Revue Roumaine de Chimie, **2007**, *52*(5), 499–507

DETERMINATION OF NIMESULIDE AND ITS ACTIVE METABOLITE IN PLASMA SAMPLES BASED ON SOLVENT DEPROTEINIZATION AND HPLC-DAD ANALYSIS

Iulia SORA,[a] Toma GALAON,[a] Victor DAVID[b] and Andrei MEDVEDOVICI[a,b*]

[b] University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, Sos. Panduri, no. 90, Bucharest - 5, 050663, Romania
[a] LaborMed Pharma S.A., Splaiul Independentei 319, Bucharest - 6, 060664, Bucharest, Romania.

Received April 5, 2006

A selective, sensitive and accurate HPLC method for the assay of nimesulide and its major metabolite (4'-hydroxy-nimesulide) in human plasma samples is presented. The sample preparation procedure was based on a simple protein precipitation with acetonitrile. The reversed phase liquid chromatography was used as separation technique followed by UV detection of analytes at 300 nm. Separation was achieved on a Chromolith Performance RP 18e column using a mobile phase consisting of aqueous 0.2% triethylamine at pH 3.0 with H_3PO_4 and methanol (1:1, v/v). A small volume of plasma is required for the sample preparation procedure (200 μL), nitrazepam being used as internal standard. The limits of quantification for nimesulide and 4'-hydroxy-nimesulide are 64 ng/mL and 41 ng/mL, respectively. Relative standard deviations characterizing intra-day and inter-day precision were less than 5%. The validated method was applied during a bioavailability study for a pharmaceutical formulation containing nimesulide (uncoated tablet) available on the Romanian market. Both nimesulide and 4'-hydroxy-nimesulide were determined from the real human plasma samples obtained from 22 volunteers, included in this study, after the administration of a single oral dose.

INTRODUCTION

Nimesulide (4-nitro-2-phenoxy-methanesulfonanilide) is a nonsteroidal drug (NSAID) with analgesic, antipyretic, and anti-inflammatory activity. NSAIDs act by inhibiting the cyclooxygenase enzyme (COX), which is responsible for the formation of prostaglandins, the most important mediators of inflammation. Nimesulide is a selective inhibitor of cyclo-oxygenase-2 (COX-2) and is chemically unrelated to the other acidic NSAIDs such as acetylsalicylic acid and indomethacin. It contains a sulphonanilide moiety which is inducing a weakly acidic character, the compound having a pK_a = 6.5 (see Fig. 1, compound 1). This is a very important advantage of the drug because it exhibits less irritative and toxic effects toward gastrointestinal mucosa and kidney. Due to the fact that increased prostaglandin synthesis was observed in some neuro-inflammatory conditions, such as meningitis, multiple sclerosis and human immunodeficiency virus-associated dementia, nimesulide is seen as a drug with a highly therapeutic potential.[1-3]

The primary biological pathway for metabolizing nimesulide involves an aromatic hydroxylation. This reaction is catalyzed by enzymes from cytochrome P-450 family.[1] The main metabolite of this drug is 4'-hydroxy-nimesulide (see Fig. 1, structure 2). Previous reported studies[4-7] indicated the existence in urine of several other metabolites resulted from reduction pathways of the parent drug and 4'-hydroxy-nimesulide (see Fig. 1, structures 4-7).

4'-Hydroxy-nimesulide can be detected in human plasma samples in the same time interval as required for plasma concentration profiles for nimesulide. Because its biological activity is similar to the parent drug, during bioavailability / bioequivalence studies (BA/BE) its concentration is also determined in plasma samples from volunteers.

* Corresponding author: avmedved@yahoo.com

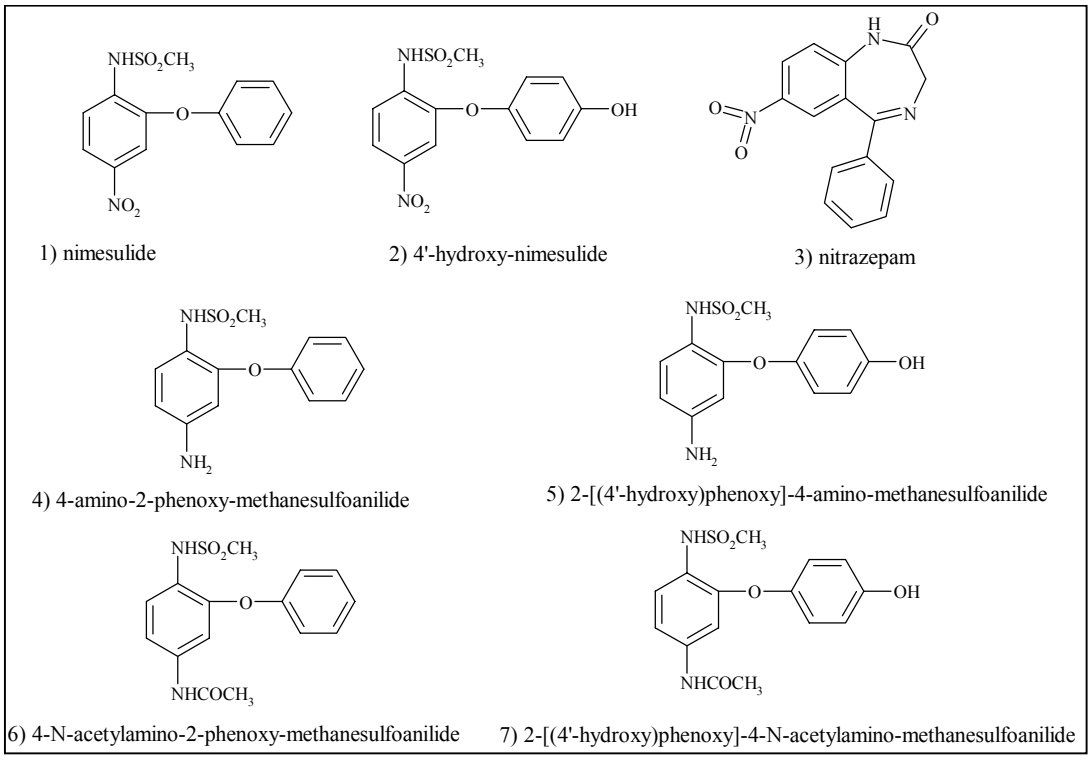

Fig. 1 – Molecular structures of nimesulide, related metabolites and nitrazepam used as Internal Standard.

Several published articles described HPLC methods for the analysis of nimesulide in plasma samples. The sample preparation procedures are based mainly on liquid-liquid extraction using toluene,[6] benzene,[6] dichloromethane[6] or diethyl ether as extraction agents. To increase selectivity of the preparation method, back-extraction was employed when using toluene.[10] The limits of quantification (LOQ) cited in these methods ranged from 100 to 500 ng/mL.

Liquid-liquid extraction is not the first choice in developing a sample preparation method for BA/BE studies, as long as it is a tedious procedure to be repeated for hundreds of real samples. A simpler method[11] based on protein precipitation with methanol and reversed phase chromatography with spectrometric detection at 404 nm was applied only for nimesulide determination in plasma samples, producing a better LOQ of about 80 ng/mL. However, this method can not be applied to the determination of 4'-hydroxy-nimesulide because of its poor selectivity, fact demonstrated by preliminary experiments involving several types of plasma.

The present study proposes an improved validated analytical method for the determination of nimesulide and 4'-hydroxy-nimesulide within small volumes of human plasma samples, based on protein precipitation by means of acetonitrile addition, to be applied to a BA study of a commercially available pharmaceutical formulation taken from the Romanian market.

EXPERIMENTAL

1. Instrumentation

Experiments were performed with an Agilent 1100 series LC/DAD (Agilent Technology, Waldbronn, Germany) system consisting of the following modules: degasser (G1322A), quaternary pump (G1311A), autosampler (G1313A), column thermostat (G1316A), and diode-array detector (G1315B). System control and data acquisition were made by means of the Agilent ChemStation Version A 10.02 software (Agilent Technologies, Waldbronn, Germany). The system was operationally qualified before and after the BA study.

2. Chromatografic method

A single monolithic Chromolith Performance RP-18e column (Merck, Germany), 100 mm length and 4.6 mm internal diameter fitted with Chromolith Guard Cartridge RP-18e (10mm x 4.6 mm) was used during the validation stage and entire BA study. Column was validated before and after study completion, by computing the lowest value corresponding to the height equivalent to the theoretical plate (HETP) in case of the fluoranthene peak (a variation from 7.06 to 7.59 μm was noticed during the whole process, meaning around 600 injected samples). The column was thermostated at 25ºC.

The mobile phase composition consists in aqueous 0.2% triethylamine (TEA) brought to pH 3 with phosphoric acid and methanol (MeOH) in the volumetric ratio of 1:1, pumped at a

flow rate of 1.5 mL/min. Injections were made automatically. The injection volume was 750 µL and the whole analytical run was 7 minutes.

In such experimental LC conditions capacity factors characterizing the target compounds are the following: 1.5 for 4'-hydroxy-nimesulide, 2.1 for the nitrazepam (I.S.) and 3.85 for nimesulide.

Column equilibration of at least 10 minutes is required when starting a new session of experiments.

3. DAD parameters

The analytical wavelength for the detection was set at 300 ± 2 nm, against a reference wavelength of 515 ± 10 nm. The detector slit was 4 nm. The response time was selected in accordance with the flow rate and fixed at 2 s.

4. Materials

Nimesulide and nitrazepam were obtained from European Pharmacopoeia (Council of Europe, Strasbourg, France); 4'-hydroxy-nimesulide was obtained from SynFine Research, Ontario, Canada. The reagents used to obtain the aqueous component of mobile phase, TEA and phosphoric acid, were purchased from Panreac (Spain) and from Riedel de Haën (Germany), respectively. Methanol and acetonitrile (HPLC grade) were manufactured by Merck (Darmstadt, Germany). Water for chromatography (having a maximum conductivity of 0.055 μScm^{-1} and a maximum TOC content of 30 ppb) was produced within the laboratory by means of a TKA Lab HP 6UV/UF instrument and used during experiments.

5. Sample preparation procedure

A 200 µL plasma aliquot was mixed with 400 µL of a solution in acetonitrile containing 1 µg/mL Nitrazepam (I.S., see Fig. 1, compound 3) and vortexed for 5 minutes at 2000 rpm. The sample was then centrifuged at 7500 x *g*. The resulting supernatant was transferred in a glass test tube and diluted with 1100 µL of aqueous 0.2 % TEA brought to pH 3 with phosphoric acid. The mixture was homogenized and injected (750 µL) in the chromatografic column.

RESULTS AND DISCUSSION

1. Choice of internal standard

The choice of the most suitable internal standard is a critical step when developing analytical methods for BA studies. Both similarity in structure and hydrophobic character with the target analytes should be considered. In this particular case the most important aspect is hidrophobicity because the sample preparation procedure involves the dissolution of both target analytes and I.S. in a water rich media. Estimated values for the octanol/water partition constants (taken as ten-base logarithm, log $K_{o,w}$) by fragment methodology of the target compounds are 1.74 for 4'-hydroxynimesulide, 2.22 for nimesulide; and 2.45 for I.S. The experimentally determined log $K_{o,w}$ for nimesulide[12] (2.6) and nitrazepam[13] (2.25) explain better the elution order and also confirms the correctness of the choice for the internal standard. All analytes of interest exhibits strong adsorption bands in the 300-400 nm interval, resulting in a sensitive and relatively selective UV detection.

2. Sample preparation procedure

Preliminary tests were focused on the following directions: *a)* liquid-liquid extraction with diethyl ether, dichlormethane and a mixture of diethyl ether:dichlormethane (1:1), from acidic, neutral and alkaline buffered plasma matrixes; *b)* protein precipitation by addition of water miscible organic solvents (methanol, acetonitrile).

Experiments pointed out that diethyl ether could be a proper solvent for the extraction of the target analytes from plasma. Recoveries from acidic buffered media are high for metabolite (91.3%) and nimesulide (93.2%), while under neutral or alkaline conditions both target compounds show lower extraction yields (below 50%) and the process exhibits poor reproducibility. It is worthwhile to note that in acidic media the I.S. exhibits hydrolytic instability. Real plasma concentration levels of nimesulide and metabolite did not justify the use of liquid-liquid extraction, considered as a labor extensive procedure, more often requiring larger sample volume to be processed.

Protein precipitation with methanol leads to strong interferences from co-isolated endogenous compounds, especially in the case of 4'-hydroxy-nimesulide. After few injections of the processed samples, some adsorption of the interfering compounds occurs in the head of the column, leading to serious shifts of retention and peak symmetry troubles.

Unlike methanol, protein precipitation with acetonitrile produces higher yields and consequently cleaner samples. Typical results of the method applied to plasma samples are illustrated in Fig. 2.

The main disadvantage of protein precipitation procedure using ACN is the consequent sample dilution (the volumetric mixing ratio between plasma and organic solvent is 1:2). In order to avoid the loss in sensitivity, injection of large volumes is required. Analytes focusing phenomena in the sample solvent on injection is usually associated with such procedures. Allowance of higher injection volumes is conditioned by the

nature of the sample solvent composition, behaving as a weaker dissolving agent compared to the mobile phase. In this specific method, the mobile phase composition imposes further dilution of the supernatant (~ 1/4 ratio) with the aqueous component, in order to achieve injection volumes 15 times higher than usual (750 μL). Thus, sample dilution is fully compensated by the increased injected volume, such solution offering a time saving, low cost, simple and direct preparation procedure. I.S. addition is achieved simultaneously with protein precipitation process.

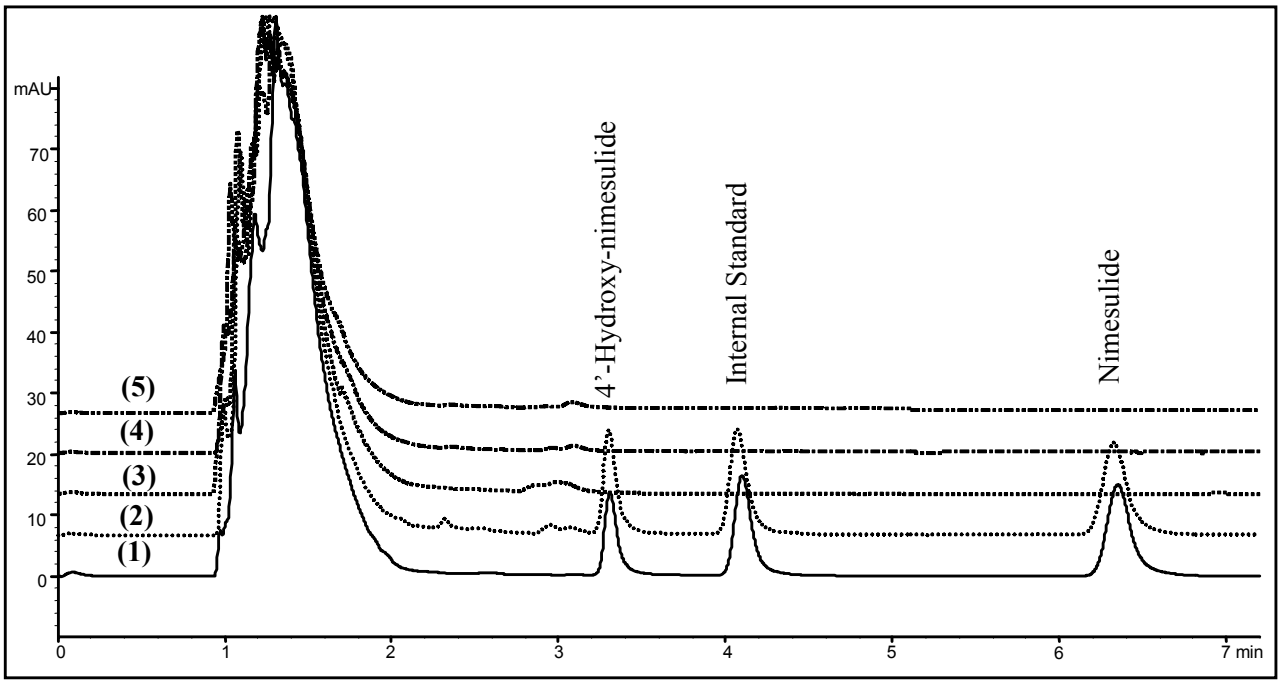

Fig. 2 – Chromatograms obtained after protein precipitation by means of ACN addition to three different blank plasma samples (3)- (5), to a real plasma sample provided from a volunteer (2) and to a spiked plasma sample used in validation (1) having concentration of 2000 ng/mL for 4'-hydroxy-nimesulide, 4000 ng/mL for nimesulide and 2000 ng/mL for I.S.

3. Recovery

The sample preparation procedure and the chromatographic method were applied to spiked plasma samples containing 2000 ng/mL I.S. and the following concentrations of nimesulide: 250; 1000; 4000 ng/mL and of 4'-hydroxy-nimesulide: 125; 500; 2000 ng/mL. The resulting peak areas were compared to those resulting after injection of aqueous solutions having the same concentrations of the target compounds. Six replicates were made at each concentration level. Calculated recoveries are presented below:

Table 1
Recoveries of the target compounds

Analyte	Concentration (ng/mL)	Recovery (%)	RSD%
4'-hydroxy-nimesulide	125	93.68	1.07
	500	99.09	1.88
	2000	88.08	0.81
	Average	**93.62**	**1.25**
I.S.	2000	**89.35**	**1.27**
nimesulide	500	90.61	4.08
	1000	92.22	3.52
	4000	88.16	0.49
	Average	**90.33**	**2.70**

4. Calibration and quantification limit

Calibration was realized over a wide range of nimesulide and 4'-hydroxy-nimesulide concentrations (up to 6000 ng/mL for nimesulide and up to 3000 ng/mL for metabolite) in spiked plasma samples, to cover the normal interval of the plasmatic concentration variability cited in literature. The concentration of the I.S. in all samples was 2000 ng/mL. Calibration procedure was repeated for each analyte in three different days. Linear regression functions (y= A + B∗x; y: analyte/I.S. peak area ratio; x: analyte concentration in plasma samples, in ng/mL) were characterized by the following parameters: for nimesulide the slope (B) was 0.00032 ± 1.52*10^{-6}, the intercept (A) was 0.006 ± 0.004, and the mean correlation coefficient (r_{xy}) was 0.9999; for 4'-hydroxy-nimesulide the slope (b) was 0.00032 ± 1.89*10^{-6}, the intercept (a) was -0.005 ± 0.003 and the mean correlation coefficient (r_{xy}) was 0.9998.

The limit of quantification (LOQ) was computed by means of the following relationship[14]: $LOQ=[2*t(s_A+s_B*C_{av})]/(B+2*t*s_B)$ were s_A, s_B are the standard deviations calculated for A and B, C_{av} the mean concentration value from the set used for the linear regression and t is the Student coefficient for a confidence degree of 90% and n-2 degrees of freedom, n is the number of calibrating solutions. The relationship between LOD and LOQ is generally taken as: LOQ = 3.33xLOD. For nimesulide the LOQ was estimated at about 64.2 ng/mL and the LOD (limit of detection) was estimated at about 19.3 ng/mL in plasma samples. For 4'-hydroxy-nimesulide the LOQ value was around 41 ng/mL, and the LOD value was estimated at about 12.3 ng/mL in plasma samples.

The same parameters can be computed on the basis of the signal/noise ratio[15] and interpolation in the calibration function: $10*s_A = A + B*LOQ$; $3*s_A = A + B*LOD$. Thus, quantification and detection limits are 49.8 ng/mL and 15 ng/mL for nimesulide, and 57.2 ng/mL and 17.2 ng/mL for 4-hydroxy-nimesulide, data being in fair agreement with previously estimated values.

The experimental confirmation of the linearity interval derived from the calibration procedure, by plotting all values of the peak area ratios mediated by concentration against concentration values and representing the experimental variation interval overlaid to the accepted one (corresponding to an RSD% of 15%). Computed individual values should fit within the allowed variation interval. It has been proved that for each concentration level i (i = 1, 2,…,7) and six replicate samples j (j = 1, 2,…,6) for each concentration level i the following ratio $[A^{(i)}_{analyte,j} / A_{I.S.} \times C^{(i)}_{analyte,j}]$ did not varied upon the $C^{(i)}_{analyte,j}$ value (see Fig. 4.). As can be observed, this ratio becomes more variable in the proximity of the quantification limit.

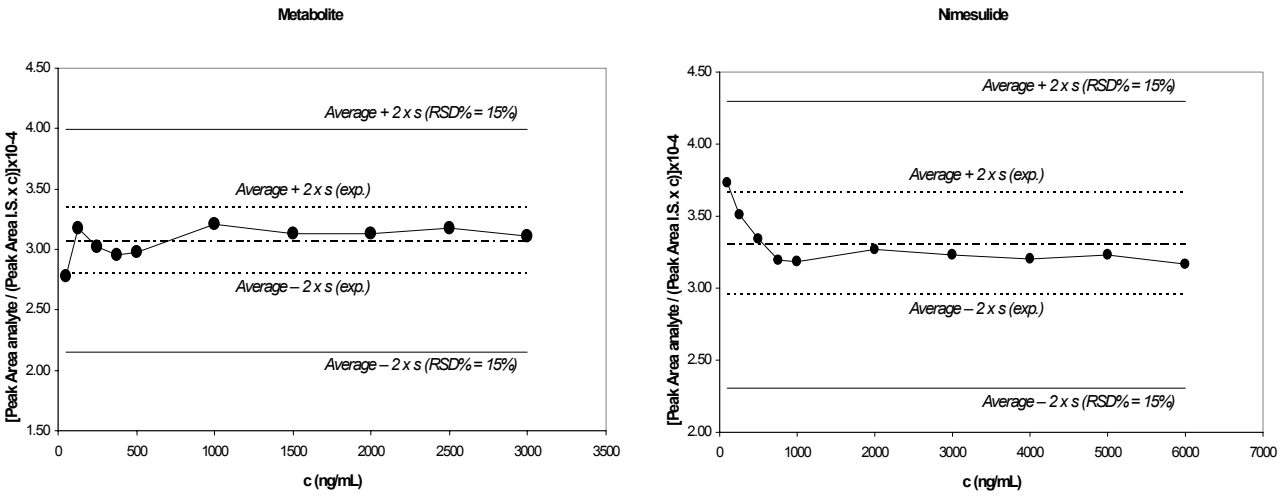

Fig. 3 – Variation interval of the mean ratio [$A_{analyte}/A_{I.S.}$* $C_{analyte}$] against concentration as a tool for evaluation of the linearity domain.

5. Precision

Intra-day and interday precisions were studied at three concentration levels (125; 500; 2000 ng/mL for 4'-hydroxy-nimesulide and 250; 1000; 4000 for nimesulide) and 2000 ng/mL I.S. Table 2 presents the experimental results obtained during the evaluation of precision, considering as

parameters the absolute peak area of nimesulide and 4'-hydroxy-nimesulide, the peak area ratio between these analytes and I.S., and the corresponding calculated concentration (resulting from the regression equation obtained under the linearity study).

Table 2

Intra- and inter-day precision for spiked plasma samples

Spiked concentration (ng/mL)	Parameter	Intra-day (n=10)		Inter-day (n=6)	
		Mean ± 2 S.D.	RSD%	Mean ± 2 S.D.	RSD%
		4'-hydroxy-nimesulide			
125	Peak Area	10.1 ± 0.7	3.6	10.6 ± 1.2	5.5
	Analyte/IS peak area ratio	0.039 ± 0.001	1.3	0.038 ± 0.004	5.2
	Experimental conc.(ng/mL)	136 ± 3.3	1.2	135 ± 12. 5	4.6
500	Peak Area	40.3 ± 4.6	5.7	38.6 ± 1.7	2.2
	Analyte/IS peak area ratio	0.163 ± 0.006	1.8	0.148 ± 0.005	1.5
	Experimental conc.(ng/mL)	526 ± 19	1.8	481 ± 14	1.5
2000	Peak Area	157.7 ± 14. 6	4.6	163.8 ± 11.3	3.5
	Analyte/IS peak area ratio	0.635 ± 0.010	0.8	0.627 ± 0.0147	1.2
	Experimental conc.(ng/mL)	2008 ± 30	0.8	1984 ± 46	1.2
		Nimesulide			
250	Peak Area	21.7 ± 2.0	4.6	24.4 ± 2.6	5.4
	Analyte/IS peak area ratio	0.083 ± 0.004	2.7	0.089 ± 0.007	4.1
	Experimental conc.(ng/mL)	241 ± 1.4	2.9	259 ± 23	4.4
1000	Peak Area	77.2 ± 8	5.2	82.4 ± 5.8	3.5
	Analyte/IS peak area ratio	0.313 ± 0.005	0.8	0.317 ± 0.009	1.4
	Experimental conc.(ng/mL)	961 ± 16	0.9	975 ± 27	1.4
4000	Peak Area	316.8 ± 29.9	4.7	335.9 ± 20.1	3.0
	Analyte/IS peak area ratio	1.275 ± 0.009	0.3	1.285 ± 0.019	0.8
	Experimental conc.(ng/mL)	3981 ± 27	0.3	4014 ± 60	0.8
		Internal Standard			
2000	Peak Area	252.6 ± 25.6	5.1	265.5 ± 40.3	7.6

6. Accuracy

The accuracy of the method can be evaluated from the calibration samples analyzed over the whole bioavailability study (11 sets). During this study a calibration was performed once at each two volunteers. Bulk plasma samples were spiked at 25, 50, 75, 250, 500, 1000, 1500, 2500 ng/mL with 4'-hydroxy-nimesulide, at 50, 100, 150, 500, 1000, 2000, 3000, 5000 ng/mL with nimesulide and at 2000 ng/mL with I.S. Eleven aliquots at each concentration level from the bulk spiked plasma samples were placed in separate vials and frozen at -40°C. One set of calibration plasma samples was thaw at the same time as the samples obtained from two volunteers, prepared in the same manner and analyzed within the same chromatographic sequence. The normal variation interval of the slopes resulting by computation of the linear regressions was $[2.99 \pm 0.15] \times 10^{-4}$ with a RSD of 2.46% for 4'-hydroxy-nimesulide and $[3.06 \pm 0.12] \times 10^{-4}$ with a RSD of 1.89% for nimesulide.

7. Stability study

Stability studies for 4'-hydroxy-nimesulide and nimesulide were made on spiked plasma samples having the following concentrations: 125, 500 and 2000 ng/mL for 4'-hydroxy-nimesulide and 250, 1000 and 4000 ng/mL for nimesulide.

The stability of the stock solution of I.S. was evaluated on a 1000 µg/mL I.S. stock solution in acetonitrile stored at room temperature for 8 days, at 48 h sampling interval. Before each analysis, the

I.S. stock solution was spiked to a blank plasma sample; samples were processed according to sample preparation procedure and injected in the chromatographic column.

Freeze and thaw stability was studied for five consecutive cycles, from -40°C to ambient (thaw process was unassisted).

Long-term stability was studied over 10 days period, at 24 h interval and a temperature of -40°C.

Short-term stability was made over a 24 h interval. Frozen spiked plasma samples were thawed unassisted at room temperature and analyzed after 2, 6, 8, 12 and 24 hours.

Post-preparative stability was evaluated by analyzing processed spiked plasma samples immediately after preparation and at 1, 3, 6, 12 and 24 h after preparation, on storage bench top, at room temperature.

Results obtained during stability evaluation study are given in Table 3.

Table 3

Results obtained during the stability study

Procedure	Concentration of 4'-hydroxy-nimesulide					
	125 ng/mL		500 ng/mL		2000 ng/mL	
	Mean	RSD%	Mean	RSD%	Mean	RSD%
Freeze/thaw stability(n=5)	123.5	3.04	496.4	0.89	1945.7	2.20
Long-term stability(n=10)	129.8	8.53	530.9	4.79	2117.9	4.95
Short-term stability(n=6)	123.9	3.30	493.0	2.39	1946.1	1.14
Post-preparative stability(n=6)	125.0	3.05	501.3	1.28	1936.2	1.82
	Concentration of nimesulide					
	250 ng/mL		1000 ng/mL		4000 ng/mL	
	Mean	RSD%	Mean	RSD%	Mean	RSD%
Freeze/thaw stability(n=5)	232.6	2.93	974.5	1.83	3937.4	0.77
Long-term stability(n=10)	242.6	4.63	1050.1	2.57	4196.4	3.45
Short-term stability(n=6)	238.1	2.95	968.0	1.88	3974.0	1.54
Post-preparative stability(n=6)	243.5	2.23	968.6	2.37	3952.7	0.76
IS stock solution stability (n=6)	Peak area			Mean	RSD%	
				253.93	7.52	

8. Robustness

The study of the robustness should evaluate the relation existing between the operational parameters of the method and the major chromatographic characteristics directly influencing the final quantitative results. A chromatographic separation characterized by a reduced variability of retention, high efficiencies and peak symmetries together with adequate resolution allows automated peak area integration, resulting in reproducible quantitative results. As the resolution provided by the chromatographic method for the two pairs of the target compounds is not critical ($R_{s\ IS/4'\text{-hydroxy-nimesulide}} = 4$ and $R_{s\ nimesulide/IS} = 9$), small variations of the operational parameters should not strongly affect the final results.

The influence of the mobile phase composition on the final results should be considered for the interval commonly accepted as the mixing accuracy of the quaternary pump. The operational qualification of the module supposes that a bias of ± 1.5% from the set value is considered as an adequate maximum acceptable limit. Consequently, a variation of ± 2% (48%-52%) methanol content in the mobile phase has been investigated. Efficiency increases with about 20% on the increase of methanol content in the mobile phase, in the studied interval. Peak symmetry remains unaffected. Retention times measured for the peaks corresponding to 4'-hydroxy-nimesulide and nimesulide show a reversed linear dependence on the increase of methanol content in the mobile phase, according to the following relationships:

t_R = 4.5535 – 0.0507 *C_{MeOH} (r_{xy}=0.998) and respectively t_R = 32.4355 – 0.5217 *C_{MeOH} (r_{xy}=0.995).

A variation of ± 0.05% of triethylamine added to the aqueous component of the mobile phase does not affect retention, efficiency and symmetry of the chromatographic peaks.

The variation of pH value in the aqueous component of the mobile phase was studied within 2.5 to 3.5 interval. An increase of about 15% in peak efficiency was observed at lower pH values. No significant changes in retention and peak symmetry were noticed.

Although the precision of a Peltier element is lower than ± 1ºC, the investigated column temperature interval was extended at 20 ÷ 30 ^oC. Retention times for both 4'-hydroxy-nimesulide and nimesulide decrease linearly on the temperature increase, according to the following relationships: t_R = -0.2535*T + 3.793 (r_{xy}=0.997) and t_R = -0.6335*T + 7.5483 (r_{xy}=0.995). Temperature variations within 25 ± 1.5ºC produce modification of the retention of the target compounds within their normal variation interval.

One can conclude that the chromatographic method is robust with respect to its main operational parameters; an automated integration method is thus applicable.

9. BA study results

The validated method was applied for a BA study of a nimesulide containing tablet available on the Romanian market. Both nimesulide and 4'-hydroxy-nimesulide were determined in real human plasma samples obtained from 22 volunteers, after administration of a single oral dose of 100 ng/mL nimesulide, on fasting conditions. Blood samples were collected at the following time intervals: before dosing (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24 hours after tablet intake. The average plasma concentration profiles of nimesulide and 4'-hydroxynimesulide are given in Fig. 4.

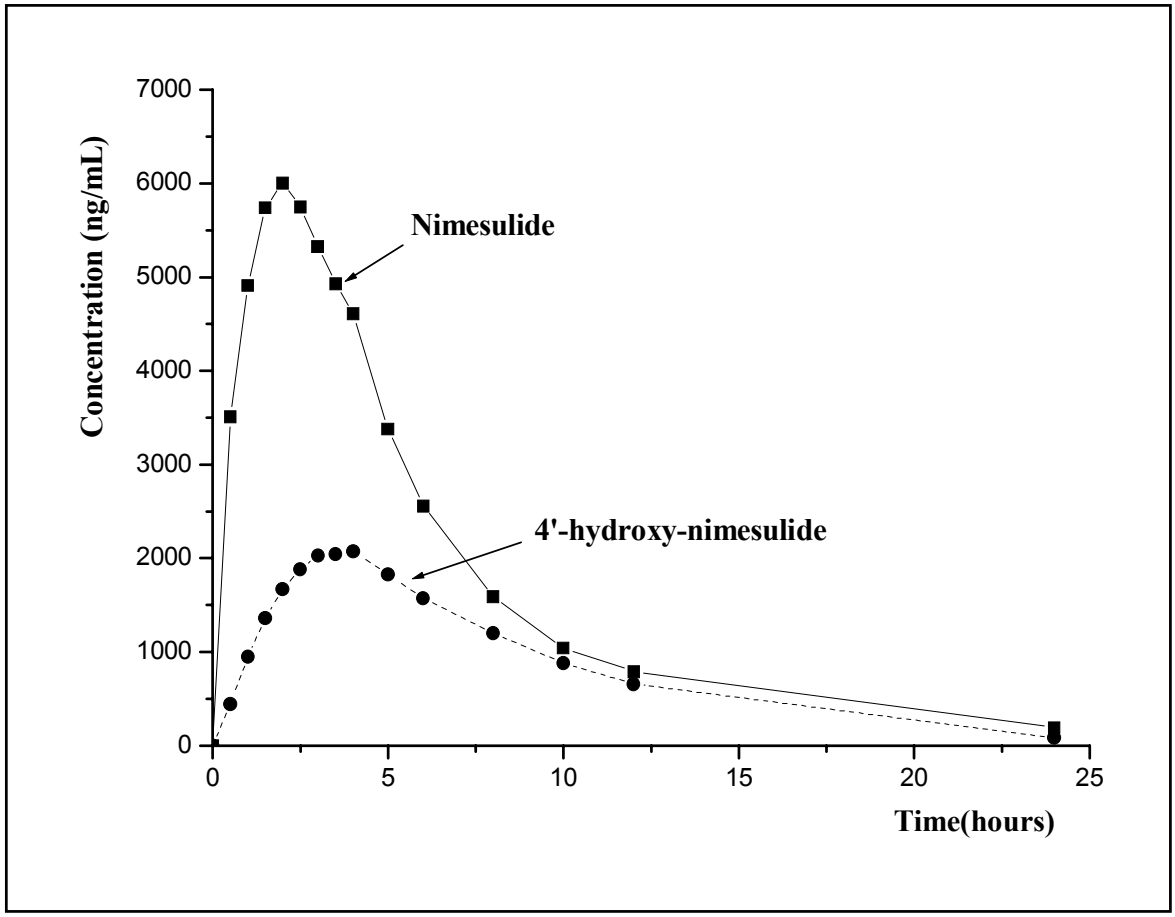

Fig. 4 – Average plasma concentration profiles of nimesulide and 4'-hydroxy nimesulide obtained during the BA study.

CONCLUSIONS

A new analytical method for the quantification of nimesulide and its major metabolite (4'-hydroxy-nimesulide) in human plasma samples is presented. The sample preparation procedure is simple, involving protein precipitation with acetonitrile. Separation of nimesulide and its main metabolite is achieved by a reversed phase mechanism followed by UV-VIS detection. The limits of quantification for nimesulide and is 4'-hydroxy-nimesulide are 64 ng/mL and 41 ng/mL, respectively. The method is also suitable for the bioequivalence studies.

REFERENCES

1. N. Seedher and S. Bhatia, *J. Pharm. Biomed. Anal*., **2005**, *39*, 257.
2. R.R. Nageswara, S. Meena and R.A. Raghuram, *J. Pharm. Biomed. Anal*., **2005**, *39*, 349.
3. B. Buecher, A. Broquet, D. Bouancheau, M.F. Heymann, A. Jany, M.G. Denis, C. Bonnet, J.P. Galmiche and H. M. Blottiere, *Dig. Liver Dis*., **2003**, *35*, 557.
4. M. Carini, G. Aldini, R. Stefani, C. Marinello and R.M. Facino, *J. Pharm. Biomed. Anal*., **1998,** *18*, 201.
5. R. Davis and R.N. Brogden, *Drugs*, **1994,** *48,* 431.
6. D. Castoldi, V. Monzani and O. Tofanetti, *J. Cromatogr*., **1988,** *425*, 413.
7. R. Maffei Facino, M. Carini, R. Stefani, C. Marinello and A. Macciocchi, *Pol. J. Pharmacol.,* **1994**, *46*, 357.
8. M. O. Pulkkinen, M. Vuento, A. Macciocchi and T. Monti, *Biopharm. Drug Dispos*., **1991,** *12,* 113.
9. G. Khaksa and N. Udupa, *J. Cromatogr. B.*, **1999,** *727*, 241.
10. D.J. Jaworowicz Jr., M.T. Filipowski and K.M.K. Boje, *J. Cromatogr. B. Biomed. Sci. Appl.*, **1999,** *723,* 293.
11. P. Ptacek, J. Macek and J. Klima, *J. Cromatogr. B.*, **2001,** *758*, 183.
12. J. Sangster, "Octanol-water Partition Coefficients: Fundamentals and Physical Chemistry", J. Wiley & Sons, Chichester, Vol.2, 1997.
13. C. Hansch, J.P. Bjorkroth and A.J. Lee, *J. Pharm. Sci.*,**1987**, 76, 663
14. J.N. Miller, *Analyst*, **1991**, *116*, 3.
15. C. Liteanu and I. Rica, "Statistical theory and methodology of trace analysis, Chapter 6 – Detection theory of analytical signals", Ellis Horwood, Chichester, 1980, p. 165.