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Stability indicating GC-FID method for estimation of Camylofin dihydrochloride and Paracetamol in pharmaceutical dosage form

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ABSTRACT

This research paper describes simple analytical method for determination of Camylofin dihydrochloride and Paracetamol in syrup formulation by Gas chromatography method. Benzoic acid was used as internal standard. Validation was carried out in compliance with the International Conference on Harmonization guidelines. The method utilized GC (Agilent Technologies 6890N Network GC system with FID detector), and RTX-5 capillary column (Crossbond 50% diphenyl-95% dimethyl polysiloxane), 30m x 0.53mm, 1.5µm as stationary phase. Helium was used as the carrier gas. The proposed method was validated for linearity, LOD, LOQ, accuracy, precision, ruggedness and solution stability. It can be conveniently adopted for routine quality control analysis.

Key Words: Validation, Gas chromatography, Pharmaceutical preparations, Camylofin dihydrochloride, Paracetamol.

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INTRODUCTION:

Camylofin dihydrochloride 3-methylbutyl 2-(2-diethylaminoethylamino)-2-phenyl-acetate hydrochloride is a drug used an antispasmodic[1]. Paracetamol also known as acetaminophen is chemically N-(4-hydroxyphenyl) acetamide, a centrally and peripherally acting analgesic and antipyretic agent [2-3]. The structure of the drug is shown in Figure 1 and 2. One such combination contains 12.5 mg of camylofin dihydrochloride and 125 mg of Paracetamol per 5 mL. The literature revealed that high performance liquid chromatography (HPLC) [3] method was used for simultaneous determination of these compounds. There are other publications for determination of these compounds but in combination with other components by other analytical techniques like HPLC, GC and spectrophotometry and colorimetric [4-17]. There are, however, no publications for simultaneous determination of these drugs in such pharmaceutical preparation by Gas chromatography. Therefore a GC method was developed for determination of camylofin dihydrochloride and paracetamol from their dosage form. The method described is simple, fast, precise and accurate for simultaneous determination of camylofin dihydrochloride and paracetamol from pharmaceutical preparation. The method is very cost and time effective since it does not require any mobile phase preparation and can be easily adapted to Quality control testing laboratory.

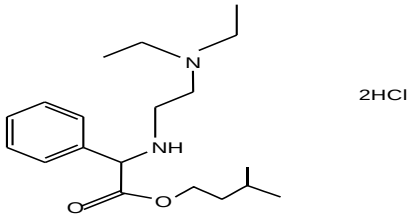

Figure 1: Structure of Camylofin dihydrochloride

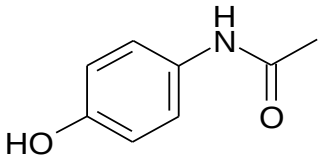

Figure 2: Structure of Paracetamol

MATERIALS AND METHODS

Reagents and Materials

Anafortan syrup manufactured by Khandelwal lab, India was procured from the market. Camylofin dihydrochloride and paracetamol were procured from Sigma Aldrich. Methanol was from Qualigens. All dilutions were performed in standard volumetric flasks.

Apparatus

The analysis was performed by using the analytical balance Mettler Toledo, the GC used is of Agilent Technologies 6890N Network GC system with FID detector. Column used in GC is a capillary column RTX-5, 30m x 0.53mm, 1.5µm. Photo stability studies were carried out in a

photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were carried out in a dry air oven (Lindberg- Blue, USA).

Experimental

Method development and optimization of chromatographic conditions:

To develop a suitable GC method for the analysis of camylofin dihydrochloride and paracetamol in their dosage form, different capillary columns were tried [18,19]. The criteria employed for selecting the columns for the analyses of the drugs were cost involve, time required for the analysis, better separation of the components. Chromatographic separation was preformed with Agilent Technologies 6890N Network Gas chromatography system, equipped with auto sampler and a flame ionization detector. Chromatograms and data were recorded by means of Empower software. RTX-5 capillary column (Crossbond 50% diphenyl-95% dimethyl polysiloxane) was used for analysis. The column dimension was 30m x 0.53mm, 1.5µm. The system was run at a flow rate of 1.5mL min^{-1}, 1 µL of sample was injected in the chromatographic system and flame ionization detector was used for simultaneous determination of camylofin dihydrochloride and paracetamol. Helium was used as a carrier gas. Oven temperature was kept 150°C and increased at a rate of 10°C min^{-1} to 300°C and held at 300°C for 13.0 minutes. Injector temperature and detector temperature were kept at 250°C and 300°C respectively. The split ratio was kept at 20:1.

Table 1: Summary of optimization of chromatographic conditions

Column used	Carrier gas	Flow rate	Observation	Result
DBWax,30mx0.53mm, 1.0µm capillary column	Helium	1.2 mL min^{-1}	No peaks observed	Method rejected
DB624,30mx0.32mm, 1.8µm capillary column	Helium	1.2 mL min-1	Peak shape for both the components not proper	Method rejected
RTX1,30mx0.53mm, 1.0µm capillary column	Helium	1.5 mL min-1	Poor resolution and low response	Method rejected
RTX5,30mx0.53mm, 1.5µm capillary column	Helium	1.5 mL min-1	Good resolution and good peak shape	Method accepted

Preparation of Standard Stock Solutions

The stock solution of camylofin dihydrochloride (750 µg mL^{-1}) was prepared by dissolving 37.5 mg of camylofin dihydrochloride (99.9 %) in methanol in a standard 50mL volumetric flask (solution A). Internal standard (benzoic acid) stock solution(500 µg mL^{-1}) was prepared by dissolving 50.2 mg benzoic acid in methanol in a 100mL standard volumetric flask (solution B).

Working Standard Solution: Weighed and transferred 30.1 mg of Paracetamol (99.8%) in a 25 mL volumetric flask. Added 5 mL of methanol and dissolved .Transferred 5.0 mL of each stock solution A and B to the same 25 mL volumetric flask and diluted up to the mark with methanol. The final concentration of Camylofin dihydrochloride and Paracetamol are 150 µg/mL and 1200 µg/mL respectively.

Sample Preparation

Weighed and transferred about 15.0 g of syrup in a 50 mL volumetric flask and diluted to the volume with Methanol. Mixed well and filtered this solution through 0.45 µm membrane filter. 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 methanol.

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. 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 1-µL standard solutions of camylofin dihydrochloride and paracetamol of strengths 150 µg mL^{-1} and 1200 µg mL^{-1} respectively using benzoic acid as an internal standard. This was repeated five times. The % RSD values were found to be satisfactory and meeting the requirements of USP 31 (RSD < 2.0 %). A typical GC chromatogram for simultaneous determination of Camylofin dihydrochloride and paracetamol from pharmaceutical formulation is shown in Figure 3,4,5,6.

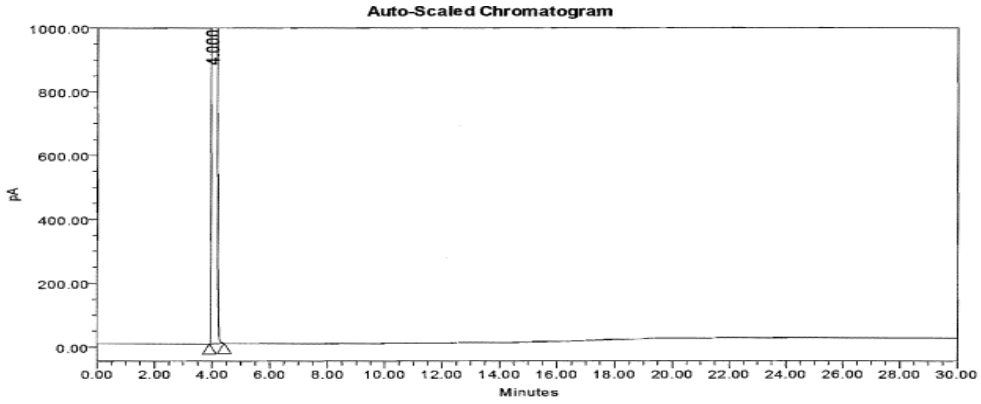

Figure 3: Chromatogram of Diluent.

Table 2: Results of System suitability

Parameters	Benzoic acid	Paracetamol	**Camylofin dihydrochloride**
Resolution	NA	30.3	16.3
Tailing factor	1.00	0.92	1.31
Theoretical plates	65474	31415	352441
% RSD	NA	0.68	0.54

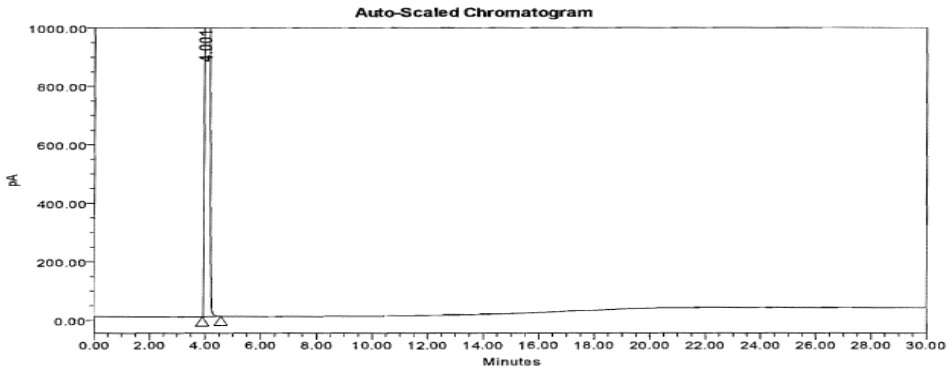

Figure 4: Chromatogram of Placebo.

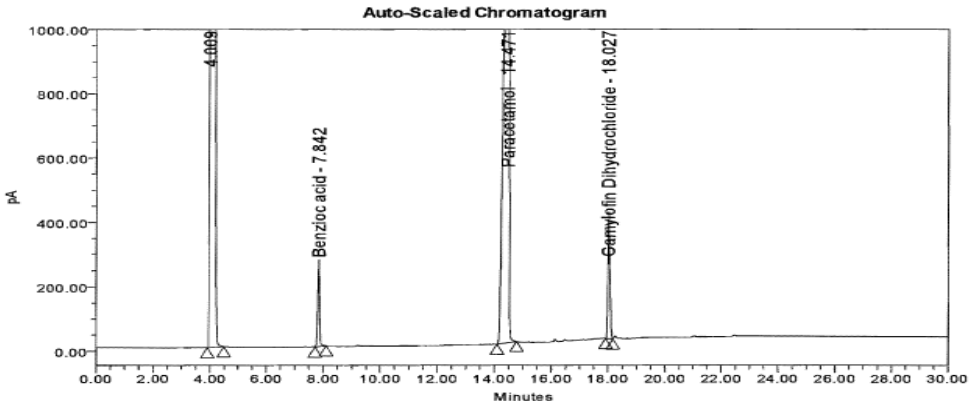

Figure 5: Chromatogram of standard preparation.

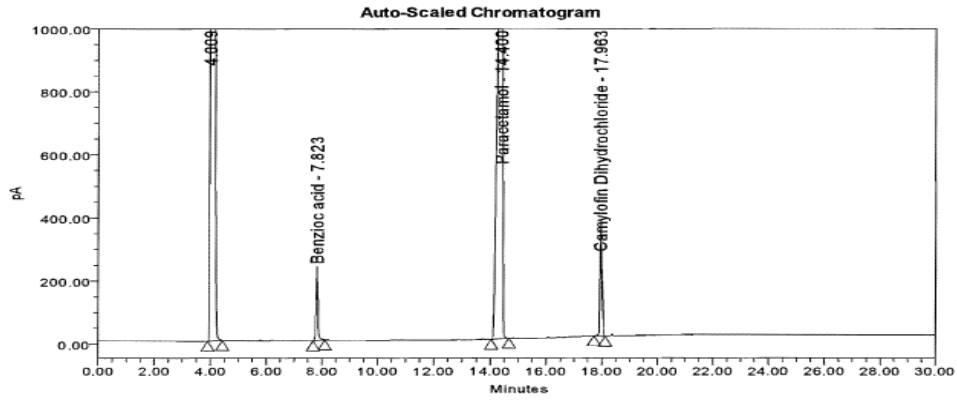

Figure 6: Chromatogram of sample preparation.

Method Validation Parameters

The method validation was carried out as per ICH guidelines [20]. Various method validation parameters ascertained are as follows.

Linearity: The Linearity of detector response is established by plotting a graph to concentration versus peak area of Camylofin Dihydrochloride and Paracetamol standards and determining the correlation coefficient. Linearity was evaluated by analysis of working standard solutions of camylofin dihydrochloride and paracetamol of seven different concentrations. The range of

linearity was from 75 µg mL^{-1} to 225 µg mL^{-1} (150 µg/mL is 100% level) for camylofin dihydrochloride and 600 µg mL^{-1} to 1800 µg mL^{-1} (1200 µg/mL is 100% level) for paracetamol. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. Figure 7 and 8 represents the linearity plots of Camylofin dihydrochloride and Paracetamol. The regression data obtained for the camylofin dihydrochloride and paracetamol is represented in Table 3. The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

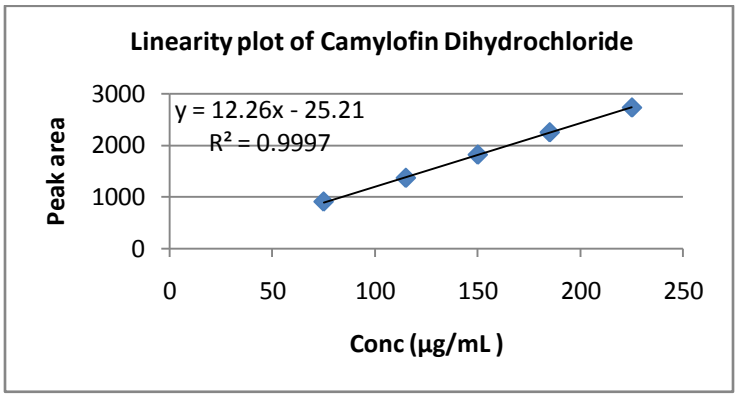

Figure 7: Linearity plot of Camylofin dihydrochloride

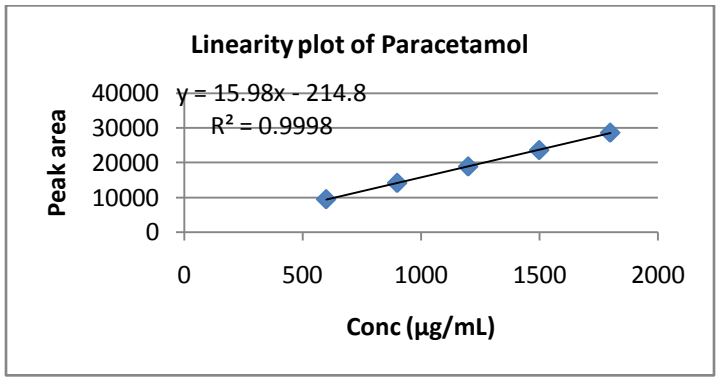

Figure 8: Linearity plot of Paracetamol

Table 3: Results of Linearity study

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n=5)
Camylofin dihydrochloride	12.26	-25.21	0.9997
Paracetamol	15.98	-214.8	0.9998

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. The LOD and LOQ of Camylofin dihydrochloride and paracetamol was experimentally determined by six injections of each drug.

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. 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 1.2 µg mL^{-1} & 1.5 µg mL^{-1} respectively. The LOQ of camylofin dihydrochloride and paracetamol was found to be 2.1 µg mL^{-1} & 2.5 µg 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. The relative standard deviations were 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 0.52% and 0.81% for Camylofin dihydrochloride and Paracetamol respectively. Refer Table 4.

Table 4: Results of Precision experiment.

Results	Camylofin dihydrochloride	Paracetamol
Drug found in mg/tab (mean)	12.05	121.69
% Mean Assay	100.41	101.41
% RSD	0.54	0.65

Table 5: Ruggedness of Assay experiment

Results	Camylofin dihydrochloride	Paracetamol
Drug found in mg/tab (mean)	11.98	121.21
% Mean Assay	99.83	101.01
% RSD	0.41	0.70
% Difference wr.t. Precision	0.13	0.05

Ruggedness (Intermediate Precision):

Ruggedness study was done by injecting six individual sample preparations at 100% of the test concentrations of Camylofin dihydrochloride and Paracetamol on different day and different GC system. The mean % Assay obtained was compared with mean % Assay of precision study. The relative standard deviation (RSD) was less than 2%. Refer Table 5.

Accuracy

Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of Camylofin dihydrochloride and paracetamol from standard stock solution was added in placebo to attain 50%, 100% and 150% of sample concentration. Each sample was prepared in triplicate at each level. Blank and standard preparations were injected and chromatograms were recorded.

Accuracy was expressed as the percentage of analytes recovered by the assay. Table 6 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.

Table 6: Accuracy of method

Analyte	Recovery Level	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	RSD (%) n= 3	(%)Recovery
Camylofin dihydrochloride	50	75.24	76.12	0.11	101.17
	100	150.48	149.56	0.25	99.39
	150	225.72	224.11	0.28	99.29
Paracetamol	50	601.12	602.69	0.21	100.26
	100	1202.24	1198.48	0.25	99.69
	150	1803.36	1795.06	0.31	99.53

Table 7: Results of Solution stability

Condition	% Assay of Camylofin dihydrochloride	% Difference w.r.t. initial assay	% Assay of Paracetamol	% Difference w.r.t. initial assay
Initial	100.92	Not applicable	101.15	Not applicable
24 hours	100.41	0.51	100.78	0.37
48 hours	100.11	0.81	100.85	0.30
72 hours	100.15	0.77	100.59	0.56

Solution stability

The solution stability of Camylofin dihydrochloride and Paracetamol was carried out by leaving the test solutions of sample in a tightly capped volumetric flask at room temperature for 72 hours. The same sample solutions were assayed for 24 hours interval up to the study period against freshly prepared standard solution.

The % assay of Camylofin dihydrochloride and Paracetamol were checked in the test solutions. The % RSD of assay of Camylofin dihydrochloride and Paracetamol during solution stability experiment was within 1.0. No significant changes were observed in the content of Camylofin dihydrochloride and Paracetamol during solution stability experiment. Sample solutions used during the experiment were stable upto the study period of 72 hours. The results are reported in table 7.

Specificity

Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Diluent and placebo was injected to determine any interference of peak at the retention time of Camylofin dihydrochloride, Paracetamol and Benzoic acid.

No peak was observed at the retention time of Camylofin dihydrochloride, Paracetamol and Benzoic acid in diluents and Placebo chromatogram.

Table 8: Summary of forced degradation results

Stress condition	Time	% Assay of Camylofin 2HCl	% Assay of Paracetamol	RRT of individual degradatant w.r.t. Internal standard	Mass balance (% assay+ % degradation products)	Remarks
Acid hydrolysis (2 M HCl)	48 h	88.25	95.23	0.94,1.17,2.05, 2.09,2.31	99.21	degradation observed
Base hydrolysis (1 N NaOH)	3 h	79.52	93.54	0.94,1.17,2.05	98.99	degradation observed
Oxidation (3% H2O2)	48 h	95.24	97.8	0.94,1.17,2.06	99.52	degradation observed
Thermal (80°C)	5 days	97.27	98.50	1.17,2.06	99.48	No degradation observed
Light (photolytic degradation)	5 days	99.21	98.93	1.90,1.94,2.35	99.72	No degradation observed

Forced Degradation study (Stress Testing)

The drug was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to the body.

To study the effect of acid, 5 mL of 2 M HCl was added to the sample and the mixture was kept for 48 hours. To study the effect of base, 5 mL of 1 N NaOH solution was added to the sample and the mixture kept for 3 hours. To study the effect of oxidizing conditions, 5 mL of 3% v/v H_2O_2 was added to the sample and the mixture was kept for 48 hours.

To study the effect of temperature sample was kept in an oven at 80°C for 5 days.

To study the effect of light sample was and kept in a photostability chamber for 5 days.

The individual % assay was calculated in comparison with the working standard solution. Refer Figure 9 to 13 for chromatograms of stress samples and Table 8 for its summary.

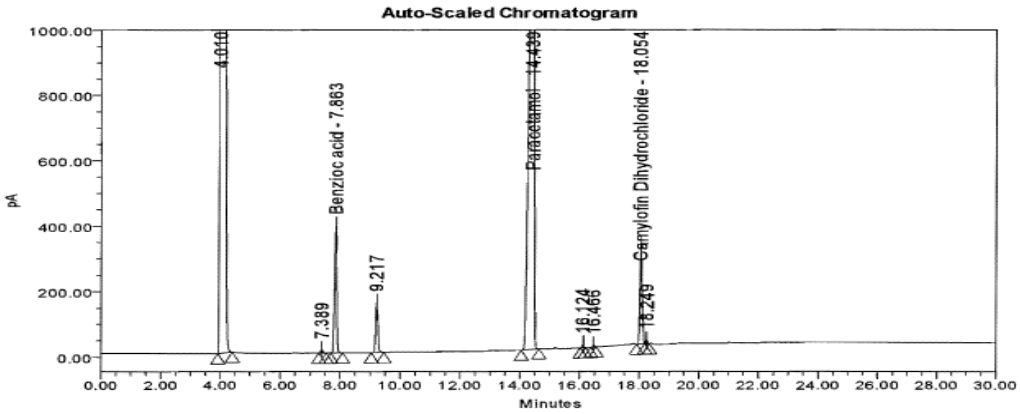

Figure 9 : Typical chromatogram obtained after degradation under acidic condition.

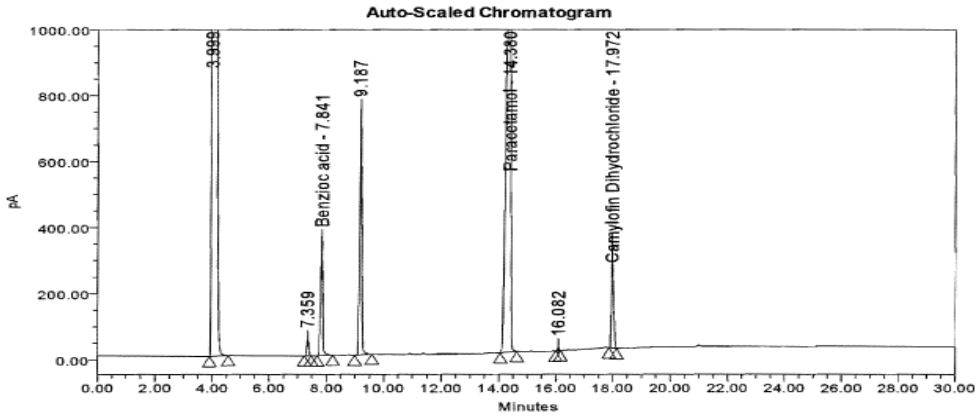

Figure 10 : Typical chromatogram obtained after degradation under basic condition.

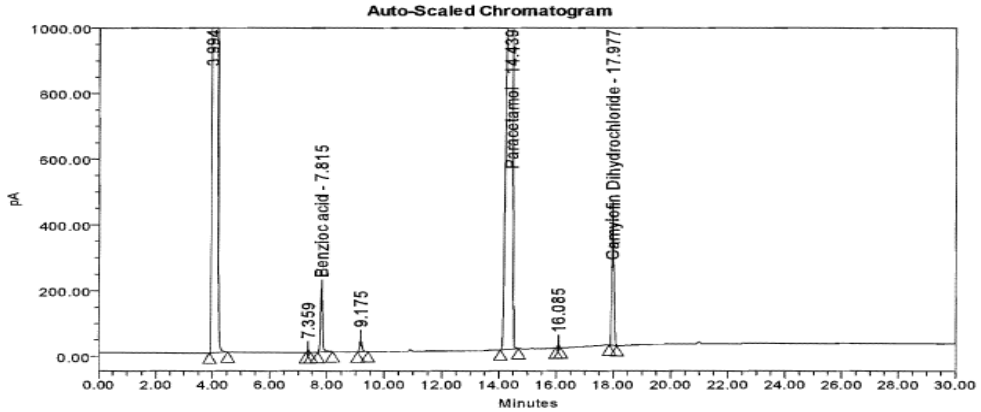

Figure 11 : Typical chromatogram obtained after degradation under oxidation condition.

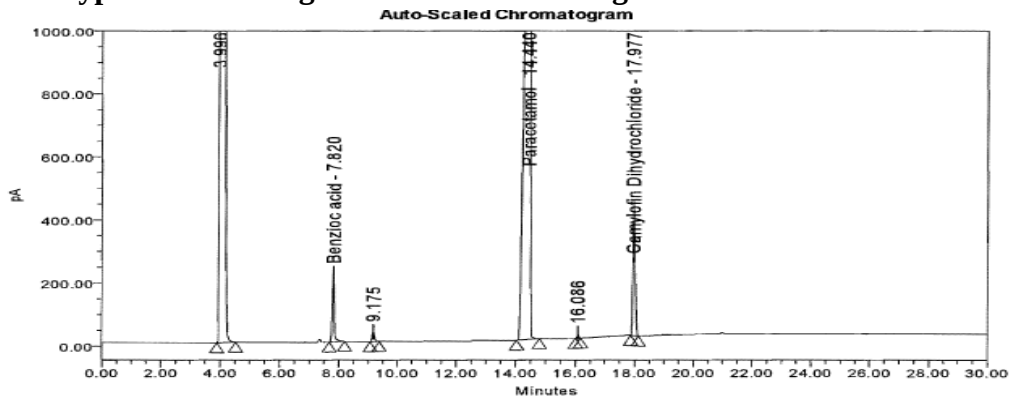

Figure 12 : Typical chromatogram obtained after Thermal degradation.

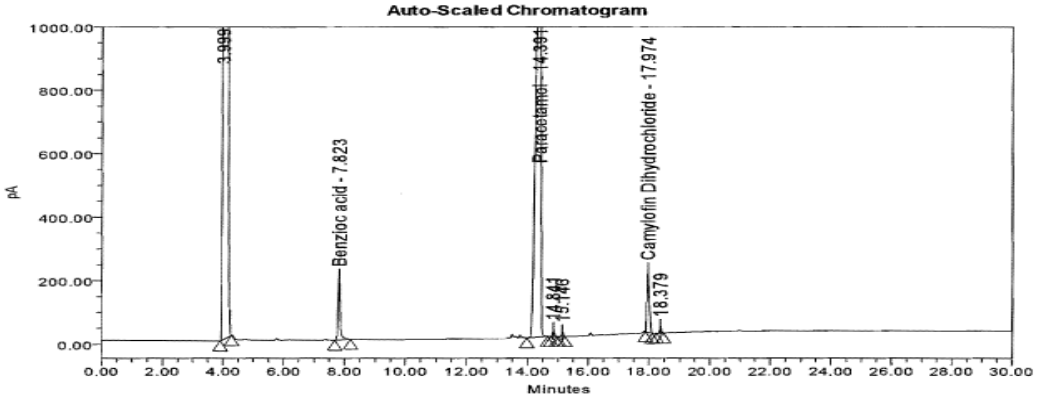

Figure 13: Typical chromatogram obtained after photostability degradation

CONCLUSION

The method after being completely validated showed satisfactory data for all the method validation parameters. Method validation study showed that the method is specific, linear, accurate, and easily reproducible and can be used for simultaneous determination of camylofin dihydrochloride and paracetamol from pharmaceutical preparations. Stress testing showed that all degradation products were well separated from Camylofin dihydrochloride and Paracetamol, confirming its stability indicating capability. The method seems to be suitable for quality control in the pharmaceutical industry because of its sensitivity, simplicity and selectivity.

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