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Determination of Camylofin Dihydrochloride and Nimesulide in Pharmaceutical Preparation by Gas Chromatography

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Abstract

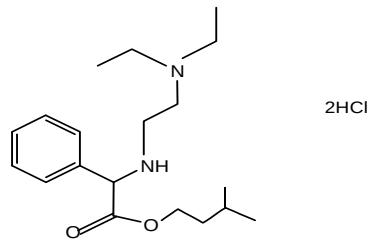
This research paper describes simple analytical method for determination of Camylofin dihydrochloride and Nimesulide in tablet formulation by Gas chromatography method. Benzoic acid was used as internal standard. Validation was carried out in compliance with the International Conference on Harmonization guidelines. The method utilized GC (Agilent Technologies 6890 N Network GC system with FID detector), and RTX-5 capillary column (5% diphenyl-95% dimethyl polysiloxane), 30 m × 0.53 mm, 1.5 µm as stationary phase. Helium was used as the carrier gas at a flow rate of 1.5 mL·min^{-1}. 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.

Keywords: Capillary Column, Gas Chromatography, Pharmaceutical Preparations, Camylofin Dihydrochloride, Nimesulide

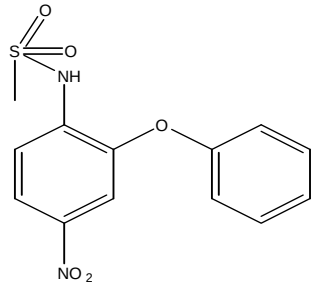
1. Introduction

Camylofin dihydrochloride is 3-methylbutyl 2-(2-diethylaminoethylamino)-2-phenyl-acetate hydrochloride is a drug used an antispasmodic [1]. Nimesulide N-(4-Nitro-2-phenoxyphenyl) methanesulfonamide.Nimesulide is a relatively COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhoea in adolescents and adults above 12 years old [2].The structure of the drug is shown in **Figure 1**. One such combination contains 50 mg of Camylofin dihydrochloride and 100 mg of Nimesulide.

The literature survey revealed that there is no method for the 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, spectrophotometry and colorimetric [4-16].There is a GC method reported for the analysis of Camylofin dihydrochloride [17]. There are, however, no publications for simultaneous determination of these drugs in such pharmaceutical preparation. Therefore a GC method was developed for



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



Nimesulide ($C_{13}H_{12}N_2O_5S$)

Figure 1. Structure of camylofin dihydrochloride and nimesulide.

determination of camylofin dihydrochloride and Nimesulide from their dosage form. The method described is simple, fast, precise and accurate for simultaneous determination of Camylofin dihydrochloride and Nimesulide 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.

2. Materials and Methods

2.1. Chemicals and Reagents

Anafortan N tablets manufactured by Khandelwal lab, India were procured from the market. Anafortan N tablets is a combination of Camylofin dihydrochloride 50 mg and Nimesulide 100 mg. Methanol was from Qualigens. All dilutions were performed in standard volumetric flasks.

2.2. Apparatus

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

2.3. Experimental

Method development and optimization of chromatographic conditions:

To develop a suitable GC method for the analysis of camylofin dihydrochloride and Nimesulide in their dosage form, different capillary columns were tried [18]. 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 6890 N 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 30 m × 0.53 mm, 1.5 µm. The system was run at a flow rate of 1.5 mL·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 Nimesulide. Helium was used as a carrier gas. Oven temperature was kept 180˚C and increased at a rate of 10˚C·min^{-1} to 280˚C and held at 280˚C for 15.0 minutes. Injector temperature and detector temperature were kept at 250˚C and 280˚C respectively. The split ratio was kept at 50:1. A summary of method development and optimization is described in **Table 1**.

2.3.1. Preparation of Standard Stock Solutions

The stock solution of Camylofin dihydrochloride (1250 µg·mL^{-1}) was prepared by dissolving 126.1 mg of Camylofin dihydrochloride (99.9%) in methanol in a standard 100 mL volumetric flask (stock solution A). The stock solution of Nimesulide (2500 µg·mL^{-1}) was prepared by dissolving 250.8 mg of Nimesulide (99.8%) in methanol in a standard 100 mL volumetric flask (stock solution B). Internal standard (benzoic acid) stock solution (5000 µg·mL^{-1}) was prepared by dissolving 500.9 mg of benzoic acid (99.6%) in methanol in a 100 mL standard volumetric flask (stock solution C).

Transferred 10.0 mL of each stock solution A, B & C to a 50 mL volumetric flask and diluted up to the mark with methanol. This is working standard solution.

2.3.2. Sample Preparation

For analysis of the tablet dosage form, twenty tablets were weighed individually and their average weight was determined. The tablets were crushed to fine homogenous powder and quantity equivalent to ten tablets were transferred in a 200 mL volumetric flask. Added about 100 mL of Methanol to the volumetric flask, shaken for 10 minutes and then sonicated for 15 minutes. The solu tion

Table 1. Summary of optimization of chromatographic conditions.

Column used	Carrier gas	Flow rate	Observation	Result
DBWax, 30 m × 0.53 mm, 1.0 µm capillary column	Helium	1.2 mL·min^{-1}	No peaks observed	Method rejected
DB624, 30 m × 0.32 mm, 1.8 µm capillary column	Helium	1.2 mL·min^{-1}	Peak shape for both components not good	Method rejected
RTX1, 30 m × 0.53 mm, 1.0 µm capillary column	Helium	1.5 mL·min^{-1}	Poor resolution and low response	Method rejected
RTX5, 30 m × 0.53 mm, 1.5 µm capillary column	Helium	1.5 mL·min^{-1}	Good resolution and good peak shape	Method accepted

was allowed to stand at room temperature for 20 - 30 minutes and filtered through Whatman no. 41 filter paper. The residue was washed with Methanol and the combined filtrate was made up to the mark with the same solvent.

5.0 mL of filtrate was quantitatively transferred to a 50 mL volumetric flask, 10.0 mL of internal standard solution was added to it, and solution was diluted up to the mark with methanol. The identities of both the compounds were established by comparing retention time of the sample solution with those of standard solution.

2.4. Validation Parameters

The method validation was carried out as per ICH guidelines [19]. Various method validation parameters were performed.

2.4.1. System Suitability Test

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. Various parameters such as tailing factor and resolution between the peaks were obtained.

2.4.2. Specificity

Specificity of the method was evaluated by injecting diluents, placebo, individual Camylofin dihydrochloride and Nimesulide and sample solution in to the GC system to check any interference to the peaks.

2.4.3. Linearity

Linearity was evaluated by analysis of working standard solutions of Camylofin dihydrochloride and Nimesulide of seven different concentrations. The range of linearity was from 250 - 750 $\mu g \cdot mL^{-1}$ for Nimesulide and 125 - 375 $\mu g \cdot mL^{-1}$ for Camylofin dihydrochloride. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients.

2.4.4. LOD and LOQ/Sensitivity

Sensitivity was determined by establishing the limit of detection (LOD) and limit of quantification (LOQ). The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively.

2.4.5. Accuracy

Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of Camylofin dihydrochloride and Nimesulide 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.

2.4.6. Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions. Method precision was determined from results from five independent determinations at 100% of the test concentrations of Camylofin dihydrochloride and Nimesulide in the product.

2.4.7. Ruggedness (Intermediate Precision)

Ruggedness study was demonstrated by injecting six individual sample preparations at 100% of the test concentrations of Camylofin dihydrochloride and Nimesulide on different day using another column and system.

2.4.8. Robustness

By deliberate change in experimental condition the resolution between Methylparaben, Camylofin dihydrochloride and Nimesulide were evaluated. To study the effect of flow rate on system suitability parameters, 0.2 units changed *i.e.* 1.3 and 1.7 $mL \cdot min^{-1}$. The effect of column temperature was studied at 170˚C and 190˚C. The injector temperature and detector temperature were kept constant.

2.4.9. Stability of Solution

The solution stability of Camylofin dihydrochloride and Nimesulide 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.

2.4.10. Stress Testing (Forced Degradation Study)

To further confirm the stability indicating nature of the method, the drug was subjected to stress conditions as per the ICH recommended test conditions [20,21].

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 at room temperature. 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 at room temperature. 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 at room temperature.

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.

3. Results and Discussion

3.1. 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 % RSD values were found to be satisfactory and meeting the requirements of USP 31 (RSD less than 2.0%). A typical GC chromatogram for simultaneous determination of camylofin dihydrochloride and Nimesulide from pharmaceutical formulation is shown in **Figure 2** and **Figure 5**. System suitability parameters are mentioned in **Table 2**.

3.2. Specificity

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

3.3. Linearity

Linearity was evaluated by analysis of working standard solutions of Camylofin dihydrochloride and Nimesulide of seven different concentrations. The range of linearity was from 125 $\mu g \cdot mL^{-1}$ to 375 $\mu g \cdot mL^{-1}$ (250 $\mu g \cdot mL^{-1}$ is 100% level) for Camylofin dihydrochloride and 250 $\mu g \cdot mL^{-1}$ to 750 $\mu g \cdot mL^{-1}$ (500 $\mu g \cdot mL^{-1}$ is 100% level) for Nimesulide. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. **Figure 6** represents the linearity plots of Camylofin dihydrochloride and Nimesulide. The regression data obtained for the Camylofin dihydrochloride and Nimesulide 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.

3.4. LOD and LOQ/Sensitivity

The LOD and LOQ of Camylofin dihydrochloride and Nimesulide was experimentally determined by six injections of each drug. The LOD of Camylofin dihydrochloride and Nimesulide was found to be 1.2 $\mu g \cdot mL^{-1}$ & 1.6 $\mu g \cdot mL^{-1}$ respectively. The LOQ of Camylofin dihydrochloride and Nimesulide was found to be 2.1 $\mu g \cdot mL^{-1}$ & 2.7 $\mu g \cdot mL^{-1}$ respectively.

3.5. Accuracy

Accuracy was expressed as the percentage of analytes recovered by the assay. **Table 4** 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 Nimesulide.

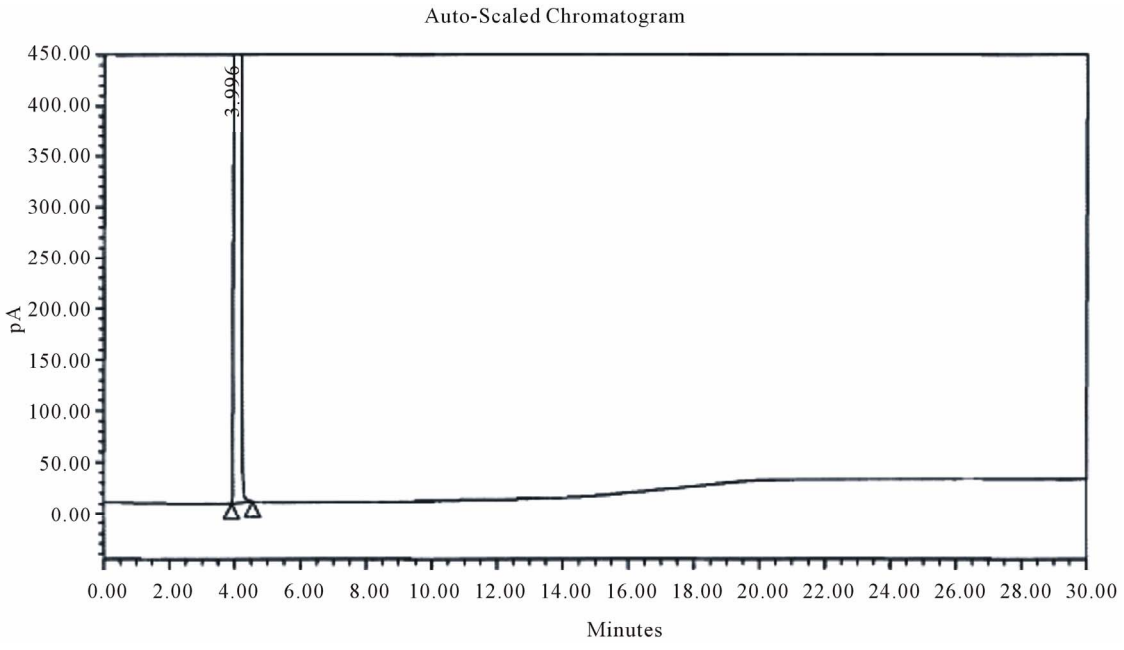


Figure 2. Chromatogram of diluent.

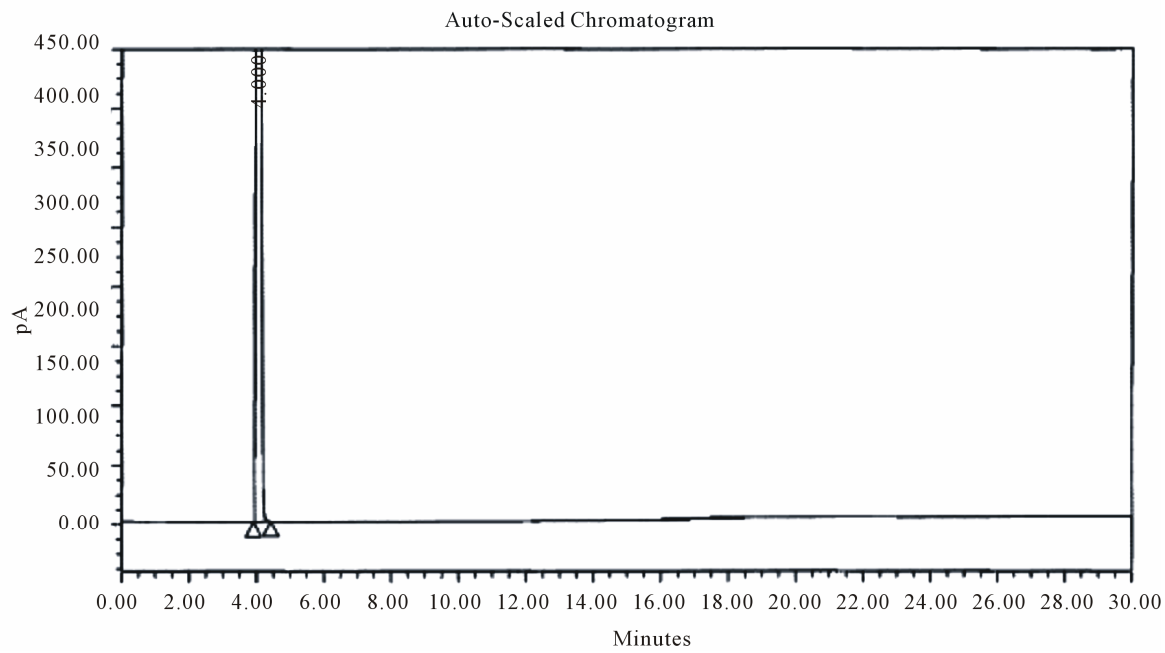


Figure 3. Chromatogram of placebo.

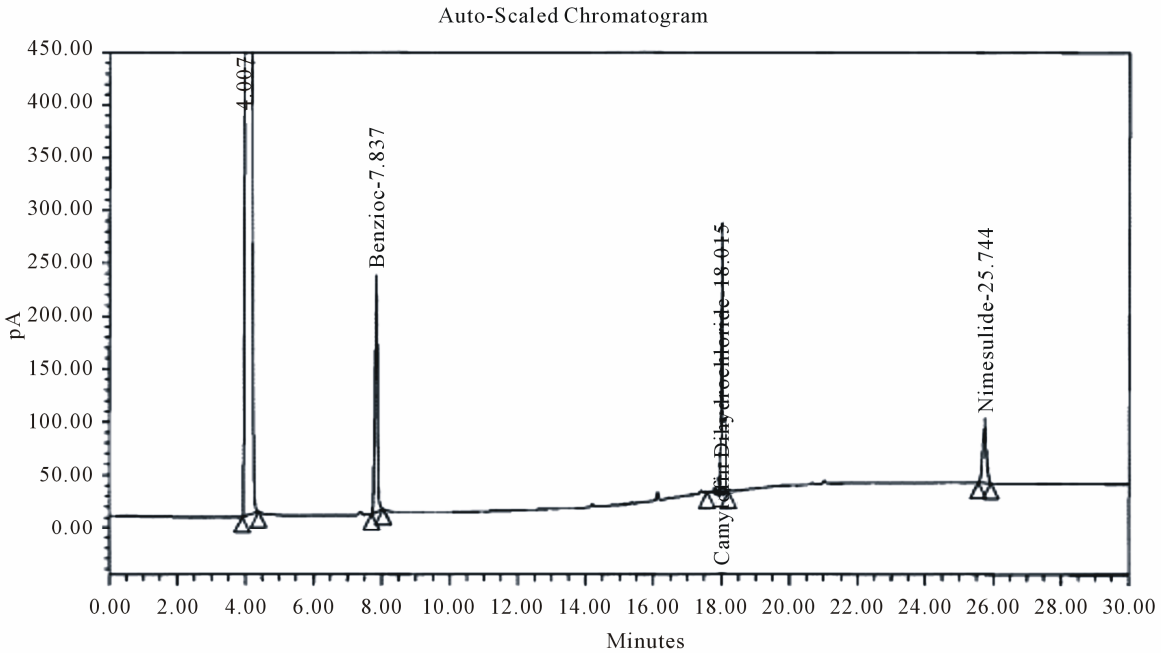


Figure 4. Chromatogram of Camylofin dihydrochloride and Nimesulide with benzoic acid (internal standard) in standard preparation. Nimesulide-25.744.

3.6. Precision

The values of the relative standard deviation of five replicate injections of the standard solution containing both the analytes of interest were within the limits of not more than 2.0%. Refer **Table 5**.

3.7. Ruggedness (Intermediate Precision) and Robustness

Ruggedness study was done by injecting six individual sample preparations at 100% of the test concentrations of Camylofin dihydrochloride and Nimesulide on different

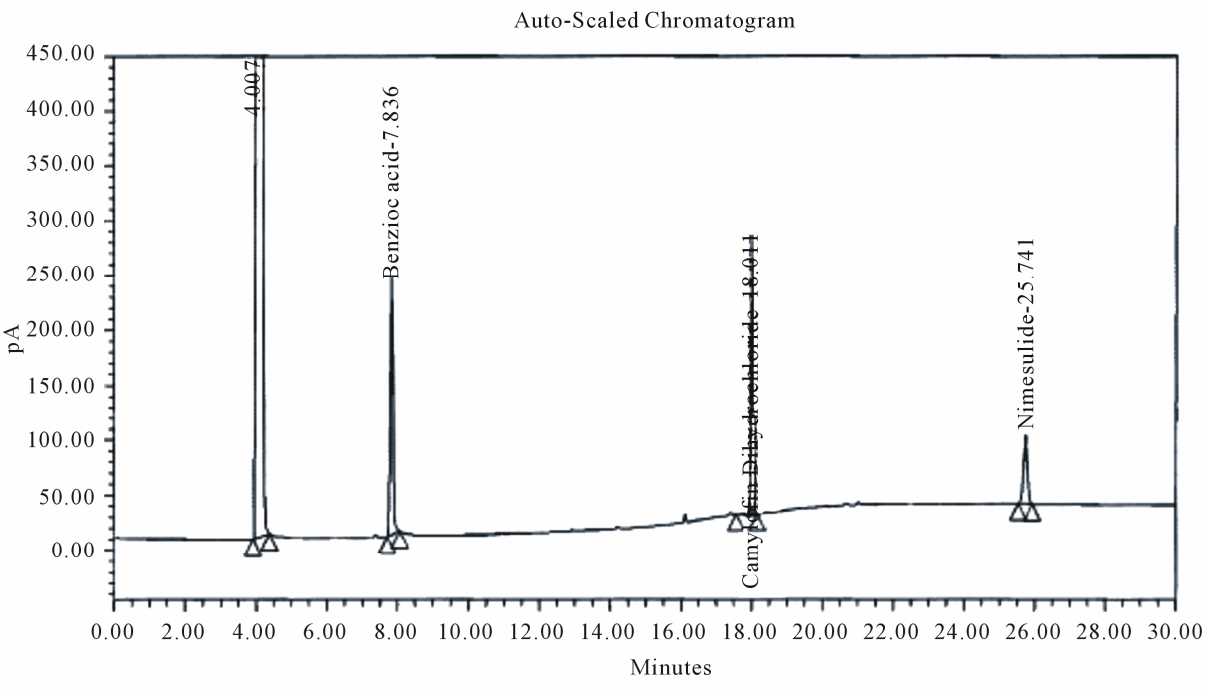


Figure 5. Chromatogram of Camylofin dihydrochloride and Nimesulide with Benzoic acid (internal standard) in sample preparation.

Table 2. Results of system suitability.

Parameters	Benzoic acid	Camylofin dihydrochloride	Nimesulide
Resolution	NA	78.2	42.8
Tailing factor	1.0	1.2	1.0
Theoretical plates	62551	375888	211127
% RSD	NA	0.45	0.51

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 6**.

The method was not affected by deliberate variations such as flow rate and column temperature.

3.8. Solution Stability

The % assay of Camylofin dihydrochloride and Nimesulide were checked in the test solutions. The % RSD of assay of Camylofin dihydrochloride and Nimesulide during solution stability experiment was within 1.0. No significant changes were observed in the content of Camylofin dihydrochloride and Nimesulide 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**.

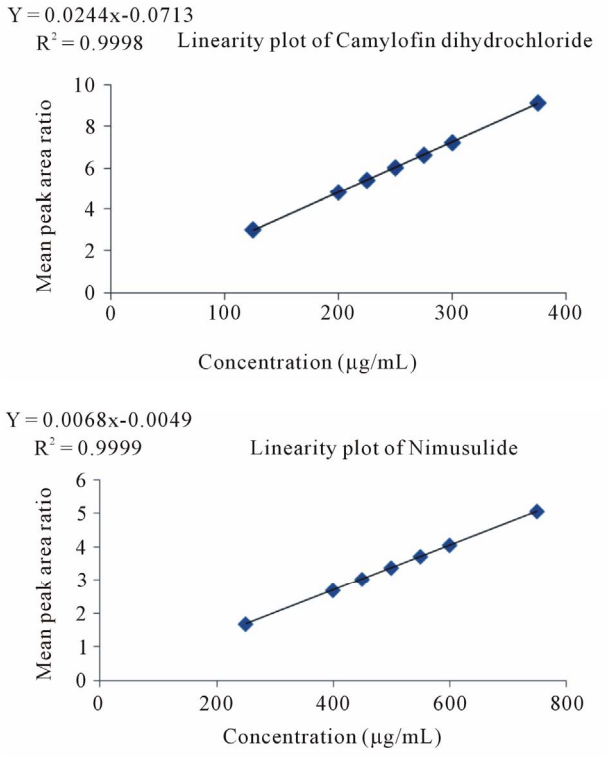


Figure 6. Linearity plot of camylofin dihydrochloride and nimesulide.

Table 3. Results of linearity study.

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n = 7)
Camylofin dihydrochloride	0.0244	–0.0713	0.9998
Nimesulide	0.0068	–0.0049	0.9999

Table 4. Accuracy of method.

Analyte	Recovery Level (%)	Amount added (µg·mL^{-1})	Amount recovered (µg·mL^{-1})	RSD (%) N = 3	(%) Recovery
Camylofin dihydrochloride	50	125.12	124.88	0.32	99.81
	100	250.24	251.91	0.22	100.67
	150	375.36	375.94	0.21	100.15
Nimesulide	50	250.74	249.15	0.25	99.37
	100	501.48	500.58	0.24	99.82
	150	752.22	751.45	0.19	99.90

Table 5. Results of precision experiment.

Results	Camylofin dihydrochloride	Nimesulide
Drug found in mg/tab (mean)	49.81	100.22
% Mean Assay	99.62	100.22
% RSD	0.47	0.29

Table 6. Ruggedness of assay experiment.

Results	Camylofin dihydrochloride	Nimesulide
Drug found in mg/tab (mean)	50.23	100.64
% Mean Assay	100.46	100.64
% RSD	0.55	0.42
% Difference wr.t. Precision	0.84	0.42

Table 7. Results of solution stability.

Condition	% Assay of Camylofin dihydrochloride	% Difference w.r.t. initial assay	% Assay of Nimesulide	% Difference w.r.t. initial assay
Initial	99.6	NA	100.2	NA
24 hours	99.4	0.2	100.0	0.2
48 hours	99.0	0.6	99.5	0.7
72 hours	98.4	1.2	99.1	1.1

3.9. Stress Testing (Forced Degradation Study)

The % degradation of Camylofin dihydrochloride in acid hydrolysis, base hydrolysis, oxidation, thermal and photolytic was 10.43, 18.20, 4.87, 1.53 and 2.20 respectively with respect to the control sample. The % degradation of Nimesulide in acid hydrolysis, base hydrolysis, oxidation, thermal and photolytic was 4.34, 21.99, 3.97, 1.97 and 7.34 respectively with respect to the control sample. The mass balance was found to be more than 97.0%. The peaks of the degradation products were well resolved from the principle peaks. The results of stress studies are tabulated in **Tables 8(a)-(b)**.

4. Conclusions

The method after being completely validated showed satisfactory data for all the method validation parameters.

Table 8. (a): Summary of forced degradation results for Camylofin dihydrochloride; (b): summary of forced degradation results for Nimesulide.

(a)

Stress condition	Time	% Assay of Camylofin 2HCl	% Degradation w.r.t control	Mass balance (% assay+ % degradation products)
Control	NA	99.74	NA	100.14
Acid hydrolysis (2 M HCl)	48 h	89.31	10.43	98.01
Base hydrolysis (1 N NaOH)	3 h	81.54	18.20	97.52
Oxidation (3% H_2O_2)	48 h	94.87	4.87	99.19
Thermal (80˚C)	5 day	98.24	1.53	98.21
Light (photolytic degradation)	5 day	97.54	2.20	98.54

(b)

Stress condition	Time	% Assay of Nimesulide	% Degradation w.r.t control	Mass balance (% assay+ % degradation products)
Control	NA	100.21	NA	100.14
Acid hydrolysis (2 M HCl)	48 h	95.87	4.34	98.01
Base hydrolysis (1 N NaOH)	3 h	78.22	21.99	97.52
Oxidation (3% H_2O_2)	48 h	96.24	3.97	99.19
Thermal (80˚C)	5 day	98.24	1.97	98.21
Light (photolytic degradation)	5 day	92.87	7.34	98.54

Method validation study showed that the method is specific, linear, accurate, easily reproducible and can be used for simultaneous determination of camylofin dihydrochloride and Nimesulide from pharmaceutical preparations. Stress testing showed that all degradation products were well separated from Camylofin dihydrochloride and Nimesulide, 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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