Q-Absorbance Ratio Spectrophotometric Method for Simultaneous Estimation of Nimesulide and Diclofenac sodium in Combined Pharmaceutical Dosage Form

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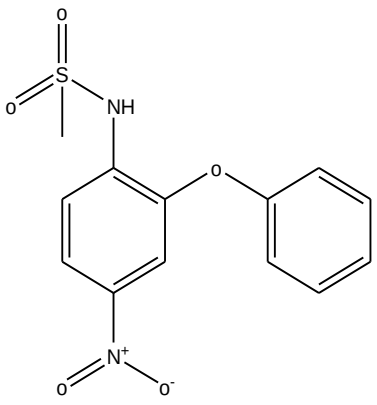
Abstract:
The objective of this work was to develop and validate a new UV spectrophotometric Q-absorption ratio method was developed and validated for the simultaneous estimation of Nimesulide and Diclofenac sodium respectively. The method involved Q-absorption ratio analysis using two wavelengths, with one being the of Nimesulide (288 nm) and the other being the isoabsorptive point of both drugs (298 nm). For spectrophotometric method, Methanol was used as a solvent. Both the drug and their mix obeyed Beer- Lamberts law at selected wavelength was observed in concentration range of 4-20 µg/ml of Nimesulide and 2-10 µg/ml of Diclofenac sodium. The results of analysis have been validated statistically and by recovery studies as per ICH guidelines. This method was found to be simple, precise, accurate, selective and rapid and can be successfully applied for the determination of pure laboratory mixtures and tablet formulations.

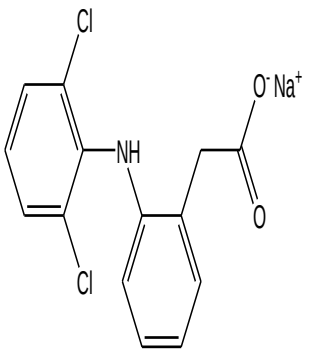
Keywords: *Q-Absorption Ratio Method, Nimesulide, Diclofenac sodium, ICH guideline, Validation*

1. Introduction

Nimesulide N-(4-nitro-2-phenoxyphenyl) methane sulfonamide, is a derivative of p-nitrophenylmethanesulfonamide. It belongs to selective COX-2 inhibitors, with a potent anti-inflammatory and analgesic activity, when administered orally, rectally, or topically. Due to its analgesic and antipyretic properties, it is widely used for the treatment of various inflammatory processes. It is approved for use in treatment of musculoskeletal disorder, thrombophlebitis, dental pain, and inflammation. Diclofenac Sodium is chemically Sodium salt of 2-[{2,6-dichlorophenyl}amino] benzene acetic acid. It is having anti-inflammatory and analgesic properties.



Structure of Nimesulide



Structure of Diclofenac sodium

Literature review revealed some HPLC and spectrophotometric method have been reported in literature for its estimation. Many methods have been reported in literature for determination of Nimesulide and Diclofenac sodium with other drugs. The present work describes a validated Simple, Precise, Accurate, Rapid & Economical UV- Spectrophotometric method for simultaneous determination of these drugs in combined tablet dosage form. To validate the developed analytical method as per ICH guideline (Q_2R1) for various parameters like accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), linearity, range, etc.

2. Materials and methods

Instrumentation

UV 1800 double beam UV Visible Spectrophotometer (Shimadzu) with a pair of 10mm path length matched quartz cells were used for the study. The UV solutions 2.42 software was used. An electronic balance used for weighing purpose was Shimadzu. Volumetric flasks and pipettes used in the study were of borosilicate glass. All the statistical calculations were carried out by using Microsoft Excel 2007.

Chemicals and Reagents

The analytical samples of Nimesulide and Diclofenac sodium were received from Camper Healthcare, Mehsana, India as gift samples. All the chemicals used were of analytical grade. The tablet formulations were procured from a local pharmacy.

Preparation of Solutions

Standard stock solution (100 µg/ml)

Accurately weighed 10 mg of Nimesulide and 10 mg of Diclofenac sodium were transferred to two separate 100 ml volumetric flask. Make up the final volume with water up to the mark to prepare a 100 µg/ml stock solution of both drugs.

Preparation of working standard solution

Standard stock solution (100µg/ml) of Nimesulide and Diclofenac sodium were used as working standard solutions. Accurately measured standard stock solution of Nimesulide (0.4, 0.8, 1.2, 1.6, & 2.0 ml) and standard stock solution of Diclofenac sodium (0.2, 0.4, 0.6, 0.8, & 1.0 ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with methanol.

Preparation of Sample solution

Twenty Tablets were weighed accurately. Quantity of the powder equivalent to about 10 mg of Nimesulide and 5 mg of Diclofenac sodium into 100 ml measuring flask and sonicate for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with methanol. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol to get a concentration of 100 µg/ml Nimesulide and 50 µg/ml of Diclofenac sodium. Take 1.2 ml from the flask and transfer into 10ml of volumetric flask and diluted up to the mark with methanol to get final conc. of 12 µg/ml Nimesulide 6 µg/ml of Diclofenac sodium.

3.Result and discussion

Spectral characteristic of Nimesulide and Diclofenac sodium

Nimesulide and Diclofenac sodium working solutions (2.0 ml Nimesulide and 1.0 ml Nimesulide) were separately transferred into a 10 ml volumetric flask and dilute to volume with methanol. Both solutions were initially scanned to determine the maximum absorption bands and Iso-absorptive point. The values were measured at 288 nm (Iso-absorptive point) and 298nm (λ max of Nimesulide). The overlapping absorption spectra of Nimesulide and Diclofenac sodium are shown in the Figure 1 which shows Iso-absorptive point and Figure 2 shows λ max of Nimesulide. The calibration curves of Nimesulide and Diclofenac sodium 298 nm and 282 nm are shown in Figure 3, 4,5,6,7 and 8.

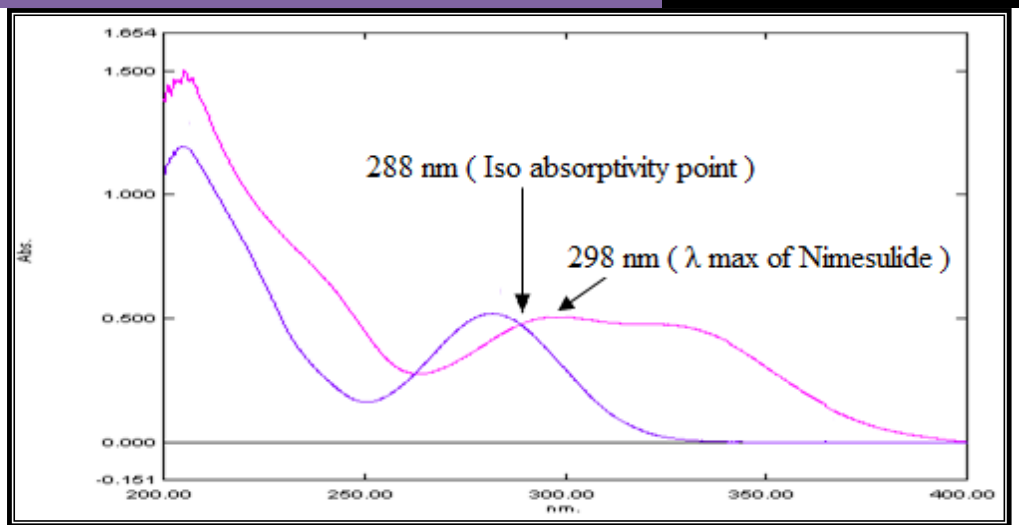


Figure 1 Overlain spectra of Nimesulide and Diclofenac sodium

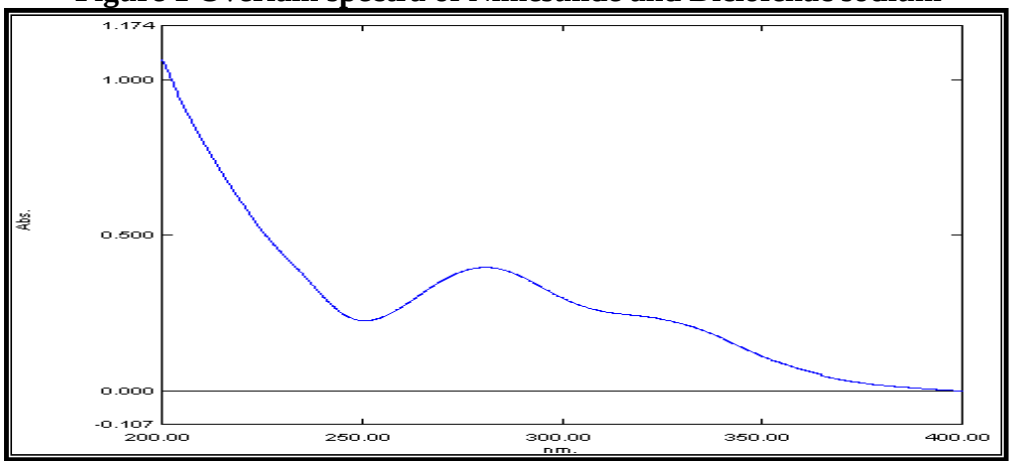


Figure 2 Absorption spectra of Sample

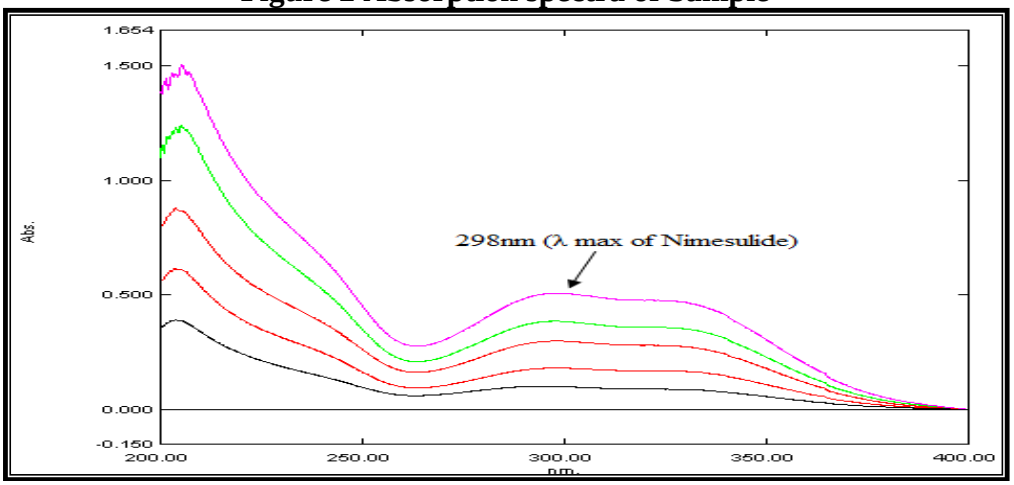


Figure 3 Absorption spectra of Nimesulide

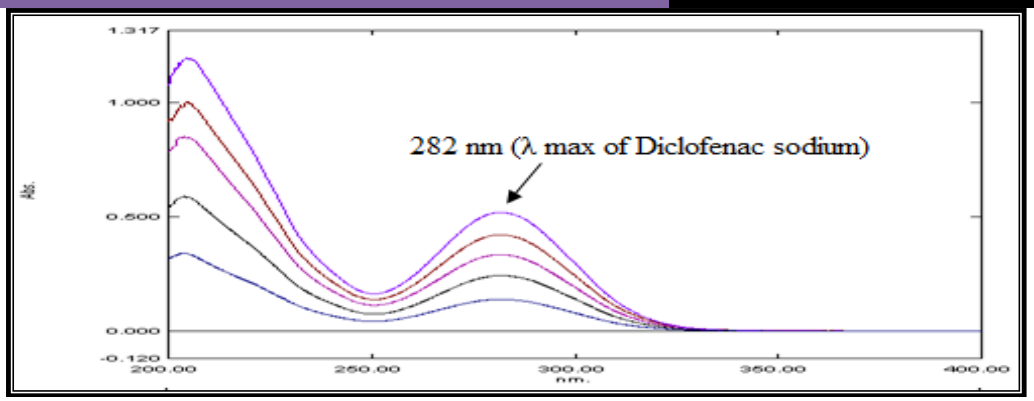


Figure 4 Absorption spectra of Diclofenac sodium

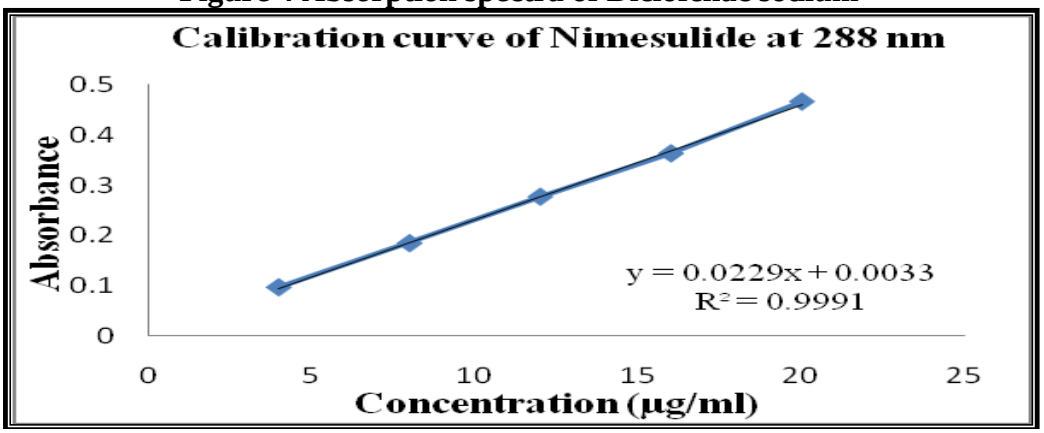


Figure 5 Calibration Curve of Nimesulide at 288 nm

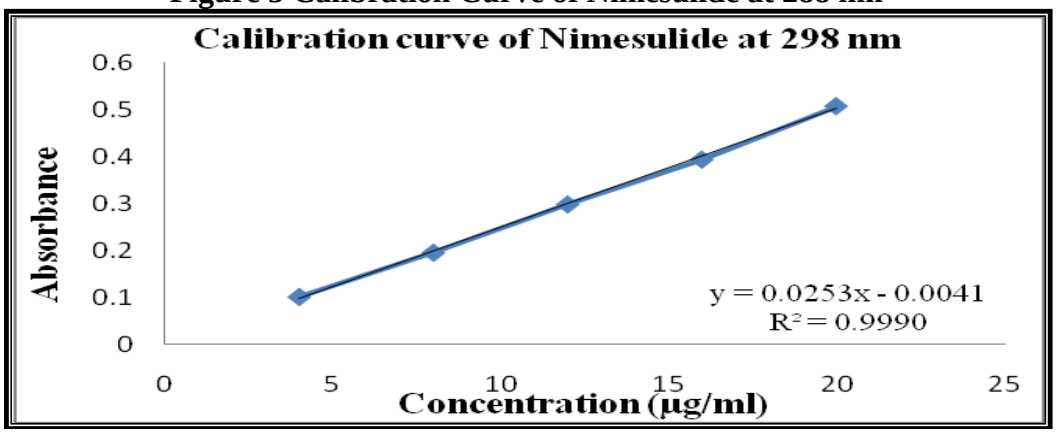


Figure 6 Calibration Curve of Nimesulide at 298 nm

A linear correlation was obtained between peak amplitude and the corresponding concentration in the range of 4 - 20 µg/ml for Nimesulide, from which the linear regression equation was computed and found to be:

Y = 0.0229x – 0.0033, r2 = 0.9991 at 288 nm

Y = 0.0253x - 0.00341, r2 = 0.9990 at 298 nm

Where Y is peak amplitude at 288/298 nm, X is the concentration in µg/ml and r2 is the correlation coefficient.

A linear correlation was obtained between peak amplitude and the corresponding concentration in the range of 2-10 µg/ml for Diclofenac sodium, from which the linear regression equation was computed and found to be:

Y = 0.0439x + 0.0468, r2 = 0.9991 at 288 nm

Y = 0.0301x + 0.0307, r2 = 0.9993 at 298 nm

Where Y is peak amplitude at 288/298 nm, X is the concentration µg/ml and r2 is the correlation

coefficient.

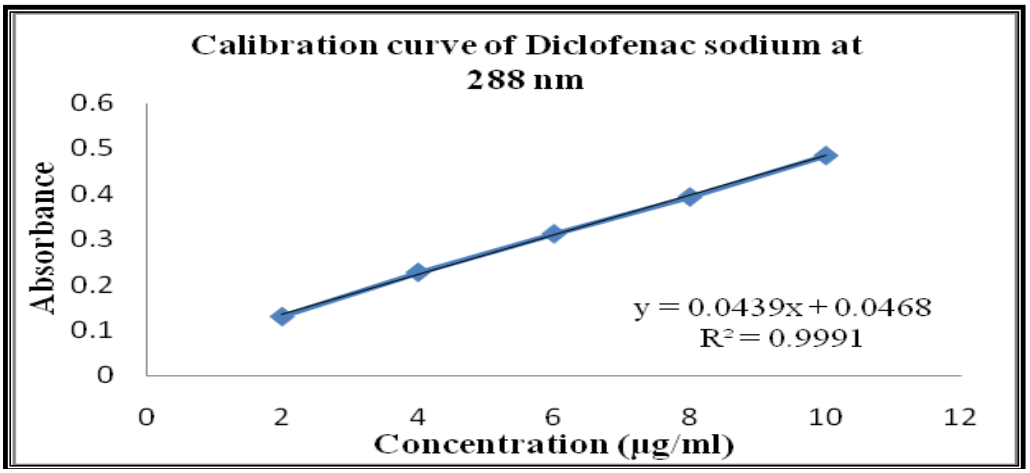


Figure 7 Calibration Curve of Diclofenac sodium at 288 nm

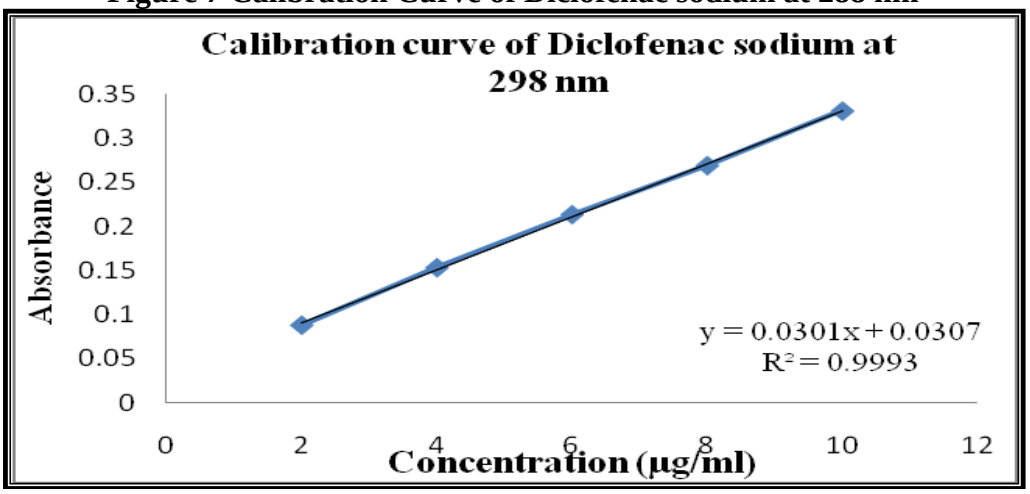


Figure 8 Calibration Curve of Diclofenac sodium at 298 nm

Accuracy (% Recovery)

Accuracy of the method was assured by use of the standard addition technique, involving analysis of formulation of the samples containing 12 µg/ml of Nimesulide and 6 µg/ml of Diclofenac sodium to which certain amounts of authentic drugs were added. The resulting mixtures were assayed and the results obtained for both drugs were compared to those expected. Good recoveries with the standard addition method (Table 3) prove the good accuracy of the proposed method.

Precision.

Method precision (Repeatability)

The precision of the instrument was checked by repeatedly (n=6) measuring the absorbance of Nimesulide (12 µg/ml) and Diclofenac sodium (6 µg/ml). The results of repeatability experiment are shown in Table 4 and 5. the developed method was found to be precise as the %RSD values for repeatability study were found to be less than 1.0%

Intermediate precision (Reproducibility)

Results of intermediate precision for both intraday and interday are shown in Table 6,7,8,9. Replicate analyses of entire concentrations range of both Nimesulide (4-20 µg/ml) and Diclofenac sodium (2-10 µg/ml) were evaluated by three replicate determinations to estimate intraday variation and inter day variation. The developed method was found to be precise as the %RSD values for reproducibility study were found to be less than 2.0%. Low %RSD values of intraday and interday precision reveal that the proposed method is precise.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ for both drugs were calculated theoretically. These data show that the method is sensitive for the simultaneous determination of Nimesulide and Diclofenac sodium.

Table 1 Linearity Data for Nimesulide

Concentration (µg/ml)	At 288 nm		At 298 nm	
	Absorbance Mean ± SD (n=3)	%RSD	Absorbance Mean ± SD (n=3)	%RSD
4	0.097 ± 0.001	0.591	0.101 ± 0.001	0.569
8	0.185 ± 0.001	0.311	0.195 ± 0.001	0.295
12	0.277 ± 0.002	0.207	0.298 ± 0.002	0.193
16	0.363 ± 0.002	0.158	0.394 ± 0.001	0.146
20	0.466 ± 0.001	0.123	0.508 ± 0.002	0.113

Table 2 Linearity Data for Diclofenac sodium

Concentration (µg/ml)	At 288 nm		At 298 nm	
	Absorbance Mean ± SD (n=3)	%RSD	Absorbance Mean ± SD (n=3)	%RSD
2	0.130 ± 0.001	0.769	0.088 ± 0.002	0.653
4	0.227 ± 0.001	0.253	0.153 ± 0.001	0.375
6	0.312 ± 0.002	0.184	0.214 ± 0.001	0.467
8	0.394 ± 0.001	0.253	0.269 ± 0.002	0.566
10	0.485 ± 0.002	0.237	0.331 ± 0.002	0.461

Table 3 Accuracy (% Recovery) of Nimesulide and Diclofenac sodium

Drug	Amount taken (µg/ml)	Amount Added (µg/ml)	Amount found (µg/ml) ± S.D (n=3)	% Recovery ± S.D (n=3)
Nimesulide	12	9.6	9.597 ± 0.037	99.978 ± 0.392
	12	12	11.983 ± 0.076	99.859 ± 0.634
	12	14.4	14.381 ± 0.128	99.917 ± 0.893
Diclofenac sodium	6	4.8	4.781 ± 0.024	99.601 ± 0.514
	6	6	5.990 ± 0.055	99.835 ± 0.931
	6	7.2	7.184 ± 0.049	99.782 ± 0.682

Table 4 Repeatability Data for Nimesulide

Concentration (µg/ml)	Absorbance		Amount found	% Amount found
	At 288 nm	At 298 nm		
12 µg /ml	0.629	0.537	12.012	100.104
	0.628	0.536	11.977	99.810
	0.627	0.536	12.052	100.438
	0.625	0.535	12.092	100.772
	0.628	0.536	11.977	99.810
	0.63	0.537	11.937	99.476
Mean	0.627	0.536	12.008	100.068
SD	0.0017	0.0007	0.0567	0.4725
%RSD	0.2743	0.1404	0.4722	0.4722

Table 5 Repeatability Data for Diclofenac sodium

Concentration (µg/ml)	Absorbance		Amount found	% Amount found
	At 288 nm	At 298 nm		
6 µg/ml	0.629	0.537	6.016	100.268
	0.628	0.536	5.996	99.940
	0.627	0.536	6.001	100.032
	0.625	0.535	6.001	100.025
	0.628	0.536	6.001	100.025
	0.630	0.537	5.997	99.957
Mean	0.627	0.536	6.002	100.041
SD	0.0017	0.0007	0.0071	0.1177
%RSD	0.2743	0.1404	0.1176	0.1176

Table 6 Intraday & Interday Precision data for analysis of Nimesulide at 288nm

Concentration (µg/ml)	Intra day Precision Mean ± S.D (n=3), %RSD	Inter day Precision Mean ± S.D (n=3), %RSD
4	0.097 ± 0.001, 0.593	0.097 ± 0.001, 0.970
8	0.184 ± 0.001, 0.543	0.185 ± 0.001, 0.621
12	0.277 ± 0.002, 0.361	0.278 ± 0.002, 0.552
16	0.364 ± 0.001, 0.274	0.363 ± 0.002, 0.420
20	0.466 ± 0.001, 0.214	0.466 ± 0.001, 0.327

Table 7 Intraday & Interday Precision data for analysis of Nimesulide at 298nm

Concentration (µg/ml)	Intra day Precision Mean ± S.D (n=3), %RSD	Inter day Precision Mean ± S.D (n=3), %RSD
4	0.101 ±0.001, 0.567	0.102 ± 0.001, 0.980
8	0.195 ±0.001, 0.512	0.194 ± 0.001, 0.784
12	0.297 ±0.002, 0.388	0.298 ± 0.002, 0.579
16	0.395 ±0.001, 0.253	0.394 ± 0.002, 0.387
20	0.508 ±0.001, 0.196	0.508 ± 0.001, 0.227

Table 8 Intraday & Interday Precision data for analysis of Diclofenac sodium at 288nm

Concentration (µg/ml)	Intra day Precision Mean ± S.D (n=3), %RSD	Inter day Precision Mean ± S.D (n=3), %RSD
2	0.131 ± 0.001, 0.763	0.131 ± 0.001, 0.883
4	0.226 ± 0.002, 0.673	0.227 ± 0.001, 0.763
6	0.313 ± 0.002, 0.486	0.313 ± 0.002, 0.665
8	0.395 ± 0.001, 0.386	0.394 ± 0.002, 0.527
10	0.485 ± 0.001, 0.314	0.485 ± 0.001, 0.357

Table 9 Intraday & Interday Precision data for analysis of Diclofenac sodium at 298nm

Concentration (µg/ml)	Intra day Precision Mean ± S.D (n=3), %RSD	Inter day Precision Mean ± S.D (n=3), %RSD
2	0.088 ± 0.002, 0.688	0.087 ± 0.001, 0.780
4	0.153 ± 0.001, 0.653	0.152 ± 0.001, 0.734
6	0.214 ± 0.001, 0.467	0.213 ± 0.002, 0.540
8	0.270 ± 0.001, 0.370	0.269 ± 0.002, 0.428
10	0.332 ± 0.001, 0.301	0.331 ± 0.001, 0.340

Estimation of Nimesulide and Diclofenac sodium in formulation

Test solution from Tablets which contain Nimesulide (4µg/ml) and Diclofenac sodium (30µg/ml) were prepared and solutions were analyzed at the λ_{max} of Nimesulide and Diclofenac sodium. The Absorptivity coefficients of these two drugs were determined using calibration curve equation. The concentration of Nimesulide and Diclofenac sodium was determined using simultaneous equations.

C_x = (Qm-Qy)*A1 / (Qx-Qy)*ax1 C_y = (Qm-Qx)*A1 / (Qy-Qx)*ay1

Where C_X and C_Y are concentrations of Nimesulide and Diclofenac sodium respectively in gm/100 ml sample solution. A1 is the absorbance of the mixture at 235 nm (Iso absorptive Point).Qx and Qy are Absorbance of Nimesulide and, Diclofenac sodium respectively in gm/100 ml sample solution.

Table 10 Assay results of Nimesulide and Diclofenac sodium

Formulation	Label Claim (mg/tablet)		Assay (Content in mg)		% Assay (Mean* ± S.D, n=6)	
	NIME	DICLO	NIME	DICLO	NIME	DICLO
DICLOPA NM	100	50	100.22	50.01	100.22 ± 0.38	100.02 ± 0.12

Table 11 Summary of Validation parameters

Parameters		Nimesulide		Diclofenac sodium	
		288nm	298 nm	288 nm	298nm
Calibration Range (µg/ml)		4 - 20	4 - 20	2 - 10	2 – 10
Molar Absorptivity ($1mole^{-1}cm^{-1}$)		233.56	249.48	543.62	370.25
Slope (m)		0.0229	0.0253	0.0439	0.0301
Intercept (c)		0.0033	0.0041	0.0468	0.0307
Correlation Coefficient(r^2)		0.9991	0.9990	0.9991	0.9993
Precision (%RSD)	Repeatability	0.2743	0.1404	0.1743	0.1404
	Intra day	0.21-0.59	0.19-0.56	0.31-0.76	0.30-0.68
	Inter-day	0.32-0.97	0.22-0.98	0.35-0.88	0.34-0.78
LOD (µg/ml)		0.062	0.094	0.034	0.062
LOQ (µg/ml)		0.190	0.285	0.104	0.188

4. Conclusion

Q-Absorbance Ratio method was developed for the determination of Nimesulide and Diclofenac sodium in combined pharmaceutical dosage form. The proposed method is Simple, Accurate, Precise and this method is suitable for routine analysis of Nimesulide and Diclofenac sodium in combined pharmaceutical dosage form. Detection and Quantification limits achieved, describe that the method is sensitive. High recoveries and acceptable %RSD values confirms accuracy and precision of developed method. Assay results show that the method can be successfully applied for routine analysis of Nimesulide and Diclofenac sodium in combined pharmaceutical dosage form.

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