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The role of the acquisition methods in the analysis of the non-steroidal anti-inflammatory drugs in Danube River by gas chromatography - mass spectrometry

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ABSTRACT

In this paper authors describe a GC-MS acquisition study, relating to the most common, non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, naproxen, ketoprofen and diclofenac. As novelties to the field, for the trimethylsilyl (TMS) oxime ester derivatives of NSAIDs, at first, a tandem mass spectrometric (MS/MS) acquisition method has been developed, and, also for the first time, the three acquisition techniques, the full scan (FS), the selective ion monitoring (SIM) and the currently optimized MS/MS ones, have been compared: all three in parallel, under strictly the same derivatization/instrumental conditions, both from model solutions and from the Danube River samples. Critical evaluation of the three acquisition protocols was collated on their analytical performances and validated with the same characteristics like the six point calibration curve, the relative standard deviation percentages (RSD%) of parallel tests, the limit of quantitation (LOQ) and the instrumental limit of quantitation (ILQ) values. Data of six point calibration ($r^2 \geq 0.997$) and RSD% (average: 5.8 RSD%) values proved to be independent on the acquisition methods, while, LOQ and ILQ values furnished considerable differences. Decreasing LOQ data, (expressed in ng/L concentrations) were obtained in the FS, SIM, MS/MS line for ibuprofen (1.0, 0.43, 0.41), naproxen (1.1, 1.0, 0.42), ketoprofen (2.6, 1.0, 0.49) and diclofenac (1.4, 0.41, 0.21), respectively. The same trend was determined in terms of the ILQ values. The practical utility of the optimized MS/MS technique was confirmed by the quantitation of the NSAID contents of the Danube River samples, determined by all three acquisition techniques. Results obtained confirmed the primary importance of the MS/MS acquisition method, even in comparison to the SIM one: avoiding the extreme overestimation of the ibuprofen (≈100%) and ketoprofen (≈400%) concentrations in the Danube River samples.

1. Introduction

During the last few decades most EU and US national water pollution control programs have been devoted to the conventional Priority Pollutants [1,2] related to the identification and quantification of various pharmaceutical and personal care products (PPCPs): including the non-steroidal anti-inflammatory drugs (NSAIDs), like ibuprofen, naproxen, ketoprofen and diclofenac. In Hungary, more than 10 million boxes of these pharmaceuticals were sold in 2006 and their production is continuously being increased [3].

Recently, in Hungary at first, we performed a detailed study on the optimum preparation protocol of these four NSAIDs prior to their FS gas chromatographic mass spectrometric analysis [4]. This optimization was related to the solid phase extraction (SPE) enrichment, to the trimethylsilyl (TMS) oxime ester derivatization and to the ionization (internal/external) processes. Optimized preparation protocol was utilized in the analysis of the NSAID contents of the effluent- and influent wastewater and Danube River samples.

Based on these experiences and on the well known fact that NSAIDs can be used as chemical markers of water contamination, we realized the need to improve the selectivity and the sensitivity in their identification and quantification possibilities, as their TMS (oxime) ester derivatives.

In the frame of an exhaustive literature evaluation [4–60] it turned out that as long as the SPE - and the derivatization conditions of the NSAIDs have been studied in detail, much less attention was paid to the comparison and criticism of their mass spectrometric acquisition methods. Table 1 represents the comparison of selected scientific papers, published in the last decade. Proposals were listed according to their preparation (analyzed as methyl [5–15,29–35,57–59], silyl [4,16–21,36–51], butyl [22],

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Table 1
Literature proposals for the analysis of the NSAID contents of environmental water and soil samples: listed according to their derivatization and GC-MS acqusition protocols.

Acquisition method	Number of analysis: [references]				Number of acquisition method
	Forms in the analysis (FA)			Underivatized	
	Methylated	Silylated	PFBBr*		
FS	**11:** [**5**–15]	**7:** [4,16–21]	**4:** [22]** [**23**–25]	**3:** [**26**–28]	25
SIM	**7:** [**29**–35]	**17:** [4,36–51]	**5:** [**23**,52–55]	**2:** [**26**,56]	31
MS/MS	**5:** [**5**,**29**,57–59]	-	-	**1:** [60]	6
FA in total	21	23	8	5	

Indications: PFBBr* = derivatized with pentafluorobenzyl bromide; ** = esterified with tetrabutylammonium hydrogen sulfate; - = no data available; underlined and bold printed references contain comparison of two acquisition methods

pentafluorobenzyl esters [23–25,52–55] or without derivatization [26–28,56,60]), and to their acquisition protocols, like full scan (FS: [4–28]) selected ion monitoring (SIM: [4,23,26,29–56]) and tandem mass spectrometry (MS/MS: [5,29,57–60]) techniques.

Compilation data revealed (Table 1) that

(1) the most common derivatization method proved to be silylation [4,16–21,36–51],

(2) the most frequently used acquisition protocol was SIM, while,

(3) MS/MS proved to be the less preferred acquisition technique: applied for the analysis of methyl [5,29,57–59] and for the underivatized [60] NSAIDs. Both single reaction monitoring (SRM) [5,29,57–60] and/or multiple reaction monitoring (MRM) [5,60] modes have been used.

It is worth to mention that the MS/MS acquisition technique, concerning the TMS (oxime) ester derivatives, has not yet been described, and the comparison of different acquisition methods are limited and performed with two protocols, only (underlined and bold printed papers in Table 1 [5,23,26,29]).

Evaluating the acquisition method comparisons from analytical point of view it turns out that

(1) all three techniques (FS, SIM, MS/MS), for the time being, were not compared under the same experimental conditions (by means of the same instrument, in the same time).
(2) The preference of the MS/MS acquisition was shown in a qualitative manner only, by the overlaid MS/MS and FS chromatograms [5] of naproxen, ketoprofen and diclofenac determined as methyl esters. In this proposal the advantage of the MS/MS acquisition was characterized with the instrument detection limit (IDL) data, given in pg values (ibuprofen 8 pg, naproxen 5 pg, ketoprofen 10 pg, diclofenac 10 pg).
(3) The MS/MS and the SIM acquisition techniques were compared in terms of their efficiency in the selection of the degradation products of ibuprofen under the wastewater treatment process, depending on their chiral properties [29].
(4) The comparison of the FS and SIM acquisition techniques [23,26] were performed for ibuprofen and diclofenac as their pentafluorobenzyl (PFB) esters [23] and for ibuprofen in its initial form [26], analyzing sewage water samples in both cases [23,26]. The advantage of the SIM protocol over the FS one was characterized with numerical data, however hardly comparable. Since,

(a) SIM quantitation of ibuprofen was compared to its FS one on the basis of the method detection limit (MDL) values, which proved to be for the SIM process less by a factor of 4 in comparison to the FS one ($MDL_{FS}/MDL_{SIM} = 4$) [26], while
(b) the comparison of the ibuprofen and diclofenac analyses, based on the limit of quantitation (LOQ) values of their PFB-derivatives, turned out considerable greater, furnishing an average factor of 54-60 ($LOQ_{FS}/LOQ_{SIM} = 54\text{-}60$) [23].

This work was undertaken in order

(1) to improve the selectivity of the quantification of ibuprofen, naproxen, ketoprofen and diclofenac as their TMS (oxime) ester derivatives by optimizing an MS/MS protocol,
(2) to perform a step by step tandem MS acquisition method, defining the most important parameters, like the selection of the ideal parent ion, the most advantageous amplitudes of the collision induced dissociation (CID), leaded for the greatest yield of daughter ions.
(3) In the frame of these studies the FS, the SIM and the MS/MS mass spectrometric acquisition methods have been critically compared.
(4) The practical utility of the developed GC-MS/MS method was planned to confirm the reliability and reproducibility of the quantification of the NSAIDs as their trimethylsilyl (oxime) ester derivatives of the Danube River and drinking water samples.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical reagent grade. Pyridine, hydroxylamine·HCl were purchased from Reanal (Budapest, Hungary). Hexane, methanol, ethyl acetate, hexamethyldisilazane (HMDS), trifluoroacetic acid (TFA) and model compounds such as, ibuprofen, naproxen, ketoprofen and diclofenac sodium salt were all from Sigma (St. Louis, MO, USA). Glass microfiber filters (GF/A 125 mm diameter, Cat No. 1820–125) were from Whatman (Maidstone, UK). Cartridges for solid-phase extraction (Oasis, HLB 200 mg) were from Waters (Milford, MA, USA).

2.2. Solid-phase extraction

SPE extractions were performed on the Visiprep DL Vacuum Manifold for 12 samples (Cat No. 57044) from Supelco (Bellefonte, PA, USA). Cartridges, prior to extractions were treated with 5 mL hexane, 5 mL ethyl acetate, 10 mL methanol and 10 mL distilled water. Before the SPE enrichment, Danube River and drinking-water samples were filtered on glass microfiber paper. Water samples (3.0 L) were adjusted to pH 4 and extractions were followed without or by adding different amounts of standard solutions, with a flow rate of 4–5 mL/min. Cartridges have been dried by vacuum and elutions were performed, in order of listing with 5 mL hexane, 5 mL ethyl acetate and 10 mL methanol. The unified eluents were reduced in volume, evaporated to dryness by means of a rotary evaporator {(Büchi Rotavapor R-200 and Büchi Vacuum pump V-700, both from Büchi

(Flawil, Switzerland)} at 30–40 ^{0}C (further on: extract). Blank tests (reagent blanks and SPE blanks) were carried out with each series.

2.3. Preparation of the TMS and TMS (oxime) ester derivatives

Model compounds (20–25 mg/100 mL, weighed with analytical precision were dissolved in water or in water/ethanol = 1/1(v/v) solution and further diluted for 10 ×, 100 ×, 1000 ×. Model solutions and the extracts were rotary evaporated to dryness at 30–40 ^{0}C. The residues were treated with 125 μL hydroxylamine·HCl containing pyridine (2.5 g hydroxylamine·HCl/100 mL) heated in oven at 70 ^{0}C for 30 min. Thereafter silylation was continued with 225 μL HMDS + 25 μL TFA and heated at 70 ^{0}C for 90 min. Samples were taken for the analysis, e.g., after dilutions with HMDS, 1 μL of the diluted solutions was injected into the GC-MS system.

2.4. Instrumentation

The apparatus consisted of a Varian CP-3800 GC connected with a Saturn 4000 MS ion trap mass spectrometer (Varian, Walnut Creek, CA, USA), equipped with a Varian CP-8400 autosampler and a Varian 1079 Programmable Temperature Vaporizing (PTV) Injector. The system worked in internal ionization mode. The column used was a product of SGE (Ringwood, Australia) SGE forte capillary: 30m × 0.25 mm; d_f = 0.25 μm. The helium carrier gas flow was set to 1 ml/min and was constant during the temperature gradient program. The temperature of the transfer line, ion trap and manifold were, in order of listing 280 ^{0}C, 210 ^{0}C and 80 ^{0}C, respectively. The injector was operated in on-column mode (as a software option). Injections were made at 100 ^{0}C and held at 100 ^{0}C for 1.0 min, then heated to 270 ^{0}C (200 ^{0}C/min), with a 3 min hold at 270 ^{0}C. The column temperature program started at 100 ^{0}C, for 1 min and then heated up to 300 ^{0}C (20 ^{0}C/min), with a 5.5 min hold at 300 ^{0}C (Total elution time 16.5 min).

2.5. MS/MS parameters

The MS/MS method for the four NSAIDs was optimized in the resonant excitation mode using the Automated Method Development (AMD) software, applying the multi-segment acquisition version, used with one segment per compound.

The general MS/MS parameters were:

Fil/Mul delay: 6.50 min; mass defect: 0 mmu/100 μ; filament current: 40 μA; Target TIC: 5000 counts; Prescan Ion Time: 1500 μs; Scan mode: Fast; Scan Time: 0.17 s/scan; Multiplier offset: Autotune + 300 V; electron energy: 70 eV.

Ion preparation method (IPM) parameters in each segment were:

isolation window: 3.0 m/z; ionization storage level: 35 m/z; high mass ejection: 35 V; excitation time: 20 ms; Modulate RF: yes; Frequency Number: 1, CID frequency offset: 0.0 kHz.

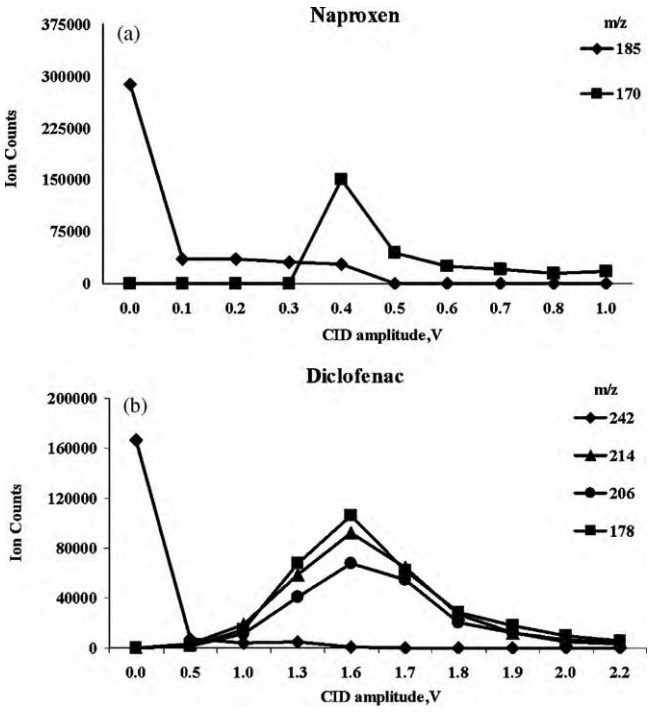

Fig. 1. Ion intensities as a function of CID voltage for parent and daughter ions: a, naproxen and b, diclofenac.

3. Results and discussion

3.1. Mass fragmentation optimization for the tandem mass-spectrometric acquisition method

MS/MS parameters were optimized, one by one, for ibuprofen, naproxen, ketoprofen and diclofenac, exhausting all the possibilities that offer our ion trap system.

Using the AMD option of the software the CID voltage was varied, scan by scan, during a single analysis. Fig. 1a and 1b show CID amplitude optimization for the TMS ester derivative of naproxen and diclofenac, providing maximum ion intensities at different CID values (0.4 V for naproxen and 1.6 V for diclofenac). CID curve profiles obtained for ibuprofen and ketoprofen derivatives furnished the optimized characteristic maximum at 1.05 V and 1.20 V, respectively (Table 2.). The greatest energy was required for the dissociation of the one chlorine atom containing parent ion of diclofenac (m/z 242, 1.6 V).

Additional parameters, like isolation window and target TIC were varied: no, or negligible impact was obtained.

One of the most important parameter was the selection of the parent ions suitable for the excitation. The chosen parent ion should have a great m/z value and an intensive ion current property to provide sufficient and reproducible fragmentation with optimum selectivity and sensitivity for identification and quantitation

Table 2
Optimized MS/MS parameters for the identification and quantification of the NSAIDs, as their TMS (oxime) ester derivatives.

Compound	t_R, min	Parent ion (PI), m/z	Excitation storage level, m/z	CID, V	Daughter ions*, m/z (relative abundance, %)
Ibuprofen	6.51	161 [M-TMSCOO]$^+$	70.9	1.05	**145** (100) [PI-CH_4]$^+$; 131 (17) [PI-C_2H_6]$^+$
Naproxen	9.09	185 [M-TMSCOO]$^+$	81.5	0.40	**170** (100) [PI-CH_3]$^+$; 153 (26) [PI-CH_3-OH]$^+$
Ketoprofen-1	9.42	324 [M-TMSO]$^+$	142.7	1.20	**250** (100); **206** (50) [PI-TMSCOOH]$^+$; **207** (50) [PI-TMSCOO]$^+$; **308** (47) [PI-CH_4]$^+$
Ketoprofen-2	9.45	324 [M-TMSO]$^+$	142.7	1.20	**207** (100); **250** (69); **206** (50); **308** (45)
Diclofenac	10.09	242 [M-TMSOH-Cl]$^+$	106.6	1.60	**178** (100) [PI-CO-HCl]$^+$; **214** (81) [PI-CO]$^+$; **206** (65) [PI-HCl]$^+$

Indication: CID = collision induced dissociation; *Bold ions were used for quantitation

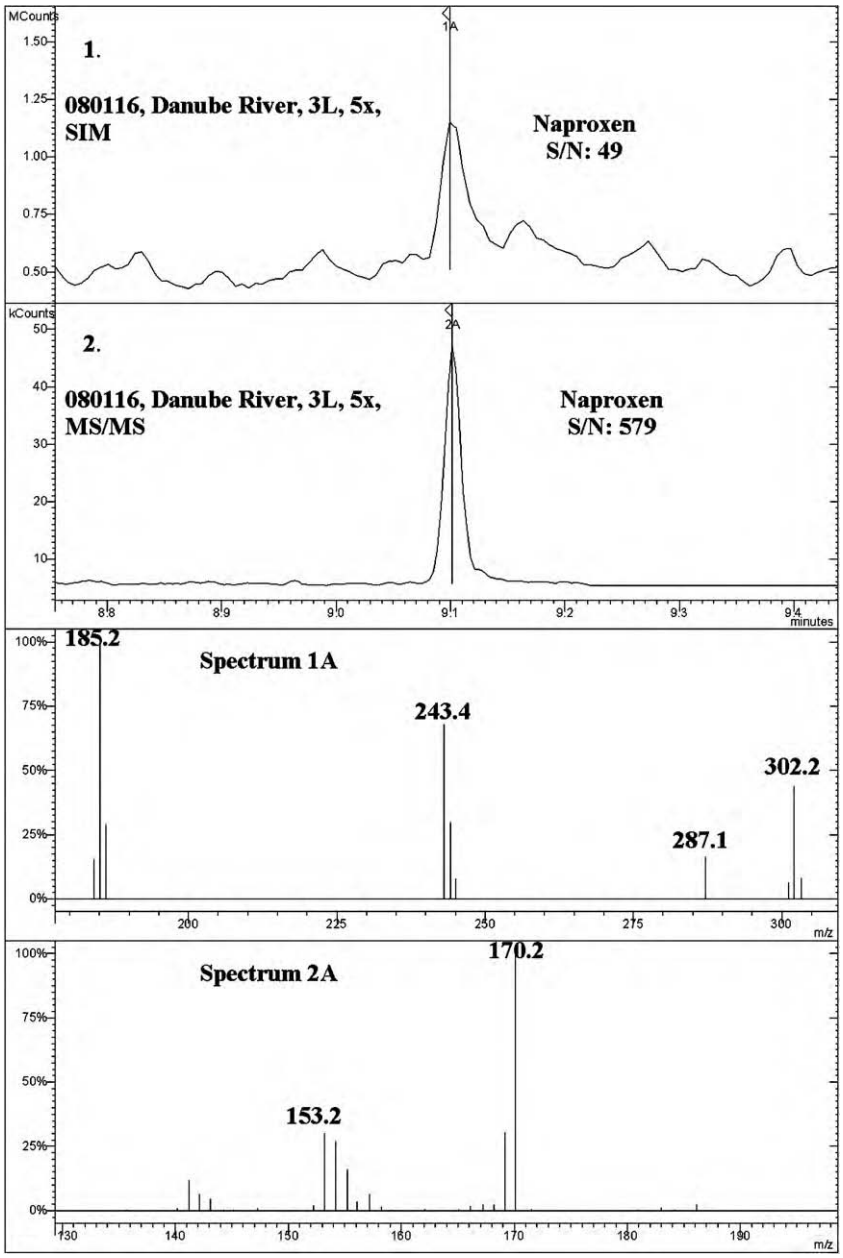

Fig. 2. Elution profile and mass spectra of the trimethylsilyl derivatives of naproxen obtained from the same Danube River (3 L samples, January 16, 2008) in parallel, one by one, with the GC-MS SIM (trace 1; spectrum 1A, 107 pg) and with the GC-MS/MS (trace 2; spectrum 2A, 99 pg) acquisition modes; S/N values were the averages of six injections (detailed data in Table 4).

purposes. Optimized MS/MS acquisition method parameters are shown in Table 2.

Both for ibuprofen and naproxen the molecular ions $[M]^{+\cdot}$ and the $[M\text{-}15]^{+}$ ions, because of their low ion current intensities [4], were unsuitable parent ions. However, their fragment ions, formed by the loss of one TMSCOO group $[M\text{-TMSCOO}]^{+}$, resulted in excellent parent ions both for ibuprofen (m/z 161) and for naproxen (m/z 185) (Table 2). Daughter ions were formed via the same way, by the loss of one methane molecule for ibuprofen (m/z 161-16 = 145), or one methyl radical for naproxen (m/z 185 -15 = 170) (Fig. 1 and Fig. 2). Both daughter ions are the most abundant ones (Table 2 and Fig. 2) and their further dissociation is negligible.

Ketoprofen, being the E and Z isomers of the TMS (oxime) esters, are eluting in two derivatives (Table 2, Ketoprofen-1, Ketoprofen-2). The MS spectra of the two oximes are identical; the same MS/MS optimization protocol was applied for both of them. The selected parent ion at m/z 324 ($[M\text{-TMSO}]^{+}$) was the second most intensive fragment ion with the greatest mass (Fig. 3, spectra 1A, 1B, 2A, 2B). Identical daughter ions were obtained after CID for the two isomers, however their relative abundances were different (Table 2, Fig. 3, spectra 3A, 3B, 4A, 4B). In order to get better sensitivity all four daughter ions (m/z 206, 207, 250 and 308) were selected for quantitation.

The fragmentation of the selected parent ion for diclofenac ($[M\text{-TMSOH-Cl}]^{+}$; m/z 242) provided three daughter ions (m/z 178, 214 and 206). All three daughter ions are formed by the losses of neutral molecules (Table 2: CO or/and HCl), and all three served for the basis of diclofenac's quantitation.

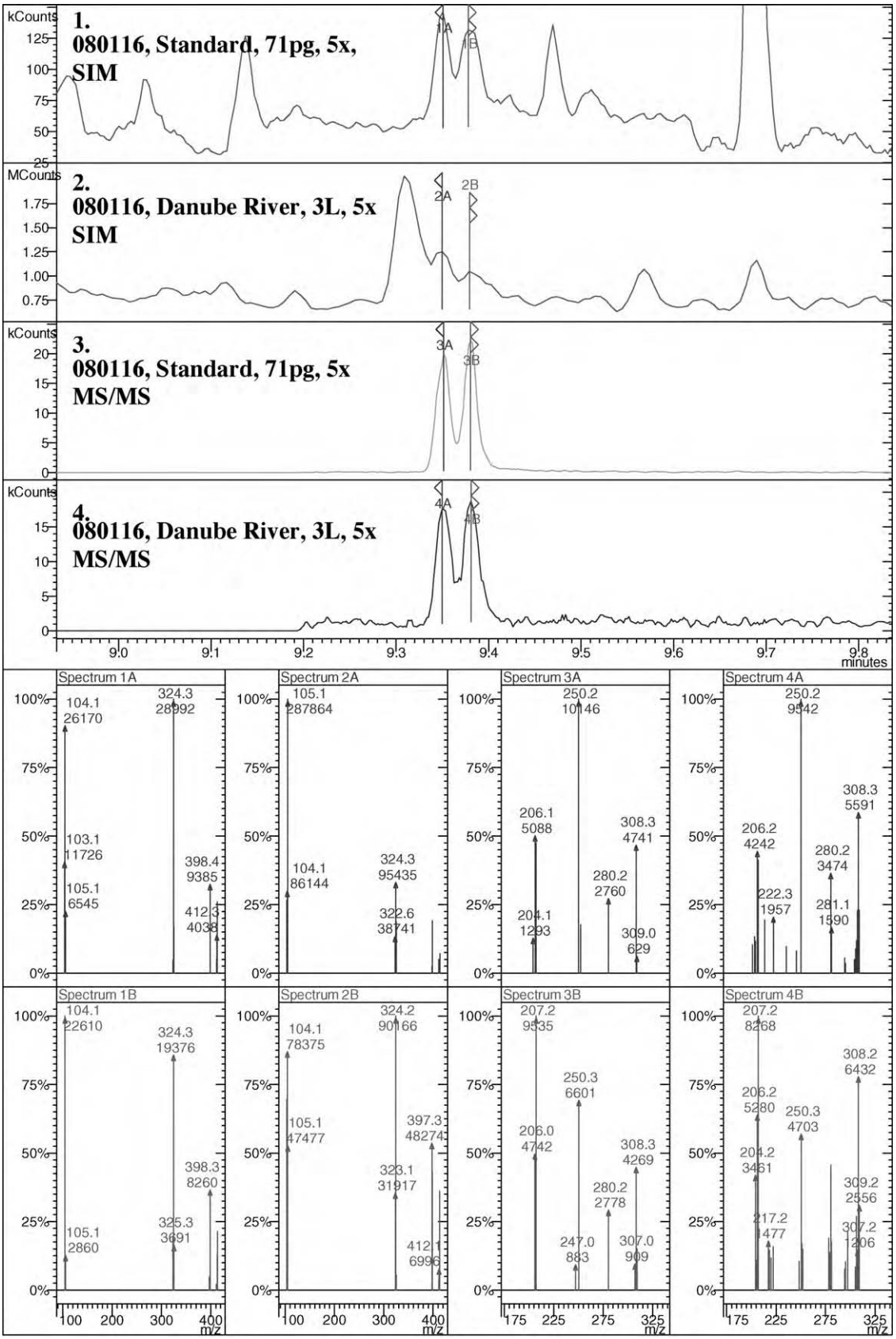

Fig. 3. Elution profile and mass spectra of the trimethylsilyl (oxime) derivatives of ketoprofen (Table 2: Ketoprofen-1, Ketoprofen-2, i.e., E and Z oximes) obtained with the GC-MS SIM (traces 1 and 2; spectra 1A, 1B, 2A, 2B) and with the GC-MS/MS (traces 3 and 4; spectra 3A, 3B, 4A, 4B) acquisition modes. Traces 1 and 3 represent 71 pg ketoprofen from standard solutions, traces 2 and 4, the calculated amounts of ketoprofen, proved to be 244 pg ketoprofen on SIM (≈ 400% overestimation) and 62 pg ketoprofen on MS/MS basis (detailed data in Table 4).

Table 3
Comparison of the analytical performances in the quantification of the NSAIDs as their TMS (oxime) ester derivatives: obtained with GC-MS FS, GC-MS SIM and GC-MS/MS acquisition methods: from model solutions (M) and from the Danube River (DR) 2008 January samples.

Compound	Acquisition method	r^2	RSD%	LOQ (ng/L)**	ILQ (pg)***	Ratios of S/N values, ****			
						SIM/FS		MS-MS/SIM	
		M				M	DR	M	DR
Ibuprofen	FS*	0.9813	9.0	1.0	2.67	7.0	1.2	1.7	5.5
	SIM	0.9959	9.8	0.43	1.15				
	MS/MS	0.9995	6.5	0.41	1.10				
Naproxen	FS*	0.9962	1.57	1.1	2.93	7.7	1.8	2.3	12
	SIM	0.9976	5.5	1.0	2.70				
	MS/MS	0.9989	6.9	0.42	1.12				
Ketoprofen	FS*	0.9984	3.67	2.6	6.83	8.0	-	1.7	23
	SIM	0.9989	9.1	1.0	2.73				
	MS/MS	0.9995	6.6	0.49	1.32				
Diclofenac	FS*	0.9982	4.20	1.4	3.73	20	2.5	2.8	17
	SIM	0.9993	4.57	0.41	1.10				
	MS/MS	0.9993	8.3	0.21	0.55				

Indications: as in Table 2, as well as: *FS = values reported in reference [17]; FS and SIM elutions were evaluated on the basis of the same ions: using m/z 161+234+263+278 for ibuprofen, m/z 185+244+288+302 for naproxen, m/z 104+324+398+413 for ketoprofen and m/z 214+242+277+368 for diclofenac; ions quantified performing MS/MS acquisition are given in Table 2; RSD% obtained from three injections of a six point calibration curve in the range of 0.5-500 ng/L; **LOQ = S/N ≥ 10; ***ILQ = injected pg/μL, considering that 1 μL sample was injected without dilution from 375 μL derivatized stock solution (LOQ ng/L = injected pg × 375); **** Ratios of the signal/noise (S/N) values: averages of six injections of each

3.2. Method validation parameters

Acquisition methods' comparison has been performed on quantitative basis, characterized with their signal to noise ratio (S/N) values.

The FS acquisition described earlier [4] was followed in improved version: background ions (products of septum/column bleed) have been excluded from the acquisition process, similarly from the SIM acquisition protocol.

The effectivity, sensitivity, reliability and reproducibility of the GC-FS, GC-SIM and GC-MS/MS acquisition methods have been compared by means of model solutions and completed by the corresponding data obtained from Danube River samples (Table 3). Critical evaluation of the three acquisition protocols was collated on their analytical performances and validated with the same characteristics, like the six point, external calibration curve, the relative standard deviation percentage (RSD%) of parallel tests, the limit of quantitation (LOQ), the instrumental limit of quantitation (ILQ), and the various S/N values. Data of six point calibration ($r^2 \geq 0.997$) and RSD% (average 5.8 RSD%) proved to be independent on the acquisition methods, while, LOQ and ILQ values furnished considerable differences. Decreasing LOQ data, (expressed in ng/L concentrations, calculated from peaks corresponding to S/N ≥ 10) were obtained in the FS, SIM, MS/MS line for ibuprofen (1.0, 0.43, 0.41), naproxen (1.1, 1.0, 0.42), ketoprofen (2.6, 1.0, 0.49) and diclofenac (1.4, 0.41, 0.21), respectively.

LOQ and ILQ values confirmed the optimum conditions and the maximum sensitivity obtained from the MS/MS acquisition mode: this experience was unambiguously associated with the S/N values compared from the three acquisition methods (Table 3: ****Ratios of the S/N values in the last four vertical columns). These ratio values were characteristic to the matrix, they have been originated from: SIM/FS and (MS/MS)/SIM ratio values obtained from model solutions (data in column M, calculated from the same injected amounts of standard compounds) and from the Danube River samples (data in column DR, obtained from the same volumes of DR samples) are considerable different. As seen, the SIM/FS ratio values obtained from model solutions (data in column M: 7.0, 7.7, 8.0, 20) guarantee the reliable, reproducible and sensitive quantitation purposes, while the same advantages in case of the Danube River sample could be achieved from the MS/MS acquisition protocol only; characterized by the matching (MS/MS)/SIM ratio values (data in column DR: 5.5, 12, 23, 17).

On the basis of data compared (Table 3, Figs. 2 and 3) it can be stated, that significant sensitivity enhancement was achieved using the MS/MS instead of the SIM mode for the analysis of the NSAIDs as their TMS (oxime) esters from the Danube River sample. Additional benefit of the MS/MS technique is the cleaner, less background ions containing chromatograms, more reliable mass spectra without any mass interference from the matrix: illustrated by the SIM and MS/MS mass spectra of naproxen (Fig. 2) and ketoprofen (Fig. 3) taken from the Danube River sample (2008 January).

Recoveries of NSAIDs have been determined with Danube River samples, fortified in the 30-66 ng/L range (detailed data not shown). Recoveries were calculated from the averages of three separate SPE extractions and three injections of each. The final results have been corrected against the corresponding blank measurements (reagent blanks and SPE blanks). Average recovery was 108% (ibuprofen 103%, naproxen 114%, ketoprofen 96% and diclofenac 119%), with an average reproducibility of 5.5% RSD. These results are in good accordance with our previous analyses in FS mode [17], with an average recovery of 99% and with an average reproducibility of 6.7% RSD.

3.3. Comparison of the NSAID contents of the Danube River samples, depending on the applied GC-MS acquisition method (FS, SIM, MS/MS) determined as their TMS (oxime) ester derivatives

In order to compare the impact of different acquisition techniques, two Danube River samples were analyzed by all three of them (FS, SIM, MS/MS). NSAID contents (Table 4, samples taken at 2008 January and at 2008 September) depending on the acquisition techniques employed reflected considerable differences. Evaluating the effect of acquisition protocols it turned out that

(1) the diclofenac contents of these two samples proved to be independent on the acquisition technique used (Table 4, ng/L values in the last horizontal line).
(2) The concentrations of naproxen are slightly dependent on the acquisition method applied (Table 4). The decreasing trend of naproxen contents obtained by FS, SIM and MS/MS methods, in order of listing, (Table 4, ng/L values in the second horizontal line), both from the 2008 January (74 ng/L, 67 ng/L, 62 ng/L) and from the 2008 September (13 ng/L, 9.7 ng/L, 9.7 ng/L) samples, seems to be unambiguous; albeit, concentration variances are

Table 4
Comparison of the NSAID contents of the Danube River samples, depending on the applied GC-MS acquisition method (FS, SIM, MS/MS) determined as their TMS (oxime) ester derivatives (five months data).

Compound⇓	NSAIDs found in the Danube River, ng/L (RSD%)*									Limit ng/L**
Sample ⇒	2008 January			2008				2009		
				September			November	April	May	
Acquisition method⇒	FS	SIM	MS/MS	FS	SIM	MS/MS				
Ibuprofen	109 (8.1)	96 (9.6)	50 (5.4)	13 (13)	9.3 (9.3)	4.4 (9.2)	25 (9.9)	6.4 (5.8)	3.7 (6.6)	**200**
Naproxen	74 (1.15)	67 (11)	62 (3.83)	13 (13)	9.7 (6.2)	8.4 (11)	27 (6.4)	8.7 (7.0)	5.7 (11)	**100**
Ketoprofen	<LOQ	305 (5.3)	77 (6.7)	<LOQ	<LOQ	<LOQ	11 (3.32)	<LOQ	<LOQ	**100**
Diclofenac	**235** (3.72)	**212** (5.8)	**224** (4.21)	21 (26)	20 (7.2)	24 (6.8)	82 (4.26)	**931** (3.89)	49 (10)	**100**

Indications as in Table 3, as well as: * (RSD%) = obtained from three separate extractions and three injections of each; ** Limit = proposed concentration limit values [61] for surface waters; bold printed values exceed the proposed limit

close to the experimental error of our analyses (in average 5.8 RSD%).

(3) Spectacular differences were obtained in the ibuprofen contents of the January and September samples, equally: confirming the unambiguous need of the MS/MS acquisition version: since, either the FS, or the SIM mode, both furnished concentrations of ≈100% overestimations: due to ibuprofen's coelution with the 4-hydroxybenzoic acid derivative [17].

(4) As to the characteristics of the TMS (oxime) esters of ketoprofen, (Ketoprofen-1 and Ketoprofen-2, which means the E/Z isomer derivatives), their coelution with the C_{18} fatty acid esters is unavoidable, even applying the SIM technique (Fig. 3, trace 2, spectra 2A, 2B). Thus, the only way of the reliable and reproducible ketoprofen quantification can be expected from the MS/MS acquisition technique: even the SIM version provided 400% overestimation (Fig. 3, trace 4, spectra 4A, 4B, Table 4, ng/L values in the third horizontal line).

(5) On the basis of the above detailed experiences {(a) - (d)}, all further analyses were performed with the MS/MS acquisition protocol (Table 4, NSAID contents in the November 2008 and in the April and May 2009 Danube River samples).

(6) As a conclusion of the practical importance of our study, relaying on the MS/MS analyses only and taking into consideration the recently proposed ng/L limit values ([61], Table 4, last vertical column), two main conclusions can be drown:
   (a) the ibuprofen, naproxen and ketoprofen contents in the Danube River revealed an acceptable scale, related to the proposed, in order of listing 200, 100 and 100 ng/L limit values [61]; varying between 3.7 - 50 ng/L (ibuprofen), 5.7 - 62 ng/L (naproxen) and 11 - 77 ng/L, or below the <LOQ (ketoprofen). While,
   (b) the diclofenac level of samples altered between 24 and 931 ng/L, out of five cases in two (224, 931 ng/L) considerably exceeding the proposed limit (100 ng/L: [61]).

(7) As to the NSAID content of the tap water in Budapest, after several trials, performed with samples of 3L volume, it has been confirmed that all four pharmaceuticals' content proved to be below of our LOQ values.

4. Conclusions

(1) To improve the selectivity of the quantification of the most common four non-steroidal anti-inflammatory pharmaceuticals (ibuprofen, naproxen, ketoprofen and diclofenac), for the first time as their TMS (oxime) ester derivatives, a tandem mass spectrometric acquisition protocol was optimized.

(2) In the frame of these studies the full-scan, the selective ion monitoring and the currently optimized, tandem mass spectrometric acquisition methods, all three at once, have been compared, on the same basis (derivatization/instrumental conditions) and characterized with the same, comparable analytical performance parameters.

(3) Data obtained revealed that the reliability and reproducibility of quantifications, in cases of naproxen and diclofenac do not depend on the acquisition protocol employed, while ibuprofen and ketoprofen can be quantitated with reliability and reproducibility, upon the tandem mass spectrometric acquisition method, exclusively.

(4) The practical utility of the currently optimized MS/MS acquisition protocol was confirmed with the analysis of Danube River samples, taken in 2008 January, September and November, as well as in 2009 April and May:

(a) the ibuprofen, naproxen and ketoprofen contents in the Danube River revealed an acceptable concentrations, in order of listing, all three being below the proposed 200, 100 and 100 ng/L limit values; varying between 3.7 - 50 ng/L (ibuprofen), 5.7 - 62 ng/L (naproxen) and 11 - 77 ng/L, or below the <LOQ (ketoprofen). While,

(b) the diclofenac level of samples altered between 24 and 931 ng/L, out of five cases in two (224, 931 ng/L) considerably exceeding the proposed limit (100 ng/L).

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