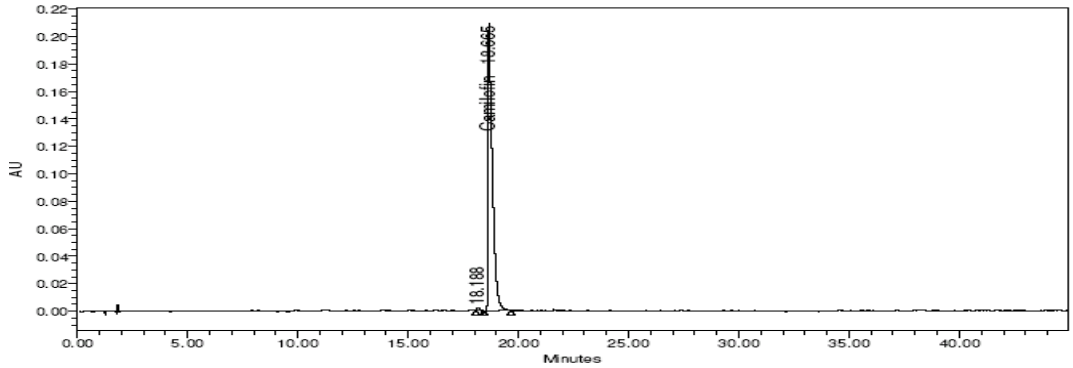

Fig.6: Chromatogram of sample exposed to Heat

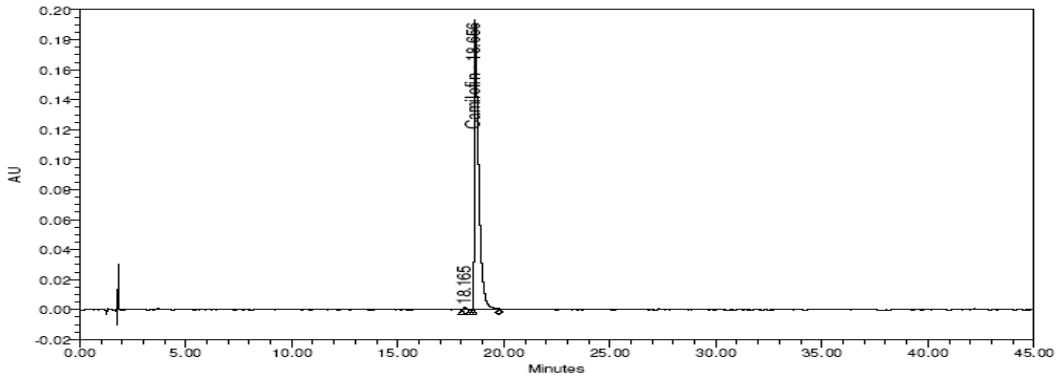

Fig. 7: Chromatogram of sample exposed to aqueous condition

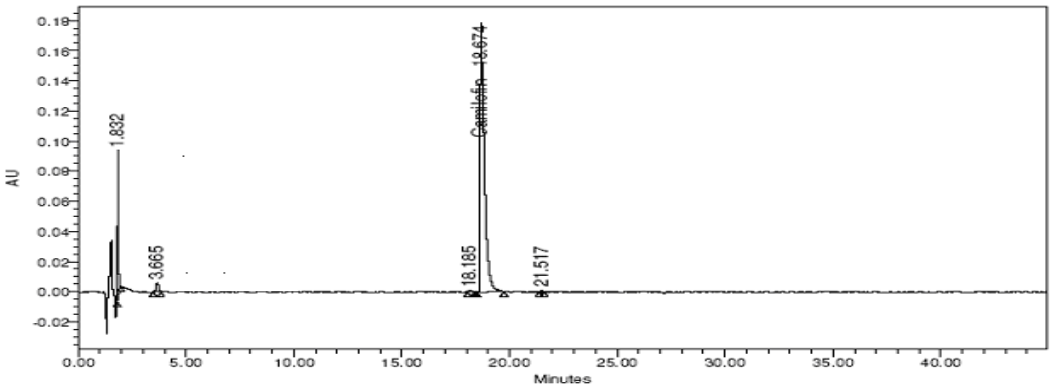

Fig. 8: Chromatogram of sample exposed to acidic condition

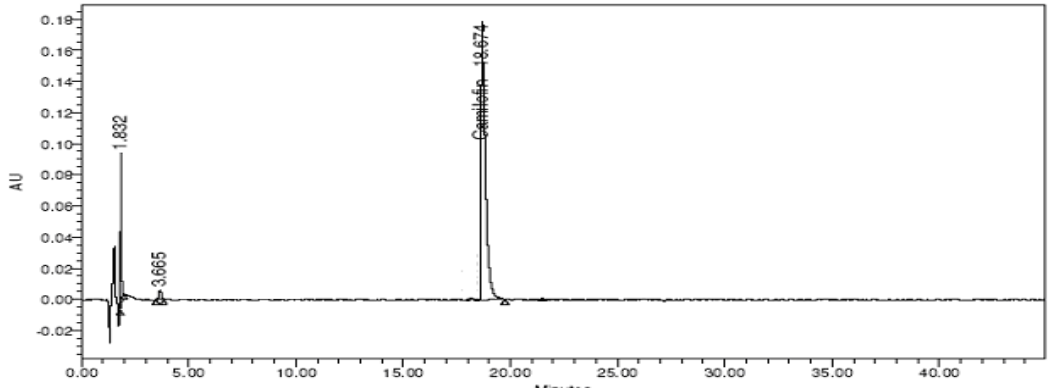

Fig.9: Chromatogram of sample exposed to basic condition

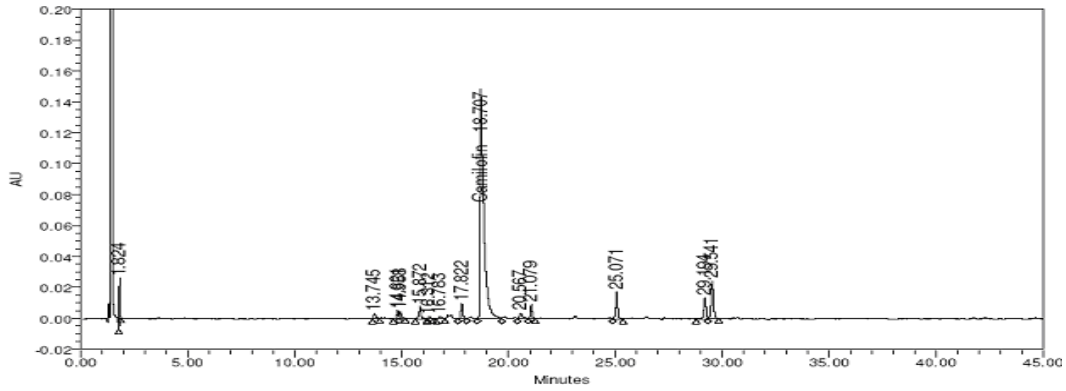

Fig. 10: Chromatogram of sample exposed to oxidation condition

Table 5: Results of accuracy experiment

Recovery Levels	Conc of Camylofin Dihydrochloride added in ppm	Conc of Camylofin Dihydrochloride recovered in ppm	Accuracy found in %	Mean Accuracy at each level (%)
50% Level	244.75	246.52	100.7	100.2
	247.74	246.09	99.3	
	239.75	240.86	100.5	
100% Level	502.49	501.11	99.7	99.8
	493.50	497.86	100.9	
	505.49	499.37	98.8	
150% Level	747.24	746.34	99.9	99.7
	743.25	747.04	100.5	
	755.24	746.28	98.8	

Robustness

By deliberate change in experimental condition the resolution between Phenyl glycine isoamyl ester and Camylofin Dihydrochloride was evaluated. To study the effect of flow rate on system suitability parameters, 0.1 units changed i.e. 0.9 and 1.1 mL min^{-1}. The effect of column temperature was studied at 20°C and 30°C. In all the above varied conditions, the components of the mobile phase were held constant. The resolution between Phenyl glycine isoamyl ester and Camylofin Dihydrochloride was greater than 2.0.

Solution stability

The solution stability of Camylofin Dihydrochloride was carried out by leaving the test solutions of sample in a tightly capped volumetric flask at room temperature for 48 hrs. The same sample solutions were assayed for 12 hrs interval up to the study period against freshly prepared standard solution. The % RSD of assay of Camylofin Dihydrochloride during solution stability was within 1.0. No significant changes were observed in the content of Camylofin Dihydrochloride during solution stability experiment. Sample solutions used during the experiment were stable upto the study period of 48 hrs.

CONCLUSION

The stability indicating RP-LC method developed for quantitative determination of Camylofin Dihydrochloride in drug substance is specific, precise, accurate and robust. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for routine analysis of production samples and also to check the stability study of Camylofin Dihydrochloride in raw material.

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