Scientific paper

Simulataneous Determination of Nimesulide and its Impurities in Pharmaceutical Formulations by Reversed-Phase High-Performance Liquid Chromatography

B. Tubić[a]*, B. Ivković[b], M. Zečević[b] and S. Vladimirov[b]

[a] Drug Agency of the Republic of Srpska, Veljka Mladjenovića bb., 78 000 Banja Luka, Bosnia and Herzegovina

[b] Institute of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, University of Belgrade 450 Vojvode Stepe St., P.O. Box 146, Belgrade 11152, Serbia

* Corresponding author: E-mail: b.tubic @spinter.net

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Abstract

A simple, rapid and reproducible reversed-phase high-performance liquid chromatography method for the analysis of nimesulide and its impurities both in the bulk drug and pharmaceutical formulations is reported. The method is suitable for monitoring the stability of nimesulide. The presence of nimesulide impurities C (2-phenoxyaniline) and D (2-phenoxy-4-nitroaniline) was observed. The best separation was achieved using an Agilent Zorbax Extend C_{18} column (150 × 4.6 mm, particle size 5 μm) at 40 °C and flow rate of 1.0 $mLmin^{-1}$. The analytes were monitored at 230 nm. The mobile phase consisted of acetonitrile – triethylamine (TEA) – water (45:0.5:54.5 v/v/v), adjusted to pH 5.2 with formic acid. Under these conditions the retention times were of 7.11, 7.98 and 8.66 min for nimesulide, D and C, respectively. The resolution of nimesulide and impurity D was 3.20 and that of impurity D and impurity C 2.40, indicating that the compounds were well separated. Evaluation of linearity, accuracy, precision, selectivity, sensitivity and robustness of the method produced satisfactory results. The developed method was successfully applied to assay nimesulide in different solid pharmaceutical formulations.

Keywords: Nimesulide, impurities, degradation products, RP-HPLC

1. Introduction

Tissue injury is associated with the release of numerous inflammatory mediators including prostaglandins synthesized from arachidonic acid via endoperoxide biosynthetic pathway, the initial step being catalyzed by cyclooxygenase (COX) that appears in two isoforms COX-1 and COX-2. The latter isoform, representing the major form of the enzyme is associated with inflammation and its expression is up-regulated in response to inflammatory stimuli in numerous tissues.[1,2]

Nimesulide (4-nitro-2-phenoxymethanesulfonanilide) is a selective COX-2 nonsteroidal anti-inflammatory drug. This compound, a derivative of *p*-nitrophenylmethanesulfonamide, is structurally a unique nonsteroidal anti-inflammatory drug. It belongs to selective COX-2 inhibitors, with a potent anti-inflammatory and analgesic activity, when administered orally, rectally, or topically.[3] Due to its analgetic and antipyretic properties, nimesulide is widely used for the treatment of various inflammatory processes.[4] Besides, it is better tolerated and causes fewer adverse effects than other currently used non-steroidal anti-inflammatory drugs.[5] Also, nimesulide seems to express less severe gastrointestinal side effects. This compound structurally differs from other new classes of COX-2 inhibitors named coxibes. Chemical structure of nimesulide is given in Fig. **a**.

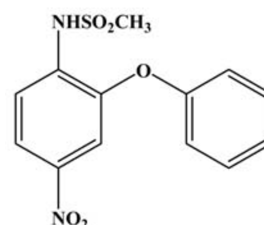

Figure a: Chemical structure of nimesulide (4-nitro-2-phenoxymethanesulfonanilide).

The literature data suggest various pK_a values (from 5.9 to 6.56) of nimesulide.[6] This compound is freely soluble in organic polar solvents, while its solubility in water was reported to be 0.01 mg mL^{-1} but it depends on the pH of the aqueous solution.[6]

Chemical structure of the main nimesulide impurities C (2-phenoxyaniline) and D (2-phenoxy-4-nitroaniline) is presented in Fig. **b**.

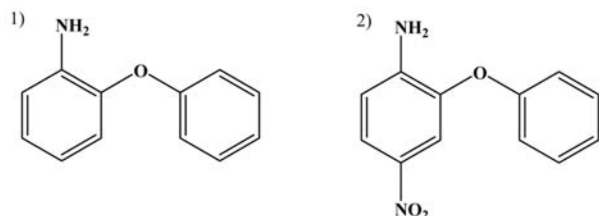

Figure b: Chemical structure of nimesulide impurities C (1) and D (2).

European Pharmacopoeia describes the tests for numesulide related substances and a potentiometric assay for nimesulide itself.[7] Tests for numesulide impurities C and D in Ph.Eur. is HPLC method with mobile phase acetonitile:1.15gL^{-1}ammonium dihydrogen phosphate (35:65 v/v) adjusted to pH 7.0 with ammonia. In addition, a reference on high speed liquid chromatography from 1977,[8] and a list of the references for the analysis of nimesulide by HPLC, the most recent being from 2007,[9–30] and by HPTLC.[31–32] are given. It is worth mentioning HPLC and TLC methods for monitoring photochemical stability of nimesulide.[33] Analytical methods reported for nimesulide also include UV spectroscopy[34] and polarography.[35]

Up to date, there are several analytical methods for determination of nimesulide in pharmaceutical dosage forms, e.g. HPLC,[28–29] high-throughput HPLC assay for nimesulide and its impurities using a short monolithic column,[30] HPTLC,[31] spectrophotometric determination of nimesulide,[36] and voltammetric determination of nimesulide in pharmaceutical formulations and human serum.[37] However, there are no reports on determination of nimesulide in the presence of its main impurities C and D in different solid pharmaceutical formulations by HPLC method. This prompted us to develop a new RP-HPLC method for simultaneous determination of nimesulide and its impurities C and D which could be used for the monitoring the stability of nimesulide in different solid pharmaceutical dosage forms. One of the tested formulations contained β-cyclodextrin. Namely, nimesulide can cause bioavailability problems in vivo that could be overcome by the formation of inclusion complexes with β-cyclodextrin.[38] The novelty of the procedure described here is based on the presentation of a new, simple, rapid (10 min), reliable and sensitive stability-indicating analytical method which could be helpful in efforts to control the quality of nimesulide in different solid pharmaceutical formulations.

2. Experimental

2. 1. Chemicals and Reagents

Standards of nimesulide and the impurities were obtained from EDQM, Council of Europe BP 907-F67029 Strasburg CEDEX 1, Batch/Lot no. 1a. TEA and formic acid (98–100%) of analytical purity were Merck (Darmstadt, Germany) products. HPLC gradient-grade acetonitrile was supplied by Lab Scan (Dublin, Ireland). All other reagents were of purity grade suitable for high-performance liquid chromatography. Water for chromatography was obtained from Millipore Simplicity 185 purification system (Billerica, Massachusetts, USA). Investigated nimesulide tablets containing 100 mg nimesulide were formulated by the following pharmaceutical companies: By Zdravlje Actavis (Serbia) (*TENAPROST*® containing lactose, maize starch, povidone K30, magnesium stearate and colloidal anhydrous silica as excipients); By Replekpharm (FYR Macedonia) (*VENTOR*® with dioctyl sodium sulfosuccinate, hypromellose, lactose monohydrate, microcrystalline cellulose and sodium starchglycolate as excipients; By Panacea Biotec (India) (*NIMULID*® with lactose monohydrate, crosscarmelose sodium, colloidal anhydrous silica, maize starch, povidone K30, polysorbate 80 and magnesium stearate as excipients); By CSC Pharmaceuticals Handels GmbH (Austria) (*AULIN*® with hydroxypropyl cellulose, docusate sodium, lactose, sodium carboxymethylstarch, microcrystalline cellulose and magnesium stearate as excipients) and by Habitpharm (Serbia) (*NIMESULID* with β-cyclodextrin, cremophore, microcrystalline cellulose, sodium starchglycolate, talc, colloidal anhydrous silica, magnesium stearate and crosspovidone as excipients).

2. 2. Instrumentation and Chromatographic Conditions

HPLC analysis was performed with an Agilent Technologies HP 1100 chromatograph (Palo Alto, CA, USA) equipped with 1100 binary pump, 1100 UV-visible detector and 20 μL loop injector. Compounds were separated on an Agilent Zorbax Extend C_{18} analytical column (150 × 4.6 mm, particle size 5 μm). Mobile phase and the solutions were degassed and vacuum filtered through 0.45 μm nylon membranes (Alltech Associates, Loceren, Belgium) before the use. Data were acquired by ChemStation software from HP.

The mobile phase consisted of acetonitrile – TEA – water (45:0.5:54.5 v/v/v) and pH adjusted to 5.2 with formic acid. Isocratic elution was performed at a flow rate of 1.0 mL min^{-1} and UV-detection at 230 nm. Before each injection, the column was equilibrated to stable baseline at a flow rate of 1.0 mL min^{-1} and 40 °C.

2. 3. Standard Solutions

The concentrations of nimesulide stock and standard solutions were 1.0 mg mL^{-1} and 0.1 mg mL^{-1}, respectively.

Solutions of the examined impurities were prepared from their own reference standards. Concentration of the stock solution of impurity C was 0.2 mg mL^{-1} and standard solution contained 0.004 mg mL^{-1}. The stock solution of impurity D contained 0.2 mg mL^{-1} and standard solution 0.004 mg mL^{-1}. To prepare standard solutions, the stock solutions were diluted with the mobile phase. All prepared solutions were stored light-protected.

For nimesulide standard calibration plot the concentrations from 0.016 to 0.160 mg mL^{-1} were used. Standard calibration plots for impurities C and D were obtained using the concentrations from 0.033 to 0.333 μg mL^{-1}.

The solutions of nimesulide and impurities C and D were stable during the described chromatographic analysis.

2. 3. 1. Sample Preparation

For the assay of nimesulide in dosage forms, 20 tablets from each formulation were randomly selected and powdered. Amount equivalent to 25 mg nimesulide from powdered tablets was accurately weighed, transferred into a volumetric flask, dissolved in approximately 15 mL mobile phase in an ultrasonic bath for 15 min and.the solution diluted to volume with the same solvent and filtered. It contained 1.0 mg nimesulide per mL. One mL of this solution was diluted to 10 mL in a volumetric flask with the same solvent. Each solution was chromatographed three times. All solutions were freshly prepared before the analysis.

3. Results and Discussion

3. 1. Development of the Method and Optimization

A new RP-HPLC method for simultaneous determination of nimesulide and its main impuites C and D has been developed and evaluated. The RP-HPLC method was tested for selectivity, linearity, sensitivity, accuracy, precision and robustness.

Resolution of nimesulide and impurity D, as well as that of impurity D and impurity C represented the main problem because of similarity of their chemical structure and polarity. In initial chromatographic conditions, mobile phase consisting of acetonitrile-water solution (60 : 40 v/v) adjusted to pH 4.3 with formic acid and TEA, was used. The compounds were separated on an Agilent Zorbax Extend C_{18} column (150 × 4.6 mm, particle size 5 μm) at ambient temperature on an Agilent Technologies HP 1100 chromatograph equipped with 1100 binary pump at a flow rate of 1.0 mL min^{-1}. The UV-detection was performed at 230 nm. Under these chromatographic conditions, a satisfactory separation of mixture compounds was achieved. All three compounds were eluted within 4 min and satisfactory values for required chromatographic parameters were obtained.

However, when nimesulide in concentration range present in final pharmaceutical products was chromatographed (individual impurities in finished pharmaceutical dosage form are allowed to be in quantity up to the 0.2% of the principal quantity),[39] nimesulide peak was partially overlapping the peak of impurity D, i.e. a poor separation resulted. Combined effects of mobile phase pH, temperature of the column and acetonitrile content in the mobile phase on reverse phase liquid chromatographic behavior of nimesulide and its impurities were also studied. Preliminary analyses revealed the following: 1. Lower pH values of the mobile phase led to a poor separation of impurities D and C. In addition, at higher pH values up to the 5.5 of the mobile phase, poorer separation of nimesulide from impurity D was observed; 2. Reduction of acetonitrile content in the mobile phase from 60% to 50% resulted in a better separation of nimesulide and impurity D, but poor resolution of impurities D and C. Further decrease of acetonitrile concentration to 45% provided a satisfactory separation of nimesulide from impurity D, but, at column temperature of 30 °C and pH 4.0 of the mobile phase, the examined impurities were completely overlapped. At pH 4.5 of the mobile phase, the impurities were partially separated and the separation was additionally improved increasing temperature of the column. Further increase of the pH value to 5.0 and column temperature to 40 °C, resulted in a satisfactory separation of the impurities.

Temperature increase reduced the retention time of the compounds. Higher temperature values of the column positively influenced separation of the compounds, regardless of mobile phase acidity and acetonitrile concentration. For the purpose of lower costs of the method, the lowest acetonitrile concentration of 45% in the mobile phase at which a satisfactory separation of the impurities was achieved, together with the correction of temperature and pH were applied.

Using a software ChemOffice 7 Ultra, 2002 (CambridgeSoft Corporation, USA) it was found that nimesulide and the impurities D and C had LogP of 3.08, 3.09 and 3.13, respectively, what is in accordance with experimental conditions, as well with the properties of the examined compounds.

Nimesulide was first eluted from the column, to be followed by the impurity D and then C. p*Ka* value of nimesulide was around 6.56[6]. It appeared in the ionized form at the tested pH values of the mobile phase. Its molecule has one methylsulfonamide group and one nitro group in *p*-position in relation to methylsulfonamide group. These groups influenced nimesulide polarity and because of that, it was shortly retained on the nonpolar stationary phase. Since it was more soluble in the polar mobile phase used here than the impurities, it was the first to be eluted from the column, i.e., it had the shortest retention time. Molecule of the impurity D contains the primary aromatic amino group instead of methylsulfonamide group of nimesulide and nitro group in *p*-position in rela-

tion to the primary amino group. Its polarity is lower comparing to nimesulide and it was eluted from the column after nimesulide. The molecule of the impurity C has one primary aromatic amino group and it was the most lipophilic component of the analyzed mixture. So, having the higher affinity for the binding at the stationary phase, the impurity C had the longest retention time and was eluted from the column at the end of chromatographic procedure.

3. 2. Method Validation

Optimal chromatographic parameters were achieved with the mobile phase consisting of acetonitrile – TEA – water (45:0.5:54.5 v/v/v), pH adjusted to 5.2 with formic acid, on an Agilent Zorbax Extend C_{18} column (150 × 4.6 mm, particle size 5 µm) at 40 °C. The pump operated in an isocratic mode at a flow rate of 1.0 mL min^{-1}. The UV-detection was performed at 230 nm. Injections were carried out using a 20 µL loop. Before each injection, the column was equilibrated to stable baseline at a flow rate of 1.0 mL min^{-1} and 40 °C. Representative chromatogram obtained from working solutions of nimesulide and its impurities C and D is shown in Fig. **c**.

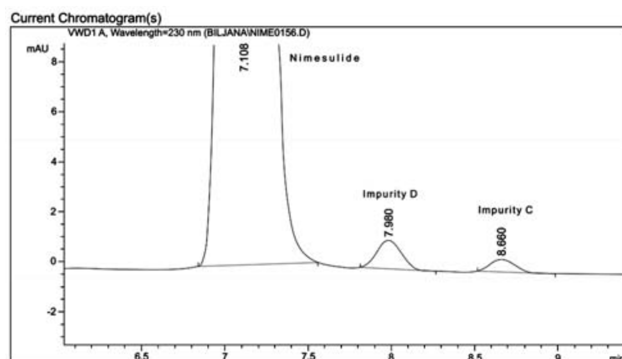

Figure c: Representative chromatogram obtained from working solutions of nimesulide (N, 0.1 mg mL^{-1}) and its impurities (C and D 0.004 mg mL^{-1}).

HPLC conditions: Mobile phase – acetonitrile – TEA – water (45:0.5:54.5 v/v/v), pH 5.2 (adjusted with formic acid). Flow rate 1.0 mL min^{-1}.

Under described conditions, RP-HPLC procedure was performed. Optimal chromatographic parameters such as retention time (t_R), retention factor ($k' = t_R - t_0/t_0$), symmetry factor ($A_s = W_{0.05}/2d$), resolution ($Rs = 1.18*(t_{R2} - t_{R1})/W_{h1} + W_{h2}$), separation factor ($\alpha = k_2'/k_1'$), plate number ($N = 5.54*(t_R/W_h)^2$) and height equivalent to one theoretical plate (HETP = L/N) are listed in Table 1.

Tests for selectivity, sensitivity, linearity, accuracy, precision and robustness of the method afforded satisfactory results. Qualitative comparison of the chromatograms obtained from standard and sample solutions showed that the selectivity of the HPLC method described here was good. Since the excipients did not interfere with the examined compounds which is shown in Fig. **d**., the method is suitable for quality and stability testing of both the active substance and the final product.

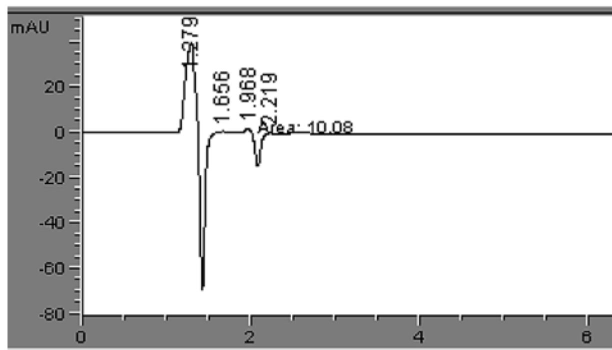

Figure d: Chromatogram of the excipients.

The calibration curves were evaluated by their correlation coefficients. Linearity for nimesulide was assessed within the concentration range 0.016–0.165 mg mL^{-1} with correlation coefficient of r = 0.9999. Linearity for impurities C and D was assessed within the concentration range 0.033–0.333 µg mL^{-1} with correlation coefficients of r = 0.9986 and r = 0.9999, respectively. Regression lines for nimesulide and the examined impurities were constructed by the method of the least squares. The results of the regression analysis of nimesulide, impurity C and impurity D are summarized in Table 2.

Both limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the equation: LOD = 3xSa/a and LOQ = 10xSa/a, where Sa represents standard deviation of the slope (a). The quantitation limit for nimesulide was 5.8 µg mL^{-1}. Minimum levels at which impurity C could be reliably detected and quantified were 0.0044 µg mL^{-1} and 0.0146 µg mL^{-1}, respectively. The detection limit of impurity D was 0.0026 µg mL^{-1} and the corresponding quantitation limit 0.0085 µg mL^{-1}. The obtained LOD and LOQ values of nimesulide and impurities C and D confirmed the sensitivity of this method.

Table 1. Chromatographic parameters of the described HPLC method (System suitability test).

Compound	t_R	k'	A_s	Rs	α	N	HETP
Nimesulide	7.11	4.21	0.928	3.20	1.15	10834.88	0.014
Impurity D	7.98	4.85	0.981	2.40	1.10	13673.37	0.011
Impurity C	8.66	5.35	0.955			13926.75	0.011

Table 2. Validation parameters of the calibration curves, limits of detection and quantitation.

Compound	Regression equation y = a*x + b	r	Sa	Sb	t_α	LOD (μg/mL)	LOQ (μg/mL)
Nimesulide	39944.274*x + 54.906	0.9999	23.39	225.05	2.35		5.8
Impurity C	68.410*x – 0.027	0.9986	0.10	0.51	0.26	0.0044	0.0146
Impurity D	46.932*x – 0.069	0.9999	0.04	0.20	1.70	0.0026	0.0085

y – peak area; *a* – the slope; *b* – the intercept; *x* – concentration of the compound; *r* – correlation coefficient; *Sa* – standard deviation of the slope; *Sb* – standard deviation of the intercept; t_α – the confidence factor.

Robustness of the analytical method was tested applying 2^3 full factorial design at two levels.[40–42] The experimental domain of the selected factors is shown in Table 3.

Table 3. Chromatographic conditions and the range investigated during method robustness testing.

Factor (Chromatographic variable)	Low level (–)	Zero level (0)	High level (+)
acetonitrile (%)	40	45	50
pH of the mobile phase	4.0	4.5	5.0
column temperature (°C)	30	35	40

Resolutions of nimesulide and impurity D ($Rs_{1,2}$) and that of impurities D and C ($Rs_{2,3}$), were choosen as the response parameters. The design matrix of 2^3 factorial design shows eight treatment combinations of a low (–) and high (+) level of the factors (Table 4).

Table 4. Model matrix for 2^3 full factorial design with zero level.

No.	Factor level			Resolution	Resolution
	A	B	C	($Rs_{1,2}$)	($Rs_{2,3}$)
1.	–	–	–	2.81	0.00
2.	+	–	–	2.79	1.71
3.	–	+	–	2.84	0.00
4.	+	+	–	2.93	2.57
5.	–	–	+	2.87	0.00
6.	+	–	+	2.95	2.24
7.	–	+	+	2.80	0.00
8.	+	+	+	2.93	2.98
9.	0	0	0	3.04	1.66

The central point in the design, experiment No. 9, was added only as a control to see whether there is a non-linear relationship between the variables and the responses. Traditional tabular presentation of the data for calculating the classic factor effects or coefficients in a 2^3 factorial design for $Rs_{1,2}$ and for $Rs_{2,3}$ are given in Table 5.

The obtained values for the factor effects (Table 5) indicate that the content of acetonitrile in the mobile phase (factor A) and the pH of the mobile phase (factor B) had the greatest impact on the chromatographic behavior of the system. The two-factor and three-factor interactions also expressed a strong influence. However, resolutions

Table 5. Estimates of factor effects (coefficients) for the resolution of nimesulide and impurity D ($Rs_{1,2}$) and impurities D and C ($Rs_{2,3}$).

Coefficients of factor efects	Estimates of factor effects for $Rs_{1,2}$	Estimates of factor effects for $Rs_{2,3}$
b_0	2.865	1.188
b_1	0.070	2.376
b_2	0.019	0.399
$b_1 b_2$	0.039	0.399
b_3	0.004	0.233
$b_1 b_3$	0.034	0.233
$b_2 b_3$	–0.064	0.028
$b_1 b_2 b_3$	–0.014	0.028

b_0 – the intercept of the linear model or the mean value of the considered response; b_1, b_2 and b_3 – the main effects of factors; b_{12}, b_{13} and b_{23} – the two-factor interactions; b_{123} – the three-factor interactions

$Rs_{1,2}$ and $Rs_{2,3}$ were less sensitive to variations at the level of temperature of the column (factor C). Based on the results of these experiments, the following theoretical equation that correlates resolution between nimesulide and impurity D ($Rs_{1,2}$) with the most important chromatographic conditions was derived:

$$Z = 53.884 - 1.934*x - 0.762*y + 0.025*x*x - 0.001*x*y + 0.012*y*y$$

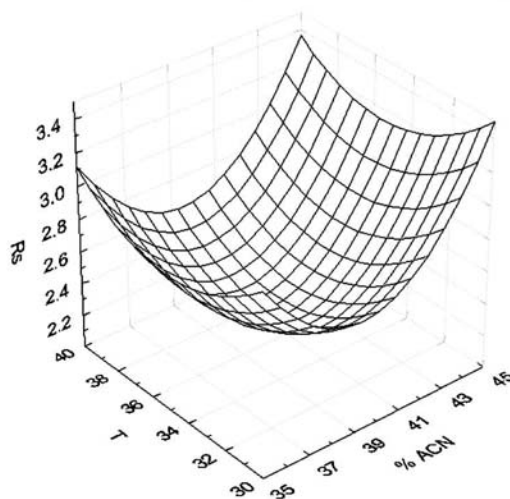

Figure e: 3D plot – The resolution $Rs_{1,2}$ response to acetonitrile content in the mobile phase (%) and temperature of the column (°C) $\alpha = f$(% ACN, t °C).

where x is the content of acetonitrile in the mobile phase (%); y – temperature of the column (°C) and z – the resolution of nimesulide and impurity D ($Rs_{1,2}$). The 3D plot is depicted in Fig. **e**.

The following theoretical equation correlating the resolution of impurities D and C ($Rs_{2,3}$) with the most important chromatographic conditions was derived:

$$Z = 35.323 + 0.337*x + 10.728*y + 0.001*x*x – 0.05*x*y – 0.857*y*y$$

where x represents acetonitrile concentration in the mobile phase (%); y is pH of the mobile phase, and z the resolution of impurities C and D ($Rs_{2,3}$). The 3D plot is depicted in Fig. **f**.

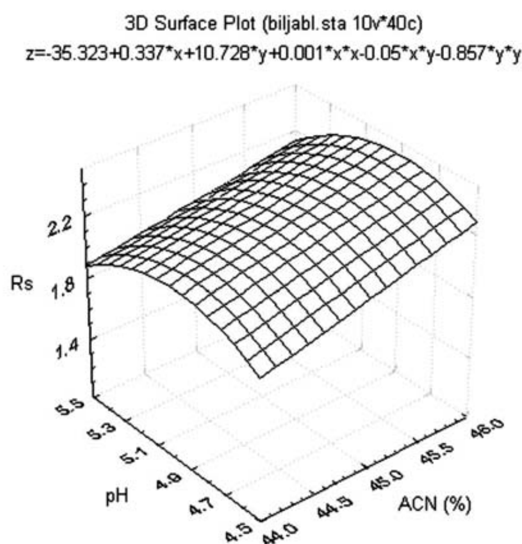

Figure f: 3D plot – The resolution $Rs_{2,3}$ response to acetonitrile concentration in the mobile phase (%), and pH of the mobile phase $\alpha = f$(% ACN, pH).

Separation of the examined impurities was poor, because the difference in their solubility was lost in more polarized mobile phase due to a low acetonitrile content.

Higher pH values of the mobile phase potentiated the expression of (–)-mesomer effect of nitro group in *p*-position of impurity D. Because of this effect, the primary aromatic amino group of impurity D was protonated to a lesser extent in relation to the aromatic amino group of impurity C. Reduced protonation of the primary aromatic amino group in impurity D led to a decrease of ionization level of the molecule and consequently, to decreased solubility of this component in polar mobile phase and its increased retention on the column. However, at higher pH values of the mobile phase, impurity C was ionized to a lesser extent than at lower pH values and as a result of decreased hydrogen ion concentration, polarity of this impurity was also reduced. Under these conditions, a better separation of the impurities was achieved than at lower pH values of the mobile phase.

At lower pH values of the mobile phase, difference in the availability of free electron pair on nitrogen atom disappeared. Thus, the influence of (–)-mesomer effect of nitro group in *p*-position in relation to the base of primary aromatic amino group was reduced. An equal protonation of the primary aromatic amino group in both analyzed impurities led to the assimilation of their polarity and they were eluted together, i.e. appeared as a single peak during spectrophotometric recording.

Temperature increase potentiated the differences in solubility of the examined compounds in polar mobile phase used and this resulted in the differences of their retention on nonpolar stationary phase.

Therefore, fine tuning of pH and acetonitrile concentration in the mobile phase can significantly improve the separation of these three analytes. A satisfactory chromatographic behavior requires resolutions to be $1 < Rs < 10$.[7] Thus, good separation and optimum run time can be assessed only with lower level of factor A and higher levels of other investigated factors. The robustness of the method was evident by the absence of overlapping peaks.

The precision of the chromatographic procedure was investigated for three concentrations from the calibration plots of each nimesulide, impurity C and impurity D. Analyzing seven solutions for the three chosen concentrations from the calibration plots of each substance assessed precision (repproducibility) of the chromatographic procedure. From the results listed in Table 6. it can be seen that relative standard deviation values (RSD < 2% for active ingredients and RSD < 5% for related compounds) and reproducibility were satisfactory.[43]

Table 6. Precision of the assay expressed as RSDs of seven samples in three concentrations.

Compound	Concentration	SD	RSD (%)
Nimesulide	0.08	1.59	0.05
(mg mL^{-1})	0.12	6.06	0.05
	0.16	13.07	0.20
Impurity C	0.05	0.02	0.44
(µg mL^{-1})	0.21	0.12	0.82
	0.33	0.10	0.45
Impurity D	0.05	0.04	1.64
(µg mL^{-1})	0.20	0.12	1.26
	0.33	0.10	0.68

Accuracy for the assay of nimesulide, impurity C and impurity D was also investigated. The closeness of the measured value to the true value for the sample (accuracy) was assessed by analyzing a sample of known concentration and comparing the measured value to the true value.[44] The accuracy results presented in Table 7. show satisfactory values of mean recovery per concentration (98.0–102.0% for active ingredients and 90.0–110.0% for related compounds) and satisfactory accuracy of the system.[43]

Table 7. Accuracy of the assay expressed as recovery values (%).

Compound	Concentration	Found concentration (μg mL^{-1}) ± SD	Recovery (%)
Nimesulide (mg mL^{-1})	0.021	0.021 ± 0.14	102.14
	0.062	0.063 ± 1.17	101.76
	0.112	0.109 ± 0.04	97.98
Impurity C (μgm L^{-1})	0.104	0.103 ± 1.37	99.04
	0.210	0.213 ± 1.08	101.43
	0.330	0.327 ± 0 .67	99.09
Impurity D μgmL^{-1})	0.102	0.101 ± 1.23	99.02
	0.204	0.204 ± 1.03	100.00
	0.326	0.328 ± 0.38	100.61

The developed HPLC method is sensitive and specific for the quantitative determination of nimesulide and its impurities C and D. Also, the method has been applied for the estimation of nimesulide drugs in five different solid pharmaceutical dosage forms. A typical chromatogram including nimesulide for the test assay is presented in Fig. **g**.

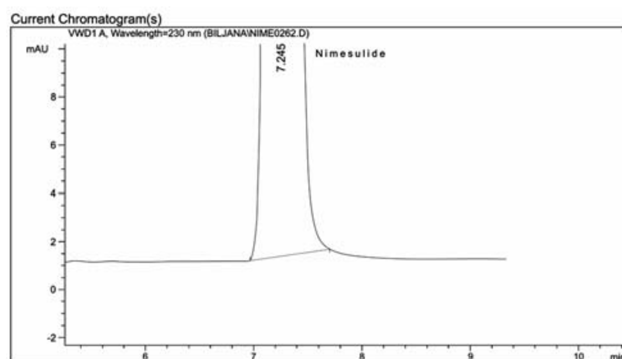

Figure g: Representative chromatogram of the sample solution under the same chromatographic conditions as in Fig. **c**.

Nimesulide tablets containing 100 mg of the active substance produced by five different manufacturers as given under **Material and methods section**, were evaluated for the content of nimesulide and its impurities C (2-phenoxyaniline) and D (2-phenoxy-4-nitroaniline) present in the formulations. Each sample was analyzed in triplicate after the extraction. The amount of nimesulide found by the described procedure is given in Table 8.

Table 8. Content of nimesulide in five different formulations.

Formulation	Nimesulide content found
TENAPROST® batch number 144-10.04-10.07	103.83%
TENAPROST® batch number 588-05.04-05.07	104.45%
VENTOR® batch number 1118	101.67%
VENTOR® batch number 51606	100.57%
VENTOR® batch number 1185	97.06%
NIMULID® batch number 0383053	99.39%
NIMULID® batch number 0384030	98.66%
AULIN® batch number 24003250	101.31%
NIMESULID batch number 0041203	102.87%

The testing tablets had impurities under the limit of detection of this method.

4. Conclusions

A simple, rapid, sensitive and accurate RP-HPLC method for simultaneous determination of nimesulide and its impurities C and D in five different solid pharmaceutical formulations has been developed. The proposed method can be used for monitoring the stability of nimesulide in pharmaceutical dosage forms, as well. The run time of 10 min and flow rate of 1.0 mL min^{-1} allows the analysis of a large number of samples with less mobile phase. The method is efficient and sensitive means to determine nimesulide and its main impurities C and D with no interference of excipients present in the pharmaceuticals demonstrating a high selectivity of this procedure. The method can be successfully used in the quality control, as well as purity and stability testing.

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Povzetek

Opisujemo enostavno in hitro metodo za določitev nimesulida in nečistoč v farmacevtskih pripravkih z dobro ponovljivostjo. Metoda je uporabna za sledenje stabilnosti. Določili smo prisotnost dveh nečistoč: C (2-fenoksianilin) in D (2-fenoksi-4-nitroanilin). Najboljšo ločitev smo dosegli z uporabo kolone Agilent Zorbax Extend C18 (150 × 4,6 mm, velikost delcev 5 μm) pri 40 °C in pretoku 1,0 mL min^{-1}. Uporabili smo UV detekcijo pri 230 nm. Mobilna faza je bila iz acetonitrila – trietilamina (TEA) – vode (45:0,5:54,5 v/v/v), s pH 5,2, kar smo dosegli z dodatkom mravljične kisline. Pod temi pogoji so bili retencijskih časi 7,11, 7,98 in 8,66 min za nimesulid, D in C z dobro ločitvijo. Evaluirali smo linearnost, točnost, pravilnost, selektivnost, občutljivost in robustnost metode in jo uporabili za določitev nimesulida v različnih farmacevtskih pripravkih.