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Determination of nimesulide in pharmaceutical dosage forms by second order derivative UV spectrophotometry☆

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Abstract

In this study, nimesulide which has been used as an analgesic, antipyretic and anti-inflammatory agent, was analyzed by using second order derivative UV spectrophotometry. The solvent, the degree of derivation, ranges of wavelength and *n*-value were chosen in order to optimize the conditions. The concentration of nimesulide in its solutions in ethanol and chloroform were determined between the wavelength ranges of 200 and 500 nm ($n = 6$, $\Delta\lambda = 21$) and in the linearity ranges of 2.0–90.0 μg ml^{-1} in ethanol and 2.0–50.0 μg ml^{-1} in chloroform by using the values obtained from the second derivative UV spectrum of the substance. The developed second derivative UV spectrophotometric method was applied to the pharmaceutical preparations such as tablet, sachet (granule) and suspension. Tablet and sachet were analysed in ethanol while the suspension was analysed in chloroform. The results obtained from derivative UV spectrophotometry were compared with those obtained by using HPLC. It was found that the difference was not statistically important between these methods. It was concluded that developed derivative UV spectrophotometric method was accurate, sensitive, precise, reproducible and could be applied directly and easily to the pharmaceutical preparations.

Keywords: Nimesulide; Derivative UV spectrophotometry; Pharmaceutical dosage forms (tablet, sachet (granule) and suspension)

1. Introduction

The chemical formula of nimesulide is 4-nitro-2-phenoxymethane sulfonanilide ($C_{13}H_{12}N_2O_5S$) (Fig. 1). Nimesulide is a new non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties [1,2] that does not induce gastrointestinal ulceration [3]. It is an inhibitor of prostaglandin synthesis from arachidonic acid and of platelet aggregation [4,5]. The pharmacokinetic profile of nimesulide has been assessed in healthy volunteers after oral and rectal administration of the sample, in tablet, sachet (granule) or suspension forms [6].

The pK_a of nimesulide in methanol-water mixtures was determined by potentiometric titration [7]. Nimesulide can be determined by spectropho-

☆ This work was taken partly from the M.Sc. thesis of Ö.Ö. Dursun.

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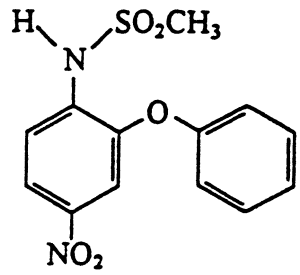

Fig. 1. Chemical structure of nimesulide.

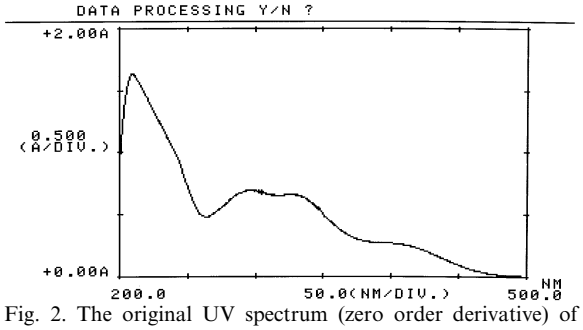

Fig. 2. The original UV spectrum (zero order derivative) of nimesulide (30 μg ml^{-1} nimesulide in ethanol).

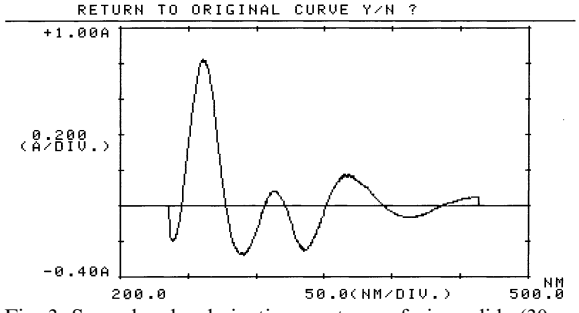

Fig. 3. Second order derivative spectrum of nimesulide (30 μg ml^{-1} nimesulide in ethanol).

tometry [8–11], chromatography [12–19], polarography [20] and fluorimetry [21].

No derivative spectrophotometric studies on nimesulide have been found in the literature.

In this study, derivative UV spectrophotometric method is developed for the determination of nimesulide. The developed method was applied to three different commercial pharmaceutical preparations of tablet, sachet (granule) and suspension. The results obtained from derivative UV spectrophotometry were compared with those obtained by using HPLC.

Derivative spectrophotometry is an analytical technique for the enhancement of sensitivity and specificity in qualitative and quantitative analysis of various compounds including pharmaceuticals. In addition, this method appears an applicable and suitable one for ultraviolet-visible region spectrophotometry, infrared, atomic-absorption and flame emission spectrophotometry and fluorimetry. The fine structural features of this derivative method are sharpened and emphasised to give an improved resolution of overlapping and are potentiated to give greater sensitivity. Besides derivative spectrophotometry presents an advantage over spectrophotometry and chromatography. In determination of substances in pharmaceutical preparations, since those formulations usually give turbid solutions, there was no need for extraction processes to eliminate the excipients which are time consuming and tedious. In addition, chromatographic technique is very expensive both in instrumentation and maintenence. The use of derivative spectrophotometry is not restricted to special cases, but may be of advantage whenever quantitative study of normal spectra is difficult. Its disadvantage is that the differentitation degrades to signal-to-noise ratio so that some

Table 1
The results of calibration curves with three methods measured in ethanol ($n = 7$)[a]

Method	Regression equations	r	Standard errors of slope	Standard errors of intercept
Peak to peak	$y = 4.03 \times 10^{-2}X - 3.1 \times 10^{-3}$	0.9999	5.14×10^{-5}	5.97×10^{-5}
Peak to zero	$y = 1.13 \times 10^{-2}X - 3.2 \times 10^{-3}$	0.9995	6.77×10^{-5}	4.71×10^{-4}
Tangent	$y = 2.62 \times 10^{-2}X - 1.9 \times 10^{-2}$	0.9998	1.98×10^{-4}	1.34×10^{-4}

[a] r, the coefficient of correlation; X, concentration of nimesulide; y, the amplitude of second order derivative spectrum.

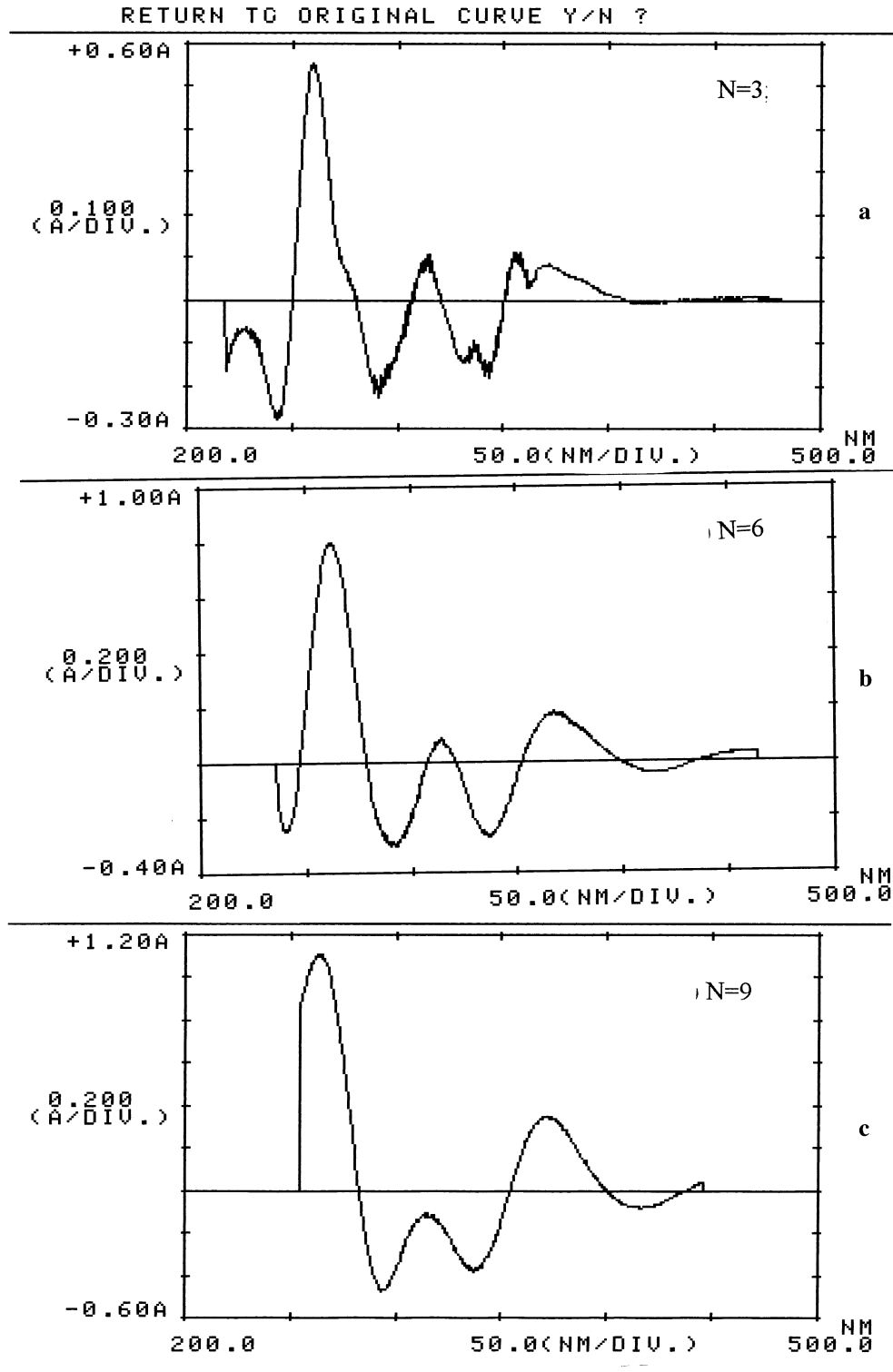

Fig. 4. Second order derivative spectrum of nimesulide (30 μg ml^{-1} nimesulide ethanol). (a) $n = 3$; $\Delta\lambda = 10.5$; (b) $n = 6$; $\Delta\lambda = 21.0$; (c) $n = 9$; $\Delta\lambda = 31.5$.

Table 2
The results of calibration curves with three methods measured in chloroform ($n = 7$)[a]

Method	Regression equations	r	Standard errors of slope	Standard errors of intercept
Peak to peak	$y = 7.57 \times 10^{-2}X - 7.6 \times 10^{-2}$	0.9999	3.61×10^{-4}	4.36×10^{-5}
Peak to zero	$y = 3.83 \times 10^{-2}X - 6.4 \times 10^{-2}$	0.9978	1.57×10^{-4}	4.04×10^{-4}
Tangent	$y = 3.96 \times 10^{-2}X - 3.6 \times 10^{-3}$	0.9998	1.45×10^{-4}	3.38×10^{-4}

[a] r, the coefficient of correlation; X, concentration of nimesulide; y, the amplitude of second order derivative spectrum.

from of smoothing is required in conjunction with the differentiation [22,23].

2. Experimental

2.1. Instrument

A Shimadzu UV-160 recording double beam UV-visible spectrophotometer with data processing system was used. UV spectra of reference and sample solutions were recorded in 1-cm quartz cells at a scan speed of 50 nm min^{-1} and fixed slit width of 3 nm. The concentrations of nimesulide in its solutions in ethanol and chloroform were determined in wavelength ranges of 200–500 nm ($n = 6$; $\Delta\lambda = 21.0$).

Varian model HPLC system with variable wavelength UV-visible detector (Star 9050) was used for the chromatographic analysis of nimesulide.

2.2. Reagents and solutions

The nimesulide standard was supplied from the Central Institute of Hygiene of Turkey. Purity of this substance was tested by controlling its melting point, UV and IR spectra. No impurities were found. All analytical and HPLC grade chemicals were supplied from Merck. Stock solutions of nimesulide (1000 μg ml^{-1}) were prepared in methanol and chloroform. A total of 100 mg nimesulide was accurately weighed and dissolved in ethanol and/or chloroform and adjusted to 100 ml with ethanol and chloroform. The solutions were kept in the dark at +4°C. Stability of nimesulide stock solutions was tested during 4 months and results showed that nimesulide solutions in ethanol and chloroform were stable.

Working standard solutions were obtained by diluting the stock solutions with concentrations ranging from 1.0 to 120.0 μg ml^{-1} in ethanol and 2.0 to 80.0 μg ml^{-1} in chloroform. Working solutions were prepared daily.

Pharmaceutical tablets contain 100 mg nimesulide and excipients. The excipients (corn starch, magnesium stearate, lactose and talc) were added to the drug for recovery studies according to manufacturer's batch formula for 100 mg nimesulide per tablet. Sachet formulation contains 100 mg nimesulide and saccharose and aromo of orange as excipients. The 1% nimesulide, pediatric

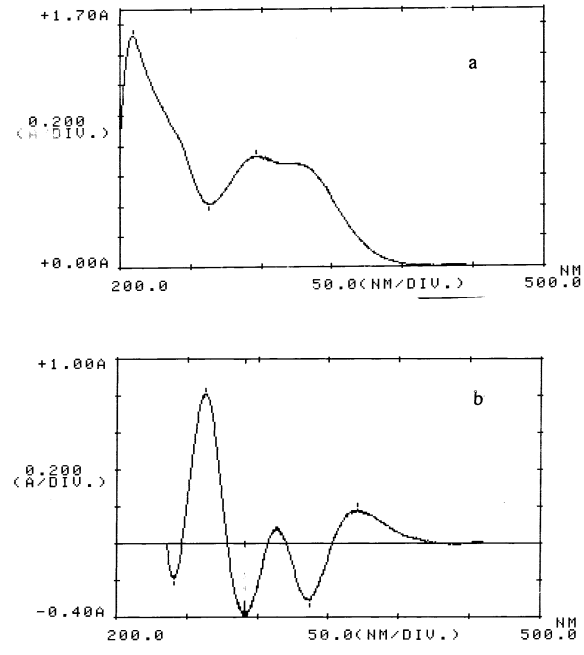

Fig. 5. The spectrum of standard and turbid pharmaceutical preparation (sachet) solution (30 μg ml^{-1} nimesulide in ethanol). (a) UV spectrum (zero order derivative). (b) Second order derivative spectrum.

Table 3
The results of analysis of pharmaceutical preparations containing nimesulide by second derivative UV spectrophotometric method[a]

Brand A® tablet (100 mg nimesulide)	Brand A® sachet (100 mg nimesulide)	Brand A® suspension (1% nimesulide)
97.44	99.51	1.043
99.51	97.44	1.043
101.58	101.00	1.006
103.65	97.44	1.023
97.44	99.51	0.968
99.51	97.44	1.006
101.58	99.51	1.023
$\bar{x} = 100.10 \pm 0.87$	$\bar{x} = 98.83 \pm 0.53$	$\bar{x} = 1.016 \pm 0.01$
S.D. = 2.30	S.D. = 1.41	S.D. = 2.60
CV = 2.30%	CV = 1.41%	CV: = 2.60%
CI: 97.97–102.23	CI: 7.53–100.13	CI: 99.23–104.05

[a] Results are means of seven separate measurements. CI, confidence intervals (95%); CV, coefficient of variation; S.D., standard deviation; $\bar{x}$, mean.

oral suspension contains prophyl-*p*-hydroxy benzoate, methyl-*p*-hydroxy benzoate, sorbitol, saccharose, and aroma of aserola as excipients besides 50 mg nimesulide per 5 ml. Recovery studies were carried out as mentioned for other preparations.

Table 5
The results of analysis from pharmaceutical preparations of nimesulide by HPLC method[a]

Brand A® tablet (100 mg nimesulide)	Brand A® sachet (100 mg nimesulide)	Brand A® suspension (1% nimesulide)
99.00	99.46	1.006
99.46	98.70	1.006
100.90	101.02	0.997
100.31	98.82	0.997
99.05	99.28	0.991
99.34	98.89	0.993
99.91	99.42	0.996
$\bar{x} = 99.71 \pm 0.27$	$\bar{x} = 99.37 \pm 0.30$	$\bar{x} = 0.998 \pm 0.002$
S.D. = 0.70	S.D. = 0.79	S.D. = 0.006
CV = 0.70%	CV = 0.79%	CV = 0.006%
CI: 99.06–100.35	CI: 97.53–100.13	CI: 99.25–100.35

[a] Result are means of seven separate measurements. CI, confidence intervals (95%); CV, coefficient of variation; S.D., standard deviation; $\bar{x}$, mean.

2.3. Procedure

A total of ten tablets or sachets of nimesulide were accurately weighed and powdered. An amount corresponding to one tablet or sachet content was weighed in a 100-ml volumetric flask

Table 4
The results of percentage recovery value in synthetic mixture of nimesulide by the developed second order derivative UV spectrophotometric methods[a]

Tablet		Sachet		Suspension	
Found nimesulide (mg)	Recovery (%)	Found nimesulide (mg)	Recovery (%)	Found nimesulide (mg)	Recovery (%)
29.80	99.33	29.85	99.51	20.46	102.30
30.19	100.63	29.23	97.44	20.50	102.30
29.60	98.66	29.89	99.63	19.56	97.80
29.85	99.51	29.46	98.20	20.87	104.35
29.65	98.85	29.87	99.56	20.88	104.40
29.34	97.82	29.85	99.51	20.52	101.00
29.85	99.51	29.80	99.33	20.48	102.40
	$\bar{x} = 99.19$		$\bar{x} = 99.02$		$\bar{x} = 102.00$
	S.D. = 0.87		S.D. = 0.86		S.D. = 2.24
	CV = 0.88%		CV = 0.87%		CV = 2.20%

[a] Added nimesulide for tablet and sachet 30 mg, for suspension 20 mg ($n = 7$). CV, coefficient of variation; S.D., standard deviation, $\bar{x}$, mean.

and 50 ml ethanol was added and the flask was sonicated for 5 min. The flask was filled to volume with ethanol. The second order derivative UV spectra were recorded against ethanol as reference solution.

A 5-ml suspension corresponding to 50 mg was put in to a 50-ml volumetric flask and 20 ml chloroform was added and the flask was sonicated for 15 min and then filled to volume with chloroform. Appropriate dilutions were made into range of calibration curve with chloroform. The second order derivative UV spectra were recorded against chloroform as reference solution.

2.4. High performance liquid chromatographic (HPLC) conditions

Stock solution of nimesulide (1000 μg ml^{-1}) was prepared in methanol. A 25-cm × 0.4-mm 10-μm particle size C_8-LiChrosorb column was used. The flow rate was 1.5 ml min^{-1}, at room temperature (25°C). The mobile phase was 530 ml methanol + 470 ml 2.5 mM anhydrous sodium acetate + 1 ml glacial acetic acid. UV detection was at a wavelength of 313 nm. HPLC studies were performed by preparing the tablet, sachet and suspension in methanol and prepared sample solutions were filtered with Whatman 42 filter papers. Then 10-μl portions were injected from 50 μg ml^{-1} nimesulide solution in methanol.

Table 6
Comparison of the results from second order derivative UV spectrophotometry and HPLC methods with non-parametric Wilcoxon's paired test[a]

	T_C	T_T ($\alpha = 0.05$, $n = 7$)	
Brand A® tablet (100 mg nimesulide)	9	2	$T_C > T_T$
Brand A® sachet (100 mg nimesulide)	9	2	$T_C > T_T$
Brand A® suspension (1% nimesulide)	3	2	$T_C > T_T$

H_0 hypothesis: no statistically significant difference exists between two methods.
$T_C > T_T$; H_0 hypothesis is accepted: $P > 0.05$.

[a] T_C, T calculated; T_T, T tabulated.

3. Results and discussion

The solubility of nimesulide in acid, water and aqueous solutions is lower than in ethanol and chloroform. Thus ethanol and chloroform were used as solvent to prepare nimesulide solutions. UV spectrum of nimesulide in ethanol gives two broad shouldered peaks at 296 and 331 nm, respectively (Fig. 2). These two shouldered peaks were separated by using derivative spectrophotometric method. In basic solutions of nimesulide the UV spectrum was not suitable for the determination of nimesulide, giving no absorption peak.

By this method, nimesulide can be determined not only in tablets but also in sachet (granule) and suspension solutions according to the high determination capacity of this method in turbid solutions [24,25].

The UV spectra of nimesulide in ethanol and chloroform were similar. The second order derivative UV spectrum analysis of nimesulide indicatedthat the novel method developed for determination of substance gave sharper and better-defined peaks when compared with the original zero order derivative spectrum (Fig. 3). The derivative wavelength difference ($\Delta\lambda$) depends on the measuring wavelength range and the key entry n (a kind of smoothing factor). Optimal wavelength range should be chosen since the broad peaks get sharper, the ratio of signal/noise elevates and the sensitivity of the method increases by controlling some degree of low-pass filtering or smoothing. Therefore, a series of n-values ($n = 1$–9) was tested by the second order UV spectrum of nimesulide in ethanol (Fig. 4). The optimum n was found to be $n = 6$ ($\Delta\lambda = 21$) in the measuring wavelength range of 200–500 nm.

In quantitative analysis of nimesulide the calibration curves were plotted using second order derivative spectra in ethanol and chloroform. The second order derivative spectra were evaluated by using peak to peak, peak to zero and tangent methods. The results of calibration curves with three methods measured in ethanol and chloroform are given in Tables 1 and 2. These results show that three derivative spectrum measuring methods can be used. The slope of the peak to peak calibration curve in ethanol was higher than

the others. The linearity ranges were found to be 2.0–90.0 μg ml^{-1} in ethanol and 2.0–50.0 μg ml^{-1} in chloroform by using the values obtained from the second order derivative UV spectrum of the substance. Peak to peak was measured between wavelengths, 262–291 nm in ethanol and 248–268 nm in chloroform. The limit of quantitation (LOQ) for nimesulide was determined as 2.0 μg ml^{-1} both in ethanol and chloroform. The limit of detection (LOD) was found to be 0.5 μg ml^{-1} in ethanol and 1.0 μg ml^{-1} in chloroform.

The developed second order derivative UV spectrophotometric method was applied to pharmaceutical preparations such as tablet, sachet (granule) and suspension (Fig. 5).

Tablets and sachets were analysed in ethanol while the suspension was analysed in chloroform. A summary of the results is shown in Table 3. Since tablets, sachets and suspensions of nimesulide yielded turbid solutions, second order derivative spectrophotometry presents an advantage for the determination of nimesulide in pharmaceutical preparations. In the proposed method there was no need for pre-separation and centrifugation procedure.

Recovery studies were performed on 30 μg ml^{-1} reference nimesulide standard solutions in ethanol and chloroform. Mean recovery and relative standard deviations were found to be 99.89% (2.04%) and 99.96% (2.97%), respectively.

The other recovery studies were performed on the synthetic mixture prepared by adding accurately weighed amounts of nimesulide to the excipient mixture and calculating the percentage recovery in each case (Table 4). The percentage recovery of nimesulide was calculated by comparing the found and added concentrations (mg found/mg added × 100). In order to detect interactions of the excipients in this method, the standard addition technique was applied to the same preparations which were analyzed by the calibration curve. There is no difference between the relative standard deviations of the two techniques. The regression equation of standard addition curve was found as $y = 0.042x + 1.2008$ ($r = 0.9998$), where y is the amplitude of second order derivative spectrum, x is concentration of nimesulide, and r is the coefficient of correlation. Since the slopes of the standard and standard addition curves were identical, it has been concluded that there was no spectral interaction in the analysis of pharmaceutical preparations. In order to determine the precision of the method, nimesulide solutions at a concentration of 30 μg ml^{-1} in ethanol were analysed ten times and the mean nimesulide value was found to be 30 ± 0.008 μg ml^{-1}. The standard deviation was found to be 0.023. It has been decided that the developed method has a good precision. In order to compare the developed UV spectrophotometric method, modified HPLC method was used for analysis of nimesulide. The linear range of the HPLC method was 0.5–100.0 μg ml^{-1}. The regression equation was found to be $y = 7570.6x + 2476.8$ ($r = 0.9994$), where y is peak square obtained from HPLC, and x is the concentration of nimesulide. The chromatographic method was applied to three different pharmaceutical preparations containing nimesulide (Table 5). The results obtained from second order UV spectrophotometry were compared with those obtained by using HPLC method. It had been found that the difference was not statistically important between these two methods (Table 6).

For ruggedness and robustness of analytical methods the tests mentioned below were carried out. Preliminary optimization experiments revealed that amongst the many operating parameters involved the solvent, the degree of derivation ranges of wavelength and N-value were chosen in order to optimize conditions. Suitable parameters on the repeatability of the method have been taken with regard to instrument set-up. HPLC conditions, such as column type, mobile phase and flow rate were also chosen. Derivative spectrophotometry and HPLC method were used by two analysts employing two different instruments to analyse the same standard and samples. The results showed no statistical differences between operators and instruments. For analysis of standard solutions, the differences between the two analysts were 0.9% for derivative UV and 1.2% for HPLC. In tablet analysis, the differences were 1.3 and 1.8%, respectively.

It was concluded that developed derivative UV spectrophotometric method was accurate, sensi-

tive, precise, reproducible and could be applied directly and easily to pharmaceutical preparations.

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