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Simultaneous RP HPLC determination of camylofin dihydrochloride and nimusulide in pharmaceutical preparations

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ABSTRACT

A simple, fast and precise reversed phase high performance liquid chromatographic method has been developed for the simultaneous determination of Camylofin dihydrochloride and Nimusulide using Methylparaben as an internal standard. Efficient chromatographic separation was achieved on Inertsil C_{18} column (250mm×4.6 mm, 5 µm) as stationary phase with a mobile phase comprising of Buffer solution pH 3.2 **:** Methanol (40:60,v/v) at a flow rate of 1.5mL min^{-1} , column temperature of 30°C and UV detection at 220 nm. The retention time of Methylparaben, Camylofin dihydrochloride and Nimusulide were about 4.2 min, 6.6 min and 10.7 min respectively. The proposed method was validated for linearity, accuracy, precision, sensitivity, robustness and solution stability. Linearity, accuracy and precision were found to be acceptable over the ranges of 250-750 µg mL^{-1} for Nimusulide and 125-375µg mL^{-1} for Camylofin dihydrochloride. The test solution was found to be stable for 48 h. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

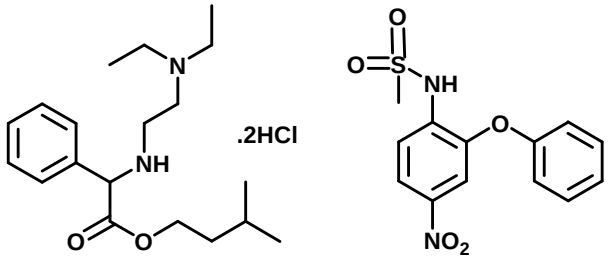
Camylofin dihydrochloride;
Nimusulide;
Validation;
Liquid chromatography;
Pharmaceutical preparations.

INTRODUCTION

Camylofin dihydrochloride is 3-methylbutyl 2-(2-diethylaminoethylamino)-2-phenyl-acetate hydrochloride is a drug used an antispasmodic[1]. Nimusulide 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 Nimusulide.

The literature revealed no method was available for simultaneous determination of this drug in such phar-



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

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

Figure 1 : Structures of camylofin dihydrochloride and nimusulide

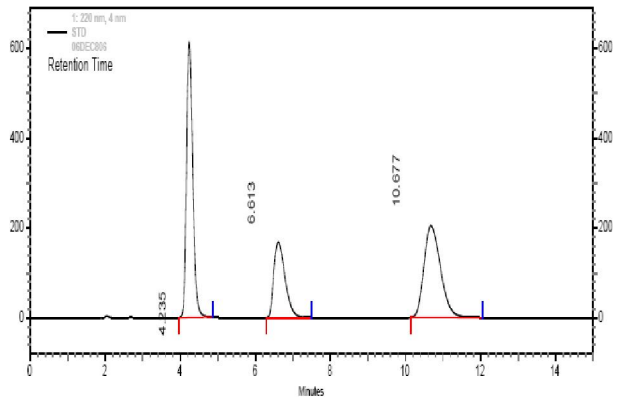


Figure 2 : A typical chromatogram of standard preparation: Methylparaben (4.235 min), Camylofin dihydrochloride (6.613 min) and Nimusulide (10.677 min)

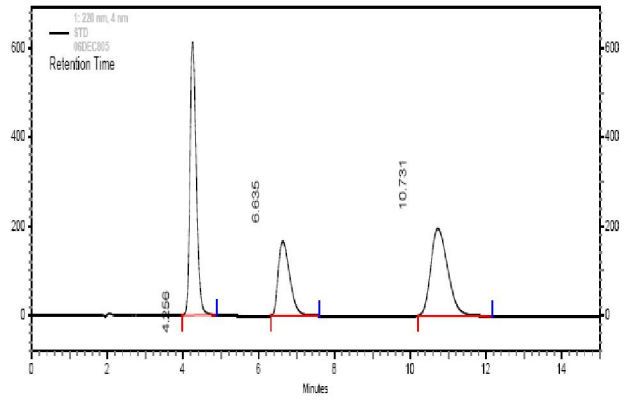


Figure 3 : A typical chromatogram of sample preparation: Methylparaben (4.256 min), Camylofin dihydrochloride (6.635 min) and Nimusulide (10.731 min)

maceutical preparation by HPLC[3-15]. Therefore an HPLC method was developed for determination of Camylofin dihydrochloride and Nimusulide from their dosage form. The method described is simple, fast, precise and accurate for simultaneous determination of Camylofin dihydrochloride and Nimusulide from pharmaceutical preparation.

EXPERIMENTAL

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 Nimusulide 100 mg. Potassium dihydrogen orthophosphate and methanol were from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

LC instrument and condition

To develop a suitable LC method for the analysis of Camylofin dihydrochloride and Nimusulide 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 involved, time required for the analysis and better separation of drugs. Chromatographic separation was preformed with Shimadzu LC 2010 High performance liquid chromatography having HPLC isocratic pump, equipped with auto sampler and a photo-diode array detector. The UV spectrums of Camylofin dihydrochloride and Nimusulide were scanned on photo diode array detector for selecting the working wavelength. Peak purity of Camylofin dihydrochloride and Nimusulide was checked using photo diode array detector. Chromatograms and data were recorded by means of Class VP software. Inertsil C_{18} column (250mm×4.6 mm, 5 µm particle) was used for the analysis. The mobile phase comprising of Buffer solution pH 3.2: Methanol (40:60, v/v) was used. 0.05 M KH_2PO_4 solution was used as the buffer solution and the pH was adjusted to 3.2 by using orthophosphoric acid. The system was run at a flow rate of 1.5mL min^{-1} and 40 µL of sample was injected in the chromatographic system. The column temperature was maintained at 30°C and detection wavelength was set at 220 nm for simultaneous determination of Camylofin dihydrochloride and Nimusulide. A typical HPLC chromatogram for simultaneous determination of Camylofin dihydrochloride and Nimusulide from pharmaceutical formulation is shown in figure 2 and 3.

Preparation of standard solutions

The stock solution of Camylofin dihydrochloride (1250 µg mL^{-1}) was prepared by dissolving 125.7 mg of Camylofin dihydrochloride (99.9 %) in methanol in a standard 100mL volumetric flask (stock solution A). The stock solution of Nimusulide (2500 µg mL^{-1}) was prepared by dissolving 250.5 mg of Nimusulide (99.8 %) in methanol in a standard 100mL volumetric flask (stock solution B). Internal standard (methyl paraben) stock solution (5000 µg mL^{-1}) was prepared by dissolving 501.6 mg of methyl paraben in methanol in a 100mL standard volumetric flask (stock solution C).

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TABLE 1 : Results of assay experiment

Results	Camylofin dihydrochloride	Nimusulide
Drug found in mg/tab (mean)	49.8	99.6
% Mean Assay	99.6	99.6
% RSD	0.67	0.71

TABLE 2 : Result of system suitability

Parameters	Methylparaben (IS)	Camylofin dihydrochloride	Nimusulide
Resolution	-	5.54	5.92
Tailing factor	1.21	1.62	1.29
Theoretical plates	3161	2282	2543

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.

Preparation of sample solution

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 equivalents to ten tablets were transferred in a 200mL volumetric flask. Added about 100 mL of Methanol to the volumetric flask, shaken for 10 minutes and then sonicated for 15 minutes. The solution 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. The amount of Camylofin dihydrochloride and Nimusulide per tablet was calculated by extrapolating the peak area from the calibration curve. The results are reported in TABLE 1.

RESULTS AND DISCUSSION

HPLC method development and optimization

Column chemistry, solvent type, solvent strength, detection and flow rate were varied to determine the chromatographic conditions for better separation.

Several mobile phases using different organic solvents as part of mobile phase were tried. Water and acetonitrile in the ratio of 500:500, v/v was chosen for initial trail with a 25 cm length, 4.6 mm ID and 5 micron particle size C-18 stationary phase. Flow rate was 1.0 mL $min^{-1.}$ When test solution was injected the resolution between Methylparaben and Camylofin dihydrochloride was less (<1.2). Results obtained with 25 cm length, 4.6 mm ID and 5 micron particle size C-8 column showed lesser resolution between Methylparaben and Camylofin dihydrochloride (<1.0).

To improve the resolution between Methylparaben and Camylofin dihydrochloride, water and methanol in the ratio 500:500, v/v was used as a mobile phase. When system suitability solution was injected in the above conditions the resolution between Methylparaben and Camylofin dihydrochloride was greater than 2.0, but the tailing factor of Camylofin dihydrochloride was greater than 2.2. To further improve the tailing factor of Camylofin dihydrochloride, the ratio of water and methanol was changed. A mixture of water and methanol in the ratio of 400:600, v/v was used. Resolution between all the peaks were achieved but the peak shape of Camylofin dihydrochloride was not satisfactory. Also the tailing factor of Camylofin dihydrochloride was ~ 2.0. To improve the peak shape and tailing factor of Camylofin dihydrochloride, a buffer solution consisting of 0.05 M K_2HPO_4 solution was used instead of water. A mobile phase consisting of 0.05 M K_2HPO_4 solution and methanol in the ratio of 400:600, v/v was used. The peak shape of Nimusulide was not good. Hence buffer solution was selected at acidic side. When acidic buffer consisting of 0.05 M KH_2PO_4 solution and methanol in the ratio of 350:650, v/v was used, good resolution between Methylparaben,Camylofin dihydrochloride and Nimusulide was observed in the system suitability solution, but the peak shape for Nimusulide was not good. Hence the pH of the Buffer solution was adjusted to 3.20 with orthophosphoric acid. The resolution was greater than 4.5 and the tailing factor was less than 2.0 for all the peaks. The total run time of the chromatogram was not more than 15 min.

HPLC columns played a major role in achieving satisfactory separation between the peaks. When C8

TABLE 3 : Results of linearity

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n=7)
Camylofin dihydrochloride	0.004	0.007	0.9999
Nimusulide	0.005	0.009	0.9999

TABLE 4 : Results of assay experiment

Results	Camylofin dihydrochloride	Nimusulide
Drug found in mg/tab (mean)	49.8	99.6
% Mean Assay	99.6	99.6
% RSD	0.67	0.71

TABLE 5 : Ruggedness of assay experiment

Results	Camylofin dihydrochloride	Nimusulide
Drug found in mg/tab (mean)	50.2	99.9
% Mean Assay	100.4	99.9
% RSD	0.58	0.87
% Difference wr.t. Precision	0.8	0.3

TABLE 6 : Results of accuracy experiment

Analyte	Amount added		% Recovery	% RSD n= 3
	%	µg mL^{-1}		
Camylofin dihydrochloride	80	200.0	99.9	0.58
	100	250.0	100.1	0.72
	120	275.0	100.1	0.44
Nimusulide	80	400.0	100.1	0.65
	100	500.0	100.0	0.44
	120	600.0	100.2	0.24

column (Inertsil C8, 4.6×250 mm, 5u) was used the resolution between Methylparaben and Camylofin dihydrochloride was less (Resolution <1.5). To improve the resolution a column with more carbon loading i.e. C18 was selected (Inertsil C18, 4.6×250 mm, 5u). Satisfactory peak shape and good resolution were observed between all the peaks.

In the optimized conditions Methylparaben, Camylofin dihydrochloride and Nimusulide were well separated with a resolution greater than 4.5 and the typical retention times of Methylparaben, Camylofin dihydrochloride and Nimusulide were about 4.2 min, 6.6 min and 10.7 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. System suitability tests were performed as per the general chapter <621> in USP 32 NF 27 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 40-µL standard solutions of Camylofin dihydrochloride, Nimusulide of strengths 250 µg mL^{-1} and 500 µg mL^{-1} us using methylparaben as an internal standard. Five replicate injections were made. The %RSD values of Camylofin dihydrochloride and Nimusulide were 0.76 and 0.44 respectively. The %RSD values were found to be satisfactory and meeting the requirements of the general chapter <621> in USP 32 NF 27 (%RSD not more than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 2.

Method validation

Method validation was performed as per ICH guidelines[16,17].

Linearity

Linearity was evaluated by analysis of working standard solutions of Camylofin dihydrochloride and Nimusulide of seven different concentrations. The range of linearity was from 250-750 µg mL^{-1} for Nimusulide and 125-375 µg 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. The regression data obtained for the Camylofin dihydrochloride and Nimusulide is represented in TABLE 3. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

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. The LOD and LOQ of Camylofin dihydrochloride and Nimusulide were experimentally determined by six injections of each drug. The LOD of Camylofin dihydrochloride and Nimusulide was found to be 0.04µg mL^{-1} & 0.07 µg mL^{-1} respectively. The LOQ of Camylofin dihydrochloride and Nimusulide was found to be 0.2 µg mL^{-1} & 0.3 µg mL^{-1} respectively.

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TABLE 7 : Results of robustness experiment

Robustness condition: Change of flow rate				
Parameters	Low flow (1.3 mL/min)		High flow (1.7 mL/min)	
	Camylofin dihydrochloride	Nimusulide	Camylofin dihydrochloride	Nimusulide
Resolution	5.62	5.98	5.45	5.85
% Assay	100.5	100.1	100.2	100.5
Robustness condition: Change of column temperature				
Parameters	Low column temperature (28°C)		High column temperature (30°C)	
	Camylofin dihydrochloride	Nimusulide	Camylofin dihydrochloride	Nimusulide
Resolution	5.58	5.81	5.61	5.86
% Assay	100.2	99.9	100.1	100.3
Robustness condition: Change of Mobile Phase composition				
Parameters	Low organic composition (Buffer solution pH3.2: MeOH ::430:570)		High organic composition (Buffer solution pH3.2: MeOH ::370:630)	
	Camylofin dihydrochloride	Nimusulide	Camylofin dihydrochloride	Nimusulide
Resolution	5.85	5.96	5.21	5.52
% Assay	99.8	100.1	100.3	100.2

TABLE 8 : Results of Solution stability

% Assay	Camylofin dihydrochloride	% Difference w.r.t. initial assay	Nimusulide	% Difference w.r.t. initial assay
Initial	99.8	Not applicable	100.1	Not applicable
24 hours	99.5	0.3	99.7	0.4
48 hours	99.4	0.4	99.6	0.5
72 hours	99.1	0.7	99.4	0.7

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 deviation (RSD) was less than 2%. Method precision was determined from results from six independent determinations at 100% of the test concentrations of Camylofin dihydrochloride and Nimusulide in the product. The % RSD for Camylofin dihydrochloride and Nimusulide was found to be 0.67 and 0.71 respectively. Refer TABLE 4.

Ruggedness

Ruggedness study was done by injecting six individual sample preparations at 100% of the test concentrations of Camylofin dihydrochloride and Nimusulide on different day and different HPLC system. The mean % Assay obtained was compared with mean % Assay of precision study. The relative standard deviation (RSD) was less than 2%. The % RSD for Camylofin dihydrochloride and Nimusulide was found to be 0.58 and 0.87 respectively. Refer TABLE 5.

Accuracy

Accuracy of the developed method was confirmed by doing recovery study as per ICH guidelines at three different concentration levels 80%, 100% and 120% by replicate analysis (n=3). The results of accuracy study were reported in TABLE 6. The results indicate the method is highly accurate for simultaneous determination of Camylofin dihydrochloride and Nimusulide.

Robustness

By deliberate change in experimental condition the resolution between Methylparaben, Camylofin dihydrochloride and Nimusulide 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 min^{-1}. The effect of column temperature was studied at 28°C and 32°C. In all the above varied conditions, the components of the mobile phase were held constant. The effect of Mobile phase was studied by changing the ratio of mobile phase composition. The organic phase composition was changed by 5%. i.e. 570 mL and 630 mL for Methanol. The resolution between the peak between Methylparaben and Camylofin dihydrochloride was greater than 4.5 and Camylofin dihydrochloride and Nimusulide was greater than 5.0. The results of resolution and % Assay are mentioned in TABLE 7.

Solution stability and mobile phase stability

The solution stability of Camylofin dihydrochloride and Nimusulide 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.

Mobile phase stability was also carried out for 72 hours by injecting the freshly prepared sample solutions for every 24 hours interval. The % assay of Camylofin dihydrochloride and Nimusulide were checked in the test solutions. Mobile phase prepared was kept constant during the study period. The % RSD of assay of Camylofin dihydrochloride and Nimusulide

during solution stability and mobile phase stability experiments was within 1.0. No significant changes were observed in the content of Camylofin dihydrochloride and Nimusulide during solution stability and mobile phase stability experiments. Sample solutions and mobile phase used during the experiment were stable upto the study period of 72 hours. The results are reported in TABLE 8.

CONCLUSION

A new, reverse phase HPLC method has been developed for the simultaneous analysis of Camylofin dihydrochloride and Nimusulide in tablet formulation. It was shown above that the method was linear, accurate, precise, selective, stable and specific proving the reliability of the method. 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 of Camylofin dihydrochloride and Nimusulide.

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