High Performance Liquid Chromatographic Method for the Determination of Nimesulide and Its Impurities

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1 Introduction

Nimesulide (**I**) 4-nitro-2-phenoxymethanesulfonanilide, an anti-inflammatory drug, belongs to the chemical class known as methanesulfananilides. A previously reported technique employing HPLC [1] has been restricted to the determination of Nimesulide in plasma. This method has inadequate resolution and limited reproducibility. Nimesulide is not described in any of the Pharmacopoeia. There are no official methods for the determination of chromatographic purity and assay of the compound.

This paper describes a high-performance liquid chromatographic method for the separation and quantification of Nimesulide and its related compounds in drug raw material. The validation of the proposed HPLC procedure [2-4] is described in terms of specificity, linearity, precision, limit of quantitation, and ruggedness.

2 Materials and Methods

2.1 Chemicals

The molecular formulas of Nimesulide and its potential impurities are shown in **Figure 1**. The structure of these compounds was confirmed by MS, NMR, and IR. Standard Analytical Reference Materials of the impurities, Internal Standard (**II**) and Nimesulide were of purity not less than 99 %. The compound (**II**) was selected as internal standard due to its structural similarity to the Nimesulide and its comparable relative elution volume. The organic solvents were of HPLC grade and all the reagents were of analytical grade.

2.2 High-Performance Liquid Chromatography

The HPLC system consisted of a Hewlett-Packard 1090M chromatograph equipped with a HP-1040 diode array detector and a Chem Station 9000. The column was a μBondapak C-18, 10 μm, 30 cm x 3.9 mm i.d. (Waters Assoc.). The mobile phase consisted of a methanol buffer pH 7.0 (550:450). The buffer was a 0.05 M solution of monopotassium phosphate (KH_2PO_4) adjusted to pH 7.0. Before chromatography, the mobile phase was filtered through a Nylon-66 membrane (0.45 μm) and degassed under vacuum. The flow rate was maintained at 1.0 mL per minute. Chromatographic separation was monitored by ultraviolet detection at a wavelength of 230 nm. Sensitivity was 0.020 and 0.080 at full scale for chromatographic purity and assay, respectively. The bandwidth was 4 nm. All analyses were performed at room temperature and the injection volume was 10 microliters.

2.3 Solutions

2.3.1 Chromatographic Purity

Transfer about 300 mg of Nimesulide to a 100 ml volumetric flask, dissolve in 20 ml of methanol and dilute with mobile phase to volume.

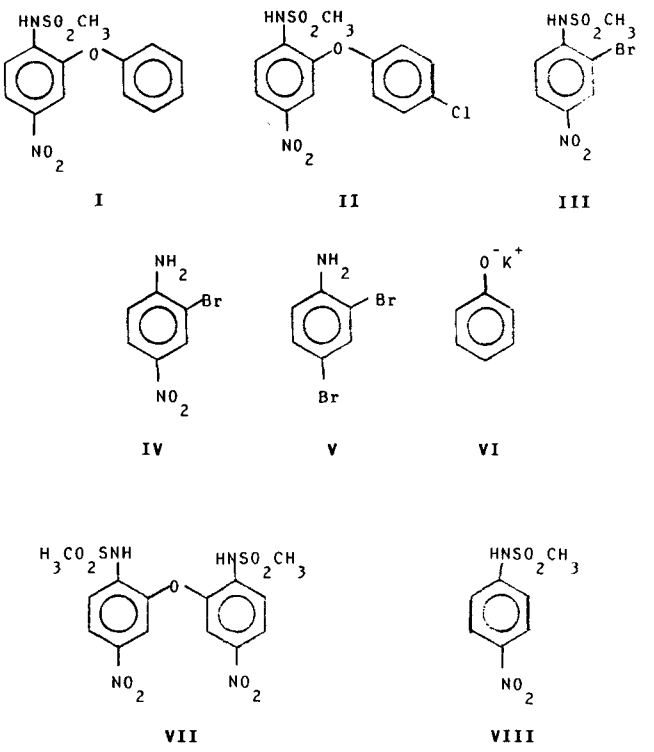

Figure 1

Molecular structures of: Nimesulide (I), internal standard (II), synthetic precursors (III), (IV), (V), and (VI). Compounds (VII) and (VIII) are impurities that were isolated and identified.

2.3.2 Assay

Internal Standard Solution:
Prepare a solution of internal standard in methanol having a concentration of about 2 mg per mL.

Standard Solution:
Transfer about 100 mg of Nimesulide reference standard to a 50 ml volumetric flask, dissolve and dilute to volume with methanol. Transfer 4.0 mL to a 50 mL volumetric flask, add 5.0 mL of internal standard solution and dilute with Mobile Phase to volume.

Sample Solution:
Prepare as directed under standard solution using Nimesulide instead of the reference standard.

System Suitability Test:
Chromatograph the standard solution and record the peak response: The resolution between the analyte and internal

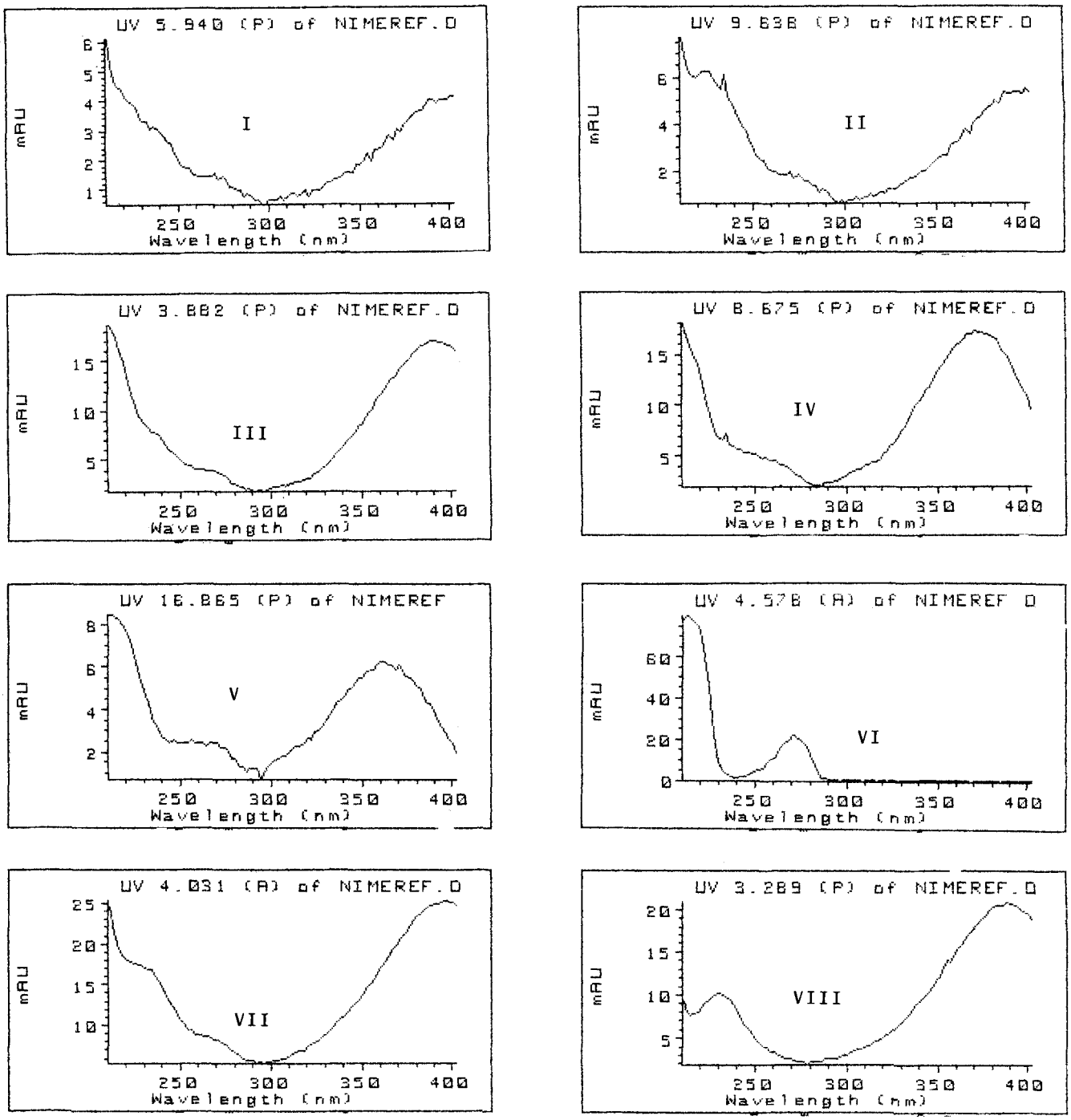

Figure 2

UV spectral scans of Nimesulide and its related compounds.

standard peaks must be not less than 5.0 and the relative standard deviation for five replicate injections must be no more than 2.0 %.

3 Results and Discussion

3.1 Evaluation of the Chromatographic System

The previous chromatographic system [1], a water/acetonitrile (50:50) mixture without pH control, gave limited specificity and reproducibility. Changing the organic modifier to methanol and the aqueous phase to potassium phosphate buffer of pH 7.0 resulted in a complete separation between Nimesulide and its related compounds.

The UV spectrum of Nimesulide shows dramatic changes with pH and the mobile phase is highly sensitive to relatively small changes in the pH value. The wavelength used in the previous technique (300 nm) is not sensitive enough for purity studies. Monitoring at 230 nm resulted in increased sensitivity, thus allowing detection less than 10 micrograms of Nimesulide directly injected onto the column. **Figure 2** shows the UV spectral scans of Nimesulide and its related compounds in mobile phase. Comparing these, at 230 nm and the respective molecular weights, indicated molar extinction differences, ruling out quantitation of the analytes by peak area normalization relative to Nimesulide (see **Table 1**).

3.2 Method Validation

The specificity of the HPLC method has been evaluated by injecting a spiked sample solution containing 2 mg/mL of I and II together with III, IV, V, VI, VII, and VIII at concentrations in the respective limits of quantitation. A chromatogram showing the resolution is given in **Figure 3**. Specificity of the procedure has been further demonstrated by using the HP-1040 array detector. The peak homogeneity is assessed by:

a) Comparison of the normalized spectral profiles recorded at the upslope and the downslope of the peak.
b) Determination of the apparent retention time relative to the detection wavelength.
c) Examination of the absorbance-ratio chromatogram.

To test the linearity of the assay procedure, a series of five standard solutions of known concentration covering 50 % to 150 % of the expected concentration range were analyzed. Each standard was injected five times and the response ratios were recorded (**Table 2**).

Table 1

Response factors and limit of quantitation.

Compound	*A* 1% / 1 cm 230 nm	Response factor	Limit of quantitation ng	%
I	635	1.00	10	-
II	570	0.90	10	0.05
III	292	0.46	20	0.10
IV	295	0.46	20	0.10
V	365	0.57	20	0.10
VI	32	0.05	100	0.50
VII	381	0.60	20	0.10
VIII	395	0.62	20	0.10

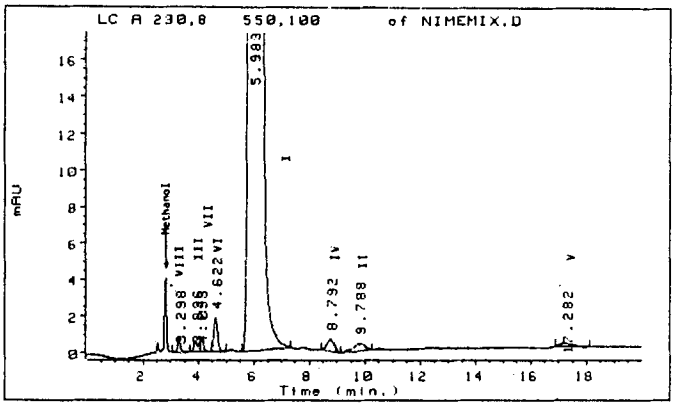

Figure 3

Chromatogram of a synthetic mixture of (I) to (VIII). (Concentrations: I: 2 mg/mL, II to VIII at the limit of quantitation.)

Table 2

Linearity of response for Nimesulide.

Std No.	Concentration of Nimesulide (µg/mL)	Relative response				
1	0.040	0.1618	0.1618	0.1632	0.1637	0.1636
2	0.080	0.3224	0.3221	0.3242	0.3223	0.3239
3	0.160	0.6509	0.6511	0.6504	0.6513	0.6518
4	0.240	0.9764	0.9767	0.9759	0.9711	0.9751
5	0.320	1.3006	1.2993	1.3008	1.2995	1.3056

Table 3

Analysis of variance (ANOVA) table.

Source	Degrees of freedom	Sum of squares	Mean squares	Variance ratio F
Regression	1	4.3431	4.3431	
R E S I D U A L Lack-of-fit	3	2.435×10^{-5}	8.1167×10^{-6}	2.9082
R E S I D U A L Pure error	20	5.582×10^{-5}	2.7910×10^{-6}	
Total:	24	4.3432		

Critical $F(0.05)[2.20] = 3.49$
The lack-of-fit ANOVA reveals that the calibration curve is not significantly non-linear.

Analysis of variance (ANOVA) was used to test non-linearity (lack-of-fit) of the relationship between the detector response and the concentration of Nimesulide (**Table 3**). Student's test was used to test for a significant intercept. The quantity "$t = b/Sb = 0.12$" was calculated (where b is the intercept and Sb is the standard error of the intercept). The calculated value for t did not

Table 4

Linearity data for Nimesulide related compounds.

Compound	*RRt*	Range (μg/mL)	Intercept (a.u.)	Slope a.u. x mL x μg^{-1}
III	0.66	2- 6	-2.5131	586.78
IV	1.50	5-15	-0.2625	209.90
V	2.90	2- 6	4.3100	350.29
VI	0.78	10-30	6.3626	18.43
VII	0.71	2- 6	-0.8173	121.23
VIII	0.56	2- 6	-3.2738	289.21

Table 5

Interday precision.

Day	1	2	3	4	5	6
% Nimesulide	100.6	100.9	99.8	100.5	99.7	100.0
in duplicate	99.5	100.6	100.6	100.1	100.7	101.3
$n = 12$	$\overline{X} = 100.4\ \%$			*RSD* (%) = 0.75		

exceed the critical value of two tailed t (20,0.95) = 2.08, which indicates that the intercept is not significantly different from zero at the 95 % confidence level. The detector responses for related compounds were also linear, as indicated in **Table 4**.

The precision of the assay method was determined by analyzing the same sample of Nimesulide on six different days. Two replicate determinations were made each day. The interday results are shown in **Table 5**.

The precision of the system was determined by making five replicate injections of a Nimesulide standard solution. The relative standard deviation of the response ratios was 0.3 %. The limits of quantitation for Nimesulide and its related compounds are shown in Table 1.

The stability of the analytical solutions was assessed by injecting fresh and aged (six hours) standard solutions. No significant statistical differences were found.

To test column-to-column variability the results were checked on a similar, second column, from the same manufacturer.

4 Conclusion

This work describes a specific, precise and sensitive HPLC method for quantitation of Nimesulide and for evaluation of its chromatographic purity. The method was applied to samples from raw material with excellent results.

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Chromatographic Purity Study in Synthetic Drugs: Buflomedil Hydrochloride

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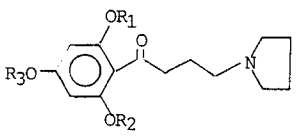
1 Introduction

Included in a general program regarding to a systematic study of purity on synthetic drugs, we performed a full chromatographic purity profile of a well known vasodilatador: Buflomedil hydrochloride *(I)*.



·HCl (*I*: $R_1 = R_2 = R_3 = CH_3$)

HPLC analysis of Buflomedil in plasma [1] and in pharmaceutical formulations [2] has been previously reported, without any mention of methods for controlling impurities. According to the synthetic pathway we proposed a set of potential impurities (*O*-demethylated compounds) shown in **Table 1**.

[1] Research and Development Department.
[2] Quality Control Department.