Drug Development and Industrial Pharmacy, 26(2), 229–234 (2000)

COMMUNICATION

New Ultraviolet Spectrophotometric Method for the Estimation of Nimesulide

Sajeev Chandran, Sachin Saggar, K. Padma Priya, and Ranendra N. Saha*

Department of Pharmacy, Birla Institute of Technology and Science, Pilani 333 031 (Rajasthan), India

ABSTRACT

Two simple and accurate ultraviolet (UV) spectrophotometric methods with better detection range for estimation of nimesulide in pure form and in solid dosage form were developed in the present studies using 50% v/v and 100% v/v acetonitrile as the solvent system. The linearity range of nimesulide in both the methods was found to be 10–50 μg/ml at a λ_{max} of 300 nm. The linear regression equations obtained by the least-square regression method are Abs = 1.33 × 10^{-1} · Conc + 1.89 × 10^{-1} in 50% v/v acetonitrile and Abs = 1.05 × 10^{-1} · Conc + 1.14 × 10^{-1} in 100% v/v acetonitrile. The detection limit as per the error propagation theory was found to be 0.46 μg/ml and 1.04 μg/ml, respectively, in 50% v/v and 100% v/v acetonitrile. The developed methods were employed with high degree of precision and accuracy for the estimation of total drug content in three commercial tablet formulations of nimesulide. The results of the analysis were validated statistically and by recovery studies.

Key Words: *Acetonitrile; Nimesulide; Ultraviolet spectrophotometry.*

INTRODUCTION

Nimesulide, a methyl sulfonamide derivative, is a relatively new nonsteroidal anti-inflammatory and analgesic drug. It is a potent selective cyclo-oxygenase A_2 inhibitor and is highly effective, with minimum drug-related side effects, in the treatment of various forms of pain and inflammatory conditions. The drug is beset with the disadvantage of poor water solubility. It is not official in USP 23 (1995), BP (1998), and IP (1996). A survey of the literature revealed that only a few ultraviolet (UV) (1,2) and visible (3,4) spectrophotometric and high-performance liquid chromatography (HPLC) (5–7) methods have been reported for the estimation of nimesulide.

* To whom correspondence and requests for reprints should be addressed.

Table 1

Selectivity of the Proposed Methods in Estimation of Standard Solution of Nimesulide by Ultraviolet Spectrophotometry at 300 nm

	Method A (50% v/v Acetonitrile)			Method B (100% v/v Acetonitrile)		
Concentration (μg/ml)	Mean Absorbance[a]	Coefficient of Variation (%)	Standard Error	Mean Absorbance[a]	Coefficient of Variation (%)	Standard Error
10	0.211 ± 0.003	1.464	0.0009	0.231 ± 0.004	1.609	0.0011
20	0.434 ± 0.004	0.898	0.0011	0.487 ± 0.003	0.616	0.0008
30	0.653 ± 0.006	0.847	0.0016	0.716 ± 0.005	0.694	0.0014
40	0.872 ± 0.007	0.781	0.0019	0.957 ± 0.013	1.179	0.0033
50	1.076 ± 0.005	0.474	0.0015	1.195 ± 0.011	0.895	0.0031

[a] Average of 10 determinations with standard deviation.

Table 2

Results of Least-Square Regression Analysis of Data for the Estimation of Nimesulide from Standard Solution by the Proposed Methods

Statistical Parameters	Method A	Method B
Regression equation[a]	$Y = 1.33 \times 10^{-1} \cdot X + 1.89 \times 10^{-1}$	$Y = 1.05 \times 10^{-1} \cdot X + 1.14 \times 10^{-1}$
Correlation coefficient *r*	0.9999	0.9998
Standard error of slope	2.12×10^{-2}	1.15×10^{-3}
Standard error of intercept on ordinate	6.39×10^{-4}	3.83×10^{-2}
Standard error of the estimate	2.02×10^{-2}	3.65×10^{-2}
95% confidence interval of slope	1.35×10^{-1}, 1.31×10^{-1}	1.09×10^{-1}, 1.02×10^{-1}
95% confidence interval of intercept	2.57×10^{-1}, 1.22×10^{-1}	2.35×10^{-1}, -0.820×10^{-2}
Slope without intercept	1.57×10^{-1}	9.34×10^{-2}

[a] Based on five calibration values; $Y =$ absorbance; $X =$ concentration of the drug in μg/ml.

Table 3

One-Way ANOVA Test for Linearity of Pure Nimesulide Solution in 50% v/v and 100% v/v Acetonitrile

Method A (50% v/v acetonitrile)					
				F	
Source of Variation	Degree of Freedom (DF)	Sum of Squares (SS)	Mean Sum of Squares (MS)	F_{Calc}	F_{Crit}[a]
Regression	1	53.24	53.24	0.0003	3.71
Lack of fit	3	0.0037	0.0012		
Within line	10	50.0370	5.0037		
Total	14	103.28			
Method B (100% v/v acetonitrile)					
				F	
Source of Variation	Degree of Freedom (DF)	Sum of Squares (SS)	Mean Sum of Squares (MS)	F_{Calc}	F_{Crit}[a]
Regression	1	33.28	33.28	0.0015	3.71
Lack of fit	3	0.1199	0.0030		
Within line	10	26.1451	2.6145		
Total	14	59.5398			

[a] Theoretical value of $F(3, 10)$ based on one-way ANOVA test at $p = .05$ level of significance.

These methods have their disadvantages, such as a narrow detection range, a complicated procedure, expense, and time consuming.

In the present work, we attempted to develop an easier, accurate, and reproducible analytical method with better detection range for estimation of nimesulide in bulk drug and in its solid dosage forms. This paper describes a UV spectrophotometric method of estimation of nimesulide in 50% v/v acetonitrile in triple-distilled water and 100% v/v acetonitrile. The developed methods were used to estimate the total drug content in three commercially available tablet formulations of nimesulide. The results of the analysis were validated by statistical methods and recovery studies.

EXPERIMENTAL

Instrument

A UV/Vis spectrophotometer (Jasco model 7800, Tokyo, Japan) with automatic wavelength correction, a wavelength accuracy of 0.5 nm, and 10-mm matched quartz cells was used for all absorbance measurements.

Materials

The acetonitrile (Merck, Mumbai, India) and methanol (Merck) used were HPLC grade. Nimesulide was obtained as a gift sample from Dr. Reddy's laboratory, Hyderabad, India. Three commercially available tablet formulation of nimesulide were selected on a random basis from the market.

Preparation of a Standard Curve

A stock solution of nimesulide was prepared by dissolving 10 mg of drug in 100 ml of 50% v/v acetonitrile (method A) and 100% v/v acetonitrile (method B) separately to get a final concentration of 100 μg/ml. The λ_{max} of nimesulide in the above two acetonitrile media was determined by scanning a suitable dilution of the stock using the UV/Vis spectrophotometer. From the stock solution, various dilutions were made to obtain solutions of 10, 20, 30, 40, and 50 μg/ml, and the absorbance was measured. The results are listed in Table 1, and the optical characteristics, accuracy, and precision parameters of the proposed methods are given in Table 2. The precision and accuracy were found by analyzing five triplicate samples containing known amounts of drug.

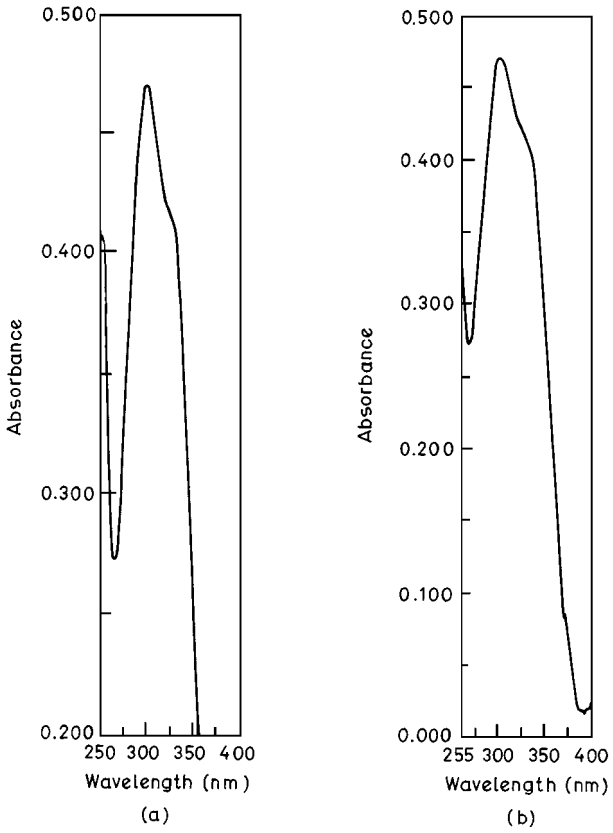

Figure 1. Ultraviolet spectrum of nimesulide in (a) 50% v/v acetonitrile and (b) 100% v/v acetonitrile.

Estimation of Drug Content in Commercial Tablets

Three commercially available tablet brands of nimesulide were taken randomly for estimation of total drug content per tablet. For each brand, 20 tablets were weighed and finely powdered. An accurately weighed aliquot amount (equivalent to 10 mg of nimesulide) was transferred to a series of 25-ml volumetric flasks (5 in each case) and dissolved in 50% v/v (method A) and 100% v/v (Method-B) acetonitrile separately. The solution was filtered through Whatman filter paper no. 1 and was diluted with 50% v/v and 100% v/v acetonitrile separately to produce a final concentration in the limits of linearity of the proposed methods (as given in Table 2). From the absorbance value, the drug content per tablet (on an aver-

Table 4

Validation Report for the Determination of Nimesulide in Standard Solutions by Ultraviolet Spectrophotometric Methods in 50% v/v and 100% v/v Acetonitrile

	Results	
Analytical Parameter	Method A (50% v/v Acetonitrile)	Method B (100% v/v Acetonitrile)
Accuracy (%)	99.87 ± 0.80	99.83 ± 0.15
Precision (%)	99.73	99.76
	99.92	100.00
	99.98	99.89
	99.87	99.88
	100.01	99.92
	RSD = 0.11	RSD = 0.09
Linearity (μg/ml)	10–50	10–50
Specificity	A 25 μg/ml solution of nimesulide will show an absorbance of 0.541 ± 0.0005	A 25 μg/ml solution of nimesulide will show an absorbance of 0.597 ± 0.0004
Limit of detection (μg/ml)	0.46	1.04
Limit of quantitation (μg/ml)	1.52	3.48
Ruggedness (%)	99.87 ± 0.80	99.83 ± 0.15

RSD = relative standard deviation.

age weight basis) was calculated. The results are tabulated in Table 3.

Recovery Studies

As an additional check on the accuracy of the developed assay methods, recovery experiments were performed by adding known amounts of pure drug to preanalyzed samples of commercial dosage forms. The percentage recovery values are also listed in Table 3.

RESULTS AND DISCUSSION

The spectra of nimesulide in 50% v/v (method A) and 100% v/v (method B) acetonitrile are shown in Figs. 1a

Table 5

Results of the Assay of Pure Nimesulide and Commercial Formulations by the Proposed Methods

		Method A (50% v/v Acetonitrile)		Method B (100% v/v Acetonitrile)	
Sample	Label Claim (mg/tablet)	Amount Found[a] (mg)	Recovery[a] (%)	Amount Found[a] (mg)	Recovery[a] (%)
Pure drug solution[b]	—	99.71 ± 0.20	99.83 ± 0.17	99.83 ± 0.16	100.01 ± 0.11
Brand A	100	99.67 ± 0.18	98.89 ± 0.30	100.01 ± 0.53	100.90 ± 0.31
Brand B	100	99.55 ± 0.13	99.84 ± 0.12	99.41 ± 0.93	100.78 ± 0.25
Brand C	100	99.66 ± 0.19	99.28 ± 0.22	99.77 ± 1.57	100.77 ± 0.18

[a] Mean and standard deviation for five triplicate determinations.
[b] 100 mg in 1000 ml.

Table 6

Two-way ANOVA Test (Without Replication) for Linearity in Estimation of Nimesulide in Various Commercial Formulations by the Proposed Methods

	Method A (50% v/v Acetonitrile)					Method B (100% v/v Acetonitrile)				
Source of Variation	SS	DF	MS	F_{Calc}	F_{Crit}	SS	DF	MS	F_{Calc}	F_{Crit}
Between the brands	0.301	3	0.060	2.733	2.901[a]	4.9514	3	0.9902	1.12	2.90[a]
Within the brand	0.088	5	0.029	1.330	3.287[b]	1.1645	5	0.3881	0.44	3.29[b]
Error	0.330	15	0.022			13.216	15	0.8811		
Total	0.719	23				19.332	23			

SS = sum of squares; DF = degree of freedom; MS = mean sum of squares.
[a] Theoretical value of $F(3,15)$ based on two-way ANOVA test at $p = .05$ level of significance.
[b] Theoretical value of $F(5,15)$ based on two-way ANOVA test at $p = .05$ level of significance.

and 1b. The λ_{max} was found to be 300 nm in both media. The statistical analysis of data obtained for the estimation of nimesulide in pure solution indicates the high level of precision of both the proposed methods, as evidenced by the low standard deviation values (Table 1). The low values of standard error and coefficient of variation (Table 1) further establish the precision of the proposed methods.

The linear regression equations obtained were $Y = 1.33 \times 10^{-1} \cdot X + 1.89 \times 10^{-1}$ and $Y = 1.05 \times 10^{-1} \cdot X + 1.14 \times 10^{-1}$ for 50% v/v and 100% v/v acetonitrile methods, respectively, where Y is the absorbance, and X is the concentration of pure nimesulide solution. The correlation coefficient values obtained are highly significant for both methods (Table 2). The reported slope values without intercept on the ordinate at 95% confidence limits suggested that the calibration lines of nimesulide solutions in 50% v/v and 100% v/v acetonitrile did not deviate from the origin as the above values lie within the confidence limits (Table 2). The precision of the fit was further confirmed from the standard error values of the intercept and slope and the estimate.

A one-way analysis of variance (ANOVA) test (8) was performed based on the separate linear calibration graph constructed with three replicates per point. The values considered were the lowest and highest variations observed from the mean absorbance value of each pure drug concentration during the replicate measurement of the standard solutions. The calculated F value F_{Calc} was found to be less than the critical F value F_{Crit} at 5% significance levels in both methods (Table 3).

The developed methods were validated according to the procedure given in the USP (9), and the results obtained are tabulated in Table 4. The reported limit of detection (LOD) and limit of quantitation (LOQ) (9,10) were calculated based on the slope of the regression equation obtained in both methods of Table 2. The Beer's law range of nimesulide in both methods was found to be 10–50 μg/ml at a λ_{max} of 300 nm. Since the reported slope values without intercept fall within the 95% confidence limits, the linearity characteristics of the proposed methods can be practically considered as 0–50 μg/ml.

The results of the estimation of nimesulide in pharmaceutical formulations by the proposed methods and analysis of reference pure drug solution are presented in Table 5. The estimated drug content with low values of standard deviation further establishes the precision of the proposed methods. The accuracy of the results of estimation was tested further by recovery experiments. Recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical excipients used in the selected formulations in the developed assay procedures. The reported F value, as given in Table 6, of a two-way ANOVA test (8) for samples suggests that there is no significant difference in the mean recoveries of the samples.

Thus, the proposed methods of estimation of nimesulide were found to be accurate, precise, and easier compared to other reported methods (1–4). Therefore, the rapidity of the proposed methods makes them useful in the routine analysis of nimesulide in bulk samples and pharmaceutical formulations. Method A can also be useful, by directly diluting the samples with acetonitrile to a 50% v/v solution, for the estimation of nimesulide in dissolution and other studies for which the studies have to be carried out in aqueous media.

ACKNOWLEDGMENTS

This work was part of a project financially supported by All India Council of Technical Education, New Delhi, India. Authors are also thankful to Dr. Reddy's laboratory, Hyderabad, India, for the generous gift samples of nimesulide.

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