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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(3): 443-448

www.thepharmajournal.com
Received: 21-01-2019
Accepted: 25-02-2019

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Method development and validation of Nimesulide and Camylofin dihydrochloride in combined tablet dosage form by UV - absorbance ratio method

Renjitha JR and Jose Kurien

Abstract
Nimesulide is a non-steroidal anti-inflammatory drug and Camylofin Dihydrochloride is an antispasmodic in combination with NSAIDS. Simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for simultaneous estimation of Nimesulide and Camylofin Dihydrochloride in tablet dosage form has been developed. Method employs formation and solving of simultaneous equation using 295 and 259nm as two analytical wavelengths for both drugs in methanol. The method was validated as per ICH guidelines. Nimesulide and Camylofin Dihydrochloride at their respective λ_{max} 295 nm and 259nm shows linearity in a concentration range 5-25µg/ml with correlation coefficient in the range of 0.9961- 0.9998. The percentage of relative standard deviation of six replicate measurements was found to be indicates the proposed method was precise. Recovery studies were conducted at three different concentrations levels and the average percentage in tablet dosage form was determined and found to be in the range of 99.33- 100.08 %. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific.

Keywords: nimesulide, camylofin dihydrochloride, UV- simultaneous equation

Introduction
Nimesulide ($C_{13}H_{12}N_2O_5S$) is chemically N-(4-Nitro-2-Phenoxyphenyl) methane sulphonamide is a non-steroidal anti-inflammatory drug (NSAID) of Sulfonanilide class with pain medication and fever reducing properties. Its approved indication is the treatment of acute pain, the symptomatic treatment of osteoarthritis, and primary dysmenorrhoea in adolescents and adults above 12 years old [13, 14]. Camylofin ($C_{19}H_{32}N_2O_2$.Hcl) Isopentyl 2-(2-(diethylamino)-2-phenyl acetate has a direct Papaverine like spasmolytic action on the smooth muscle hence it is used as antispasmodic in biliary colic, renal and ureteric colic, dysmenorrhoea, peptic ulcer and chronic enterocolitis along with NSAIDs. Nimesulide and Camylofin Dihydrocloride are available in tablet dosage form (Anafortan N). Many methods have been reported in literature for determination of Nimesulide with other drugs individually and in combination **[1-8, 10- 12]**. However there is no UV spectrophotometric method for study of Nimesulide and Camylofin dihydrochloride in tablet dosage form. This communication forms the first report of simple, sensitive, and reproducible methods for the simultaneous estimation of Nimesulide and Camylofin dihydrochloride.

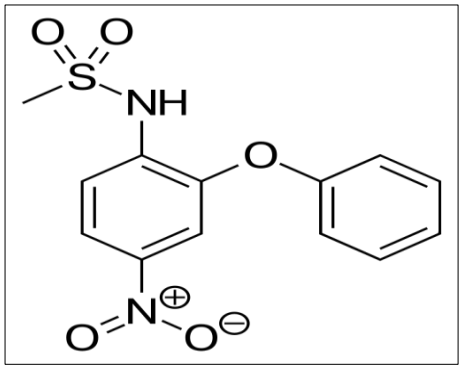

Fig 1: Structure of Nimesulide

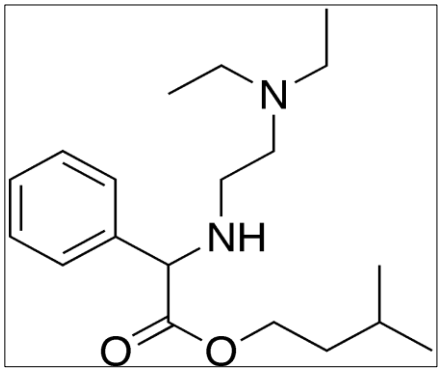

Fig 2: Structure of Camylofin Dihydrochloride

Materials and Methods

Instruments

Spectral runs were made on Jasco V 560 double beam spectrophotometer with a pair of 10mm quartz cells.

Reagents and Chemicals

Nimesulide and Camylofin Dihydrochloride reference standard provided by Yarrow Chem Laboratories, Mumbai and Sigma Aldrich respectively. Methanol HPLC grade purchased from Merck specialities (P) Ltd Mumbai. The commercially available Anafortan N tablets (Containing Nimesulide 100 mg and Camylofin dihydrochloride 50 mg), marketed by Abbott health care private Limited procured from local market.

Preparation of standard drug solution

Standard stock solution containing Nimesulide (NIM) and Camylofin dihydrochloride (CAM) were prepared individually by dissolving 50 mg of Nimesulide RS and 50 mg Camylofin dihydrochloride RS separately in 25 ml methanol. It was then sonicated for 10 minutes and the final volume of both solutions was made up to 50 ml in a 50 ml standard flask to obtain a concentration of 1 mg/ml. (solution A). From the above solution, accurately pipetted out 5.0 ml into a 50 ml standard flask and the volume were made up to the mark using methanol. The resulting solution had a concentration of 100 µg/ml.

Determination of absorption maxima

Solution containing 10µg/ml of Nimesulide and Camylofin Dihydrochloride were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both drugs. NIM and CAM showed absorbance maxima at 295 nm (λ1) and 259nm (λ2) respectively and the isoabsorbtive point was found to be 270nm.

Absorbance ratio Method

Two wavelengths selected for the method are 295 nm and 270 nm that are absorption maxima's of NIM and Iso absorptive point of CAM respectively in methanol. The stock solutions of both drugs were further diluted separately with methanol to get a series of standard solution of 5-25µg/ml of NIM and CAM. The absorbance were measured at the selected wavelengths and absorptivity's (A1%, 1cm) for both the drugs at both wavelengths were determined as mean of six independent determinations. Concentrations in the sample were obtained by using following equations [9].

$$Cx = \frac{Qm-Qy}{Qx-Qy} \times \frac{A1}{ax1} \quad \text{…. Eq. (i)}$$

$$Cy = \frac{Qm-Qx}{Qy-Qx} \times \frac{A1}{ay1} \quad \text{….Eq. (ii)}$$

Where A_1 and A_2 are absorbance's of sample at 295 nm and 270 nm respectively, a_{x1} and a_{x2} are absorptivity's of NIM at 295 and 270 nm respectively, a_{Y1} and a_{Y2} are absorptivity's of CAM at 295 nm and 270 nm respectively. C_x and C_y are the concentrations of NIM and CAM respectively in the diluted sample.

Application of the proposed method for the determination of NIM and CAM in Tablets

Twenty tablets of Anafortan N were weighed; average weight of one tablet was calculated and finely powdered with the help of a mortar and pestle. A quantity of powder equivalent to 50 mg of Nimesulide (containing 25 mg of Camylofin Dihydrochloride) was weighed accurately and transferred to a glass stoppered flask. The powder was extracted initially with 15 ml of methanol by sonication for 10 mints and filtered through whatmann no:1 filter paper to a 50 ml standard flask. The residue was further extracted twice with 10 ml of methanol and transferred to the same standard flask through the same filter paper. The volume was made up to the mark using methanol. The resulting solution had a concentration of 500 µg/ml of Camylofin Dihydrochloride and 1000 µg/ml Nimesulide. From the above solution, accurately pipetted out 1ml and transferred to a 50 ml standard flask. Then the volume was made up to the mark using methanol to obtain a concentration of 10 µg/ml of Camylofin Dihydrochloride and 20µg/ml of Nimesulide. The absorbances of resulting solution were measured at 295 and 259 nm. Values are substituted in the respective formula to obtain concentrations.

Results

The absorption spectra were observed with maximum absorption at 295 nm and 259 nm for Nimesulide and Camylofin Dihydrochloride respectively. The spectra obtained are shown in figure.

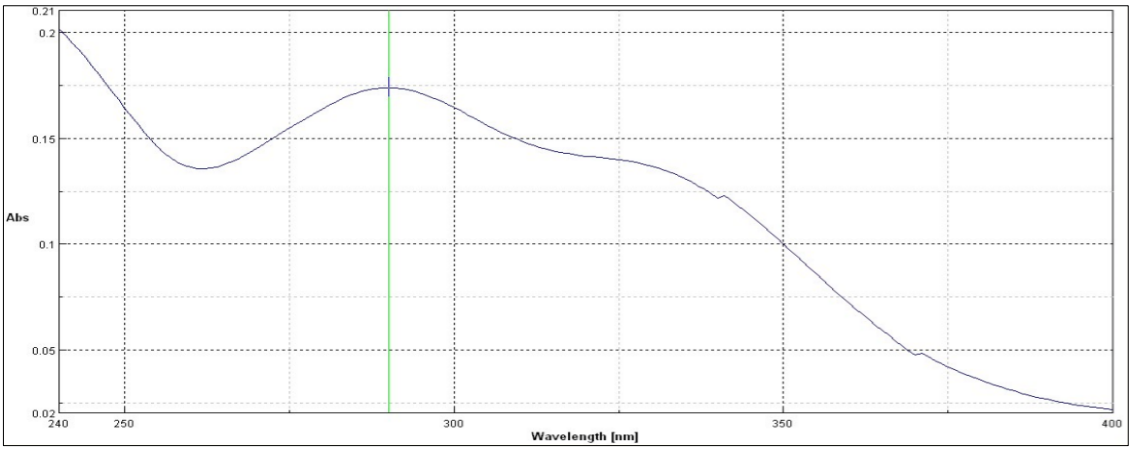

Fig 3: UV Absorption spectra of Nimesulide RS in methanol with absorption maximum at 295 nm

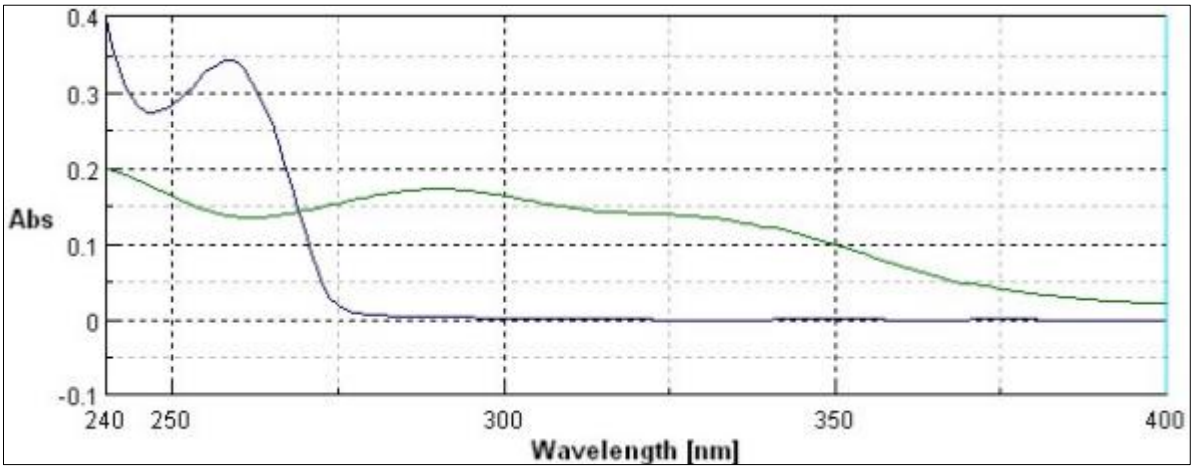

Fig 5: Overlay spectrum of Nimesulide and Camylofin dihydrochloride

Method validation [13]

The developed method was validated as per ICH guidelines for Linearity, precision, accuracy, specificity, LOD and LOQ.

1. Linearity

The linear response of Nimesulide and Camylofin Dihydrochloride was determined by analysing five different concentration of standard solution ranging from 5-25µg/ml. The absorbance of each solution was measured at 295 nm and 270 nm with methanol as blank. The calibration curve of absorbance v/s concentration was plotted and correlation co-efficient and regression line equation was determined. The linear plot of Nimesulide is given in figure 7- 10.

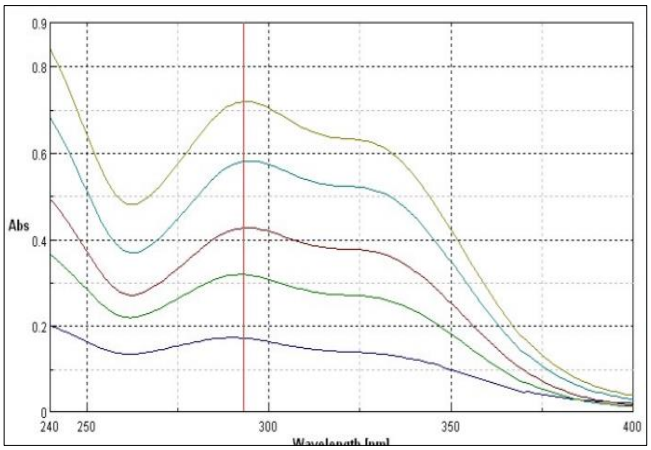

Fig 5: Zero order spectra of Nimesulide overlaid

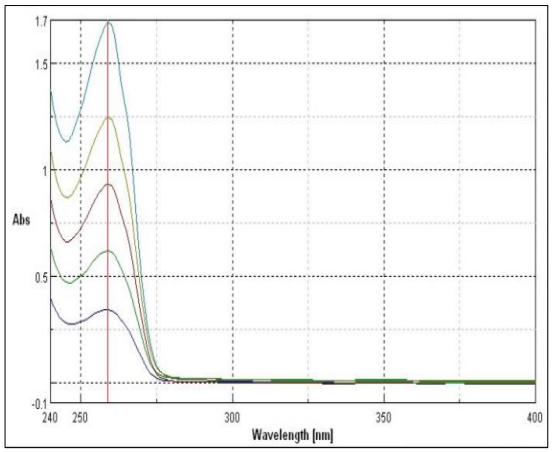

Fig 6: Zero order spectra of Camylofin dihydrochloride overlaid

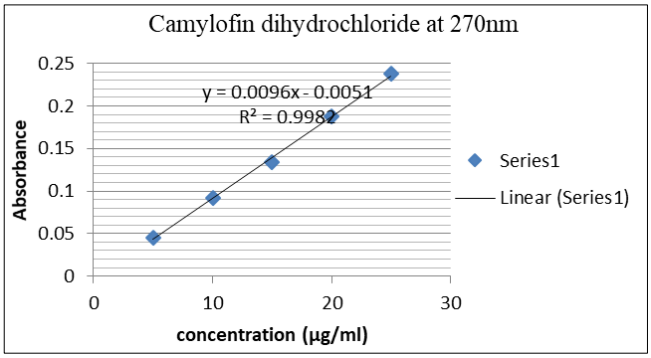

Fig 7: Calibration curve of Camylofin Dihydrochloride at 270 nm

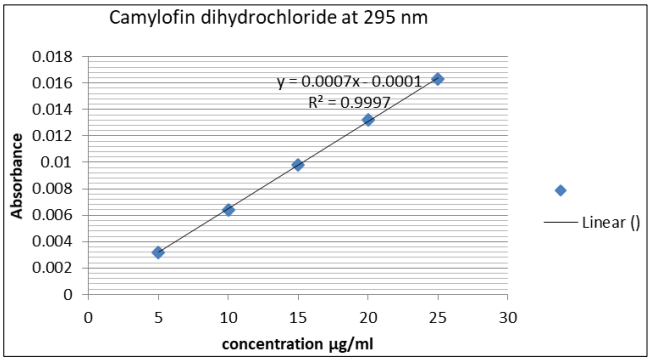

Fig 8: Calibration Curve of Camylofin Dihydrochloride at 295nm

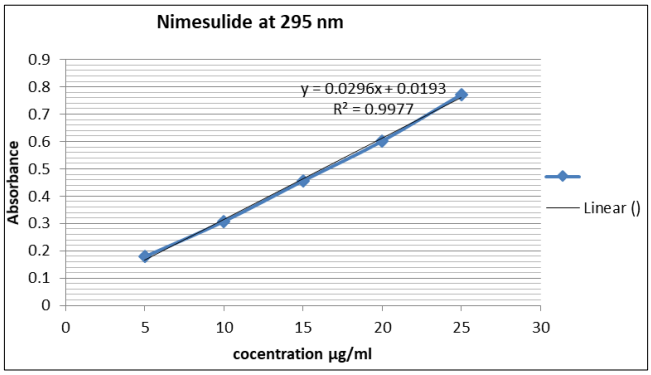

Fig 9: Calibration Curve of Nimesulide at 295 nm

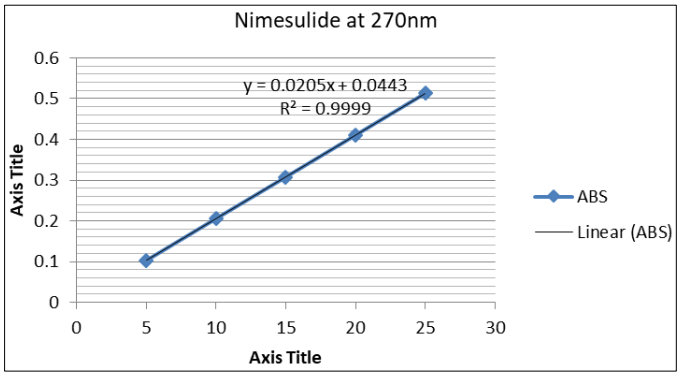

Fig 10: Calibration Curve of Nimesulide at 270 nm

2. Precision

Precision was determined in two levels –Repeatability and Intermediate Precision

➢ **Repeatability**

The repeatability of the method was studied by using six determinations at 100 % test concentration i.e. mixture of 10 µg/ml of CAM and 20µg/ ml of NIM. The results are tabulated in table 1 and the statistical validation is given in table 2.

Table 1: Results of Repeatability Study

S. No	Amount present (label claim) mg/ tablet		Amount obtained mg/tablet		Percentage label claim	
	NIM	CAM	NIM	CAM	NIM	CAM
1	20	10	19.9576	9.9781	99.7	99.6
2	20	10	19.9481	9.9789	99.76	99.81
3	20	10	19.9512	9.9794	99.77	99.81
4	20	10	19.9608	9.9763	99.82	99.78
5	20	10	19.9639	9.9752	99.84	99.77
6	20	10	19.9576	9.9781	99.7	99.6

Table 2: Repeatability Study- Statistical Validation

Components	Mean of %label claim	Standard deviation (SD)	Relative standard deviation(% RSD)	Coefficient of variation
NIM	99.77	0.0183	0.0184	0.0002
CAM	99.73	0.0292	0.0292	0.0002

➢ **Intermediate Precision**

The intermediate precision was studied by using six determinations of the mixture of 10µg/ ml of CAM and 20µ/ml of NIM. The stock solution was prepared and analysed at the same time on three consecutive days. The absorbance of the resulting solution was measured at 295 nm and 259 nm. The variations of the results on three days were analysed and the statistical validation was done. The results are furnished in table 3 and the assay results of tablet formulation are given in table 4.

Table 3: Results of Intermediate Precision

Components	Mean of % label claim (n= 18)	Standard deviation (SD)	Relative standard deviation (%RSD)	Coefficient of variation (CV)
NIM	99.80	0.0153	0.0153	0.0002
CAM	99.89	0.0179	0.0179	0.0002

Table 4: Assay result of formulation

Formulation	Label claim(mg)	Amount found (mg)	% label claim
Anafortan N Tablets			
Nimesulide	100	99.8001	99.80% w/w
Camylofin dihydrochloride	50	49.8623	99.72 w/w

3. Limit of detection and quantitation (LOD and LOQ)

LOD and LOQ were determined by linearity curve method and by using the equations.

LOD = 3.3 (σ/S)

LOQ =10 (σ/S)

Where σ is the standard deviation of the response and 'S' is the slope of the linearity curve.

Table 5: LOD and LOD data

Method parameters	NIM		CAM	
	295 nm	270 nm	295 nm	270 nm
LOD (µg/ml)	0.0394	0.0804	0.4573	0.2327
LOQ (µg/ml)	0.1193	0.2435	1.3857	0. 7052

Summary of Results

Table 6: Table show results of parameters

Parameters	Results	
UV Detection Wavelength (nm)	Nimesulide	Camylofin Dihydrochloride
	295 nm	270 nm
Linearity range	5- 25µg/ml	5- 25µg/ml
Regression equation		
at 295 nm	Y= 0.0296x + 0.0183	Y= 0.0007x + 0.0002
at 270 nm	Y= 0.0205x + 0.0443	Y= 0.0096x + 0.0051
Percentage Recovery(% w/w)		
15%	99.33	99.69
20%	99.92	99.83
25%	100.08	100.08
Precision % RSD		
Inter day	0.0153	0.0179
Repeatability	0.0184	0.0292
LOD (µg/mL)		
at 295 nm	0.0394	0.4573
at 270 nm	0.0508	0.0106
LOQ (µg/mL)		
at 295 nm	0.1193	1.3857
at 270 nm	0.1538	0.0322

Discussion

The overlain spectra of Nimesulide and Camylofin dihydrochloride exhibit λmax at 295 and 270 nm respectively. Standard calibration curves for Nimesulide and Camylofin Dihydrochloride were linear with correlation coefficient (r^2) values in the range of 0.9961- 0.9998 at the selected wavelengths and the values were average of six reading with standard deviation in the range of 0.0315- 0.1153. The calibration curves were repeated three times in a day and the average % RSD was found to be 0.0160 for NIM and 0.3876 for CAM indicate the linearity of the method.

Conclusion

The most striking feature of this method is its simplicity, economy, and rapidity. The method gives accurate and precise results for the analysis of Nimesulide and Camylofin Dihydrochloride in dosage forms.

Acknowledgement

The authors are thankful to Yarrow Chem laboratories and sigma Aldrich for providing Reference Standard, Govt medical college Trivandrum for providing necessary facilities to carry out this research work.

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