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Simultaneous method development and validation of nimesulide and camylofin dihydrochloride in tablet dosage form by uv spectrophotometric method

Renjitha J R *, Jose Kurien

College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram, Kerala, India.

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***Corresponding author:**

Email : renjurenjithajr94@gmail.com
Tel.: +91 - 9744984011

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 formhas 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.

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 [1- 4]. 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[5- 15]. 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.

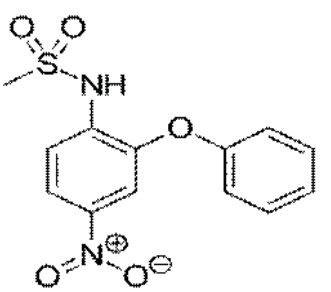

Figure 1 : Structure of Nimesulide

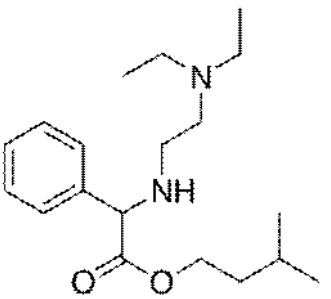

Figure 2 : Structure of Camylofin Dihydrochloride

Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The official test methods that result from these processes are used by quality control laboratories to ensure

the identity, purity, potency, and performance of drug products. As per the literature review, there are no analytical methods are reported for the simultaneous estimation of Nimesulide and Camylofin Dihydrochloride in combined dosage form. Various publications are available regarding the UV simultaneous estimation and RP-HPLC method development of Nimesulide and Camylofin Dihydrochloride, either alone or in combination with other drugs in pharmaceutical dosage form. So it is essential to develop accurate, simple, rapid, and sensitive analytical methods for the simultaneous estimation of this combination.

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 YarrowChem Laboratories, Mumbai and Sigma Aldrich respectively. Methanol HPLC grade purchased from Merckspecialities (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 5.0ml 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.

Simultaneous Equation Method

Two wavelengths selected for the method are 295 nm and 259 nm that are absorption maxima's of NIM and CAM in respectively 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. [16-20]

$$C_x = \frac{A_2 a_{y1} - A1 a y2}{a_{x2} a_{y1} - ax1 ay2} \ldots\text{Eq. (i)}$$

$$C_y = \frac{A_1 a_{x2} - A2 ax1}{a_{x2} a_{y1} - ax1 ay2} \quad \ldots\text{Eq. (ii)}$$

Where A_1 and A_2 are absorbance's of sample at 295 nm and 259 nm respectively, a_{x1} and a_{x2} are absorptivity's of NIM at 295 and 259 nm respectively, a_{Y1} and a_{Y2} are absorptivity's of CAM at 295 nm and 259 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

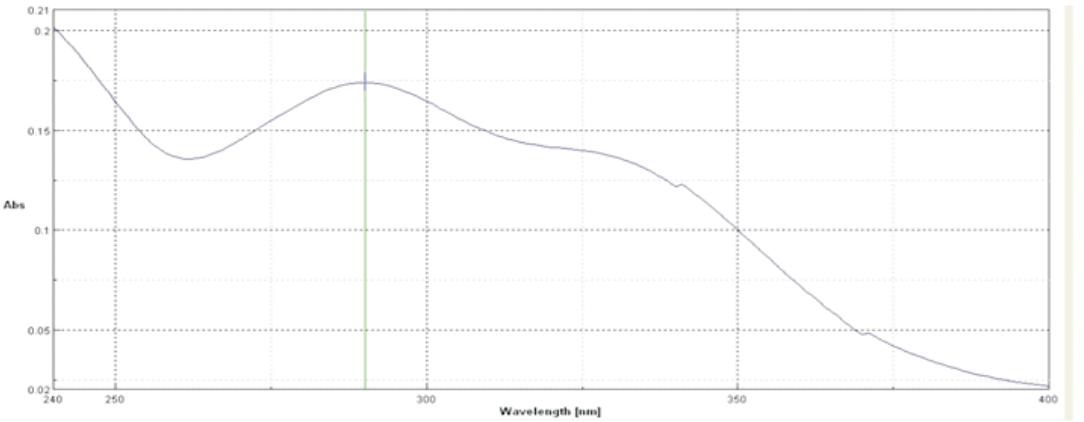

Figure 3 : UV Absorption spectra of Nimesulide RS in methanol with absorption maximum at 295 nm

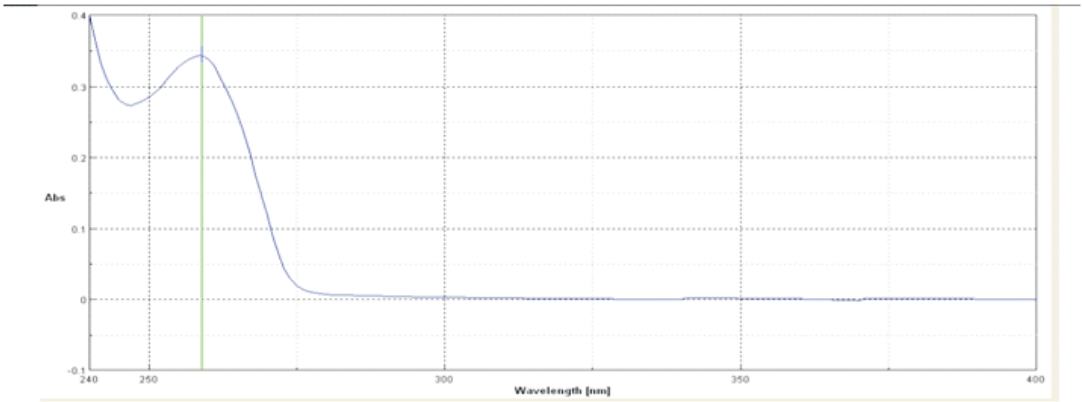

Figure 4 : UV Absorption spectra of Camylofin Dihydrochloride RS in methanol with absorption maximum at 259 nm

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.

METHOD VALIDATION[21-24]

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 259 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.

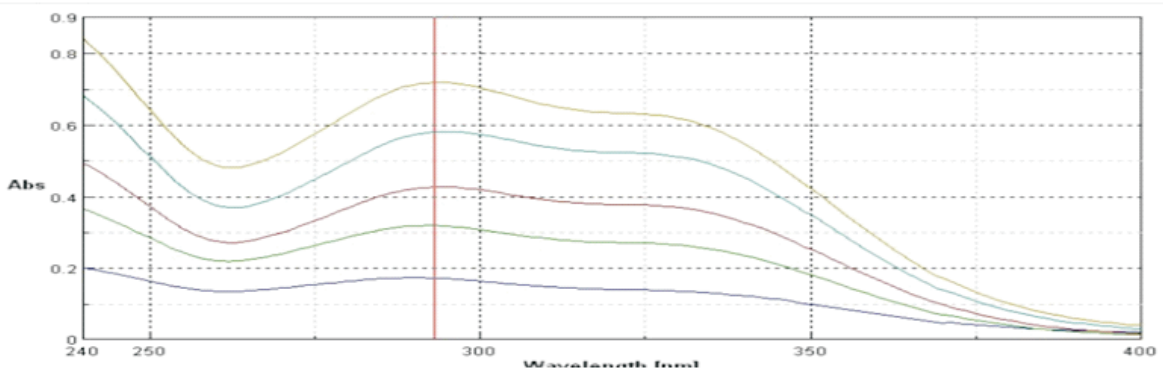

Figure 5 : Zero order spectra of Nimesulide overlaid

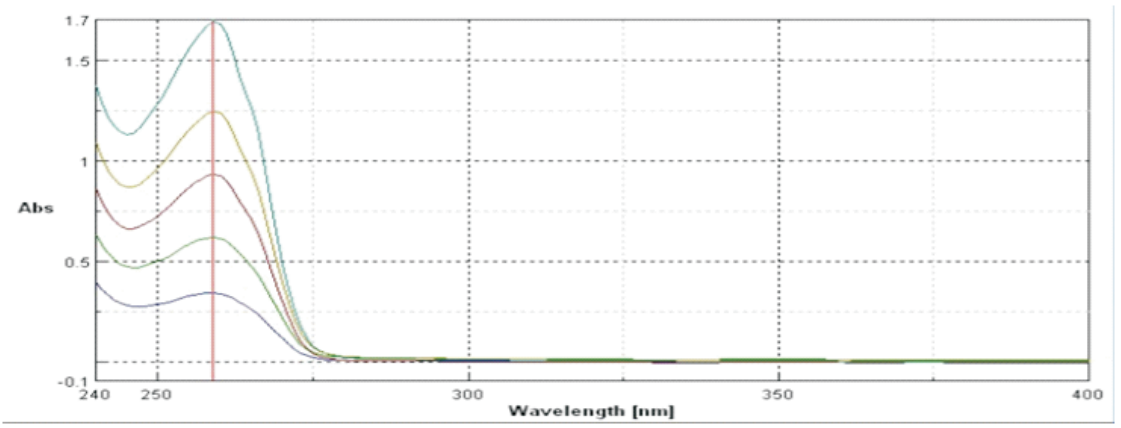

Figure 6 : Zero order spectra of Camylofin Dihydrochloride overlaid

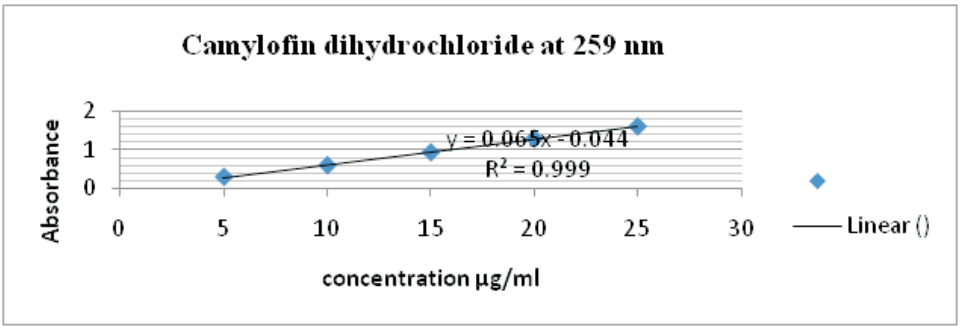

Figure 7 : Calibration curve of Camylofin Dihydrochloride at 259 nm

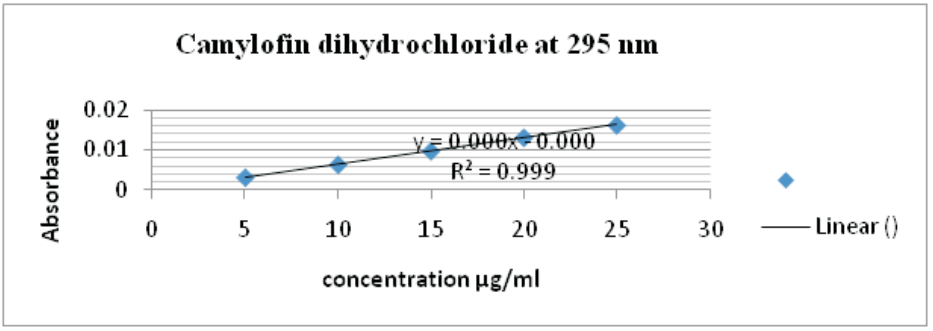

Figure 8 : Calibration Curve of Camylofin Dihydrochloride at 295nm

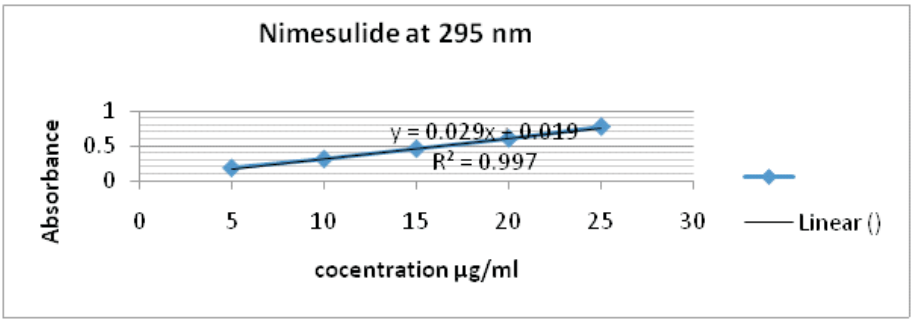

Figure 9 : Calibration Curve of Nimesulide at 295 nm

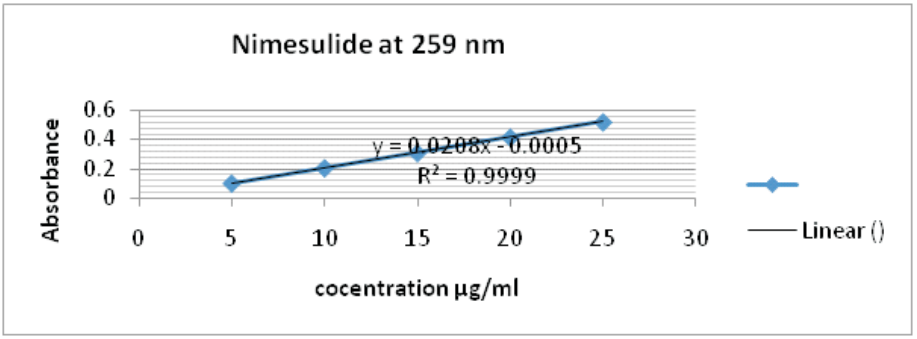

Figure 10 : Calibration Curve of Nimesulide at 259 nm

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.

Table 1 : Results of Repeatability Study

Sl.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

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	99.85	0.0153	0.0153	0.0002
CAM	99.83	0.0179	0.0179	0.0002

The results are tabulated in table 1 and the statistical validation is given in table 2.

➢ 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.

3. ACCURACY

Accuracy of the method was determined by recovery studies. To the formulation (pre-analysed sample), the reference standards of the drugs were added at the level of 15%, 20%, 25%.The assay results are shown in the table 5. The statistical validation data is shown in table 6.

Table 4 : Assay result of formulation

Formulation	Label claim(mg)	Amount found (mg)	% label claim
Anafortan N Tablets			
Nimesulide	100	99.7601	99.76% w/w
Camylofin dihydrochloride	50	49.8623	99.72 % w/w

Table 5 : Results of Recovery Study

Level of % recovery	Amount present (mg)		Amount of standard added (mg)		Drug recovery			
					Mg		Percentage	
	NIM	CAM	NIM	CAM	NIM	CAM	NIM	CAM
15%	50	25	7.5	3.75	7.4402	3.7484	99.2	99.95
	50	25	7.5	3.75	7.4480	3.7379	99.3	99.67
	50	25	7.5	3.75	7.4640	3.7288	99.5	99.44
20%	50	25	10	5	9.9951	4.9998	99.95	99.99
	50	25	10	5	9.9790	4.9893	99.79	99.7
	50	25	10	5	10.0032	4.9893	100.03	99.79
25%	50	25	12.5	6.25	12.510	6.2520	100.08	100.03
	50	25	12.5	6.25	12.5026	6.2589	100.02	100.14
	50	25	12.5	6.25	12.518	6.2537	100.14	100.06

4. 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 7 : LOD and LOD data

Method parameters	NIM		CAM	
	295 nm	259 nm	295 nm	259 nm
LOD (µg/ml)	0.0394	0.0508	0.4573	0.0106
LOQ (µg/ml)	0.1193	0.1538	1.3857	0.0322

Table 8 : SUMMARY OF RESULTS

PARAMETERS	RESULTS	
UV Detection Wavelength (nm)	Nimesulide	Camylofin Dihydrochloride
	295 nm	259 nm
Linearity range	5- 25µg/ml	5- 25µg/ml
Regression equation at 295 nm at 259 nm	Y= 0.0296x + 0.0183 Y= 0.0208x + 0.0014	Y= 0.0007x + 0.0002 Y= 0.0658x + 0.045
Percentage Recovery(% w/w) 15% 20% 25%	 99.33 99.92 100.08	 99.69 99.83 100.08
Precision % RSD Inter day Repeatability	 0.0153 0.0184	 0.0179 0.0292
LOD (µg/mL) at 295 nm at 259 nm	 0.0394 0.0508	 0.4573 0.0106
LOQ (µg/mL) at 295 nm at 259 nm	 0.1193 0.1538	 1.3857 0.0322

DISCUSSION

The overlain spectra of Nimesulide and Camylofin dihydrochloride exhibit λmax at 295 and 259 nm respectively and the UV spectra was shown in figure 3 and figure 4. Standard calibration curves for Nimesulide and Camylofin Dihydrochloride were linear and the linearity of the calibration curve and overlain spectrum was given in the figure (5-10). The correlation coefficient (r^2) values in the range of 0.9961- 0.9998 at the selected wavelengths and the regression equation is shown with in the calibration curve of Nimesulide and Camylofin Dihydrochloride (figure 7-8). 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 and the results are given in the table3. The accuracy of method was confirmed by recovery studies from tablet at three different levels 15%, 20 % and 25%; recovery in the range of 99.33- 100.08 % justifies the accuracy of method. The relative standard deviation of Nimesulide and Camylofin Dihydrochloride was in the range of 0.0003- 0.1153 and the results are given in the table 5 -6. The limit of detection was found to be 0.0394 and 0.0508 for Nimesulide and 0.4573 and 0.0106 for Camylofin Dihydrochloride at 295nm and 259nm respectively.the limit of quantitation was found to be 0.1193 and 0.1538 for Nimesulide and 1.3857 and 0.0322 for Camylofin Dihydrochloride respectively at 295 and 259nm.

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.

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