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Determination of non-steroidal anti-inflammatory drug (NSAIDs) residues in water samples

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Abstract

Pharmacologically active substances used to treat human and animal illnesses can enter the aquatic environment via effluents from wastewater treatment plants or in the case of veterinary drugs directly through liquid manure discharge. Some of these substances enter the environment either as the parent compound or as active/inactive metabolites. Due to their pharmacological activity, their determination and understanding their behavior and fate in the environment are important.

The scope of this paper was to develop an analytical procedure to determine common pharmaceutical residues in wastewaters. Pharmacologically active substances were chosen according to their wide spread application in Slovenia and Central Europe and are members of analgesics, e.g., non-steroidal anti-inflammatory drugs: ibuprofen, naproxen, ketoprofen and diclofenac. Selected compounds were isolated from synthetic water using a novel SPE sorbent Strata™ X. Due to the non-volatile nature of these compounds they were first silylised prior to gas chromatographic-mass spectrometric detection. The developed procedure was tested with synthetic wastewaters and their extraction efficiency (>84%) and method limits of detection (2–6 ng L^{-1}) were determined. Our procedure has been adopted and optimised for "real" water samples and applied to eleven drinking and ten river water samples from Slovenia. The results showed no traces of NSAIDs in all potable water samples and low-range contamination (ng L^{-1}) of Slovene rivers. These results show that NSAIDs contamination of Slovene waters is comparable with published results of water contamination in Central Europe.

Keywords: Pharmaceutical residues; NSAIDs; Water; Contamination; Solid phase extraction; Gas chromatography-mass spectrometry

1. Introduction

In recent years increasing attention has been directed toward the discharge, presence and potential effects of pharmaceuticals in the environment. Thousands of tons of pharmacologically active substances are used yearly to treat or prevent illnesses, or to help people face the stresses of modern life. The discharge of therapeutic agents from production facilities, hospitals and private household effluent as well as improper disposal of unused drugs pose a burden on the environment (Christensen, 1998). Pharmaceuticals are released into the environment either as the parent compound or as active/inactive metabolites. Thus, often it is not only the parent compound which should be the subject for a risk assessment but also the active metabolites (Christensen, 1998; Halling-Sørensen et al., 1998). Concentrations of pharmaceutical residues measured in water may give rise to human exposure in the ng per day range, which is at least three to four orders of magnitude lower than that required to produce a pharmacological effect. Risks arising from acute exposure can therefore be regarded as unlikely. However, possible effects of life-long exposures have still to be determined (Christensen, 1998).

Pharmaceuticals have been selected or designed due to and because of their biological activity. In respect to their purpose they should be considered as suspicious environmental contaminants (Christensen, 1998). Furthermore, they often have low biodegradability, and can accumulate,

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reaching detectable and biologically active amounts (Zuccato et al., 2000). Quantitative evaluation of the fate of pharmaceuticals in the aquatic environment, proper risk assessment and improvement of the efficiency of sewage treatment plants need sensitive and reliable analytical methods.

There are no data regarding pollution with pharmaceutical residues in Slovenia. Therefore, the aim of our study was to develop an analytical procedure, which allows the quantification of pharmaceuticals in water at the ng L^{-1} level. By analysing tap, well and river samples from around Slovenia, we hope to gauge the extent of pharmaceutical residues in Slovene waters. Model compounds were selected among the pharmaceuticals, which predominate in the analyses of environmental samples, as well as on the lists compiled from prescription data. Most of these pharmaceuticals belong to the class of analgesics (non-steroidal anti-inflammatory drugs, NSAIDs), antibiotics, antihypertensives, antiasthmatics, diuretics and psycholeptics (Kümmerer, 2001a). For this reason, the following four pharmaceuticals from the class of NSAIDs were chosen as model compounds: ibuprofen, naproxen, diclofenac and ketoprofen. The four investigated drugs belong to a group of the most commonly prescribed drugs between non-steroidal anti-inflammatory drugs. Data from annual reports (Oražem and Pečar-Čad, 2000, 2001, 2002) show a quantity of the drugs dispensed by prescriptions from health-centres. However, these data underestimate the total use of pharmaceuticals in Slovenia, because drugs dispensed over-the-counter and those spent in hospitals also contribute to the total. The quantities of annually prescribed pharmaceuticals have been published (Oražem and Pečar-Čad, 2000, 2001, 2002). The quantity of naproxen, together with other NSAID representatives, is the most outstanding in the group of investigated drugs and is reported to be between 1.9 and 2.6 tons/year. Furthermore, naproxen is eliminated partly unmetabolised (60%) and is persistent in the environment. Naproxen is therefore expected to pose the biggest load (among the four investigated drugs) on the Slovenian aquatic environment.

Pharmaceutical residues are usually present in environmental water samples in trace levels. The most common sample isolation and pre-concentration technique is solid-phase extraction (SPE) (Rodríguez et al., 2003) where as well as isolation and pre-concentration, the matrix-solvent (water) is exchanged with a more volatile organic solvent suitable for gas chromatography (GC). Due to low vapour pressure, gas chromatographic separations of selected NSAIDs can be performed only after derivatisation of the native compounds to less polar species (Rodríguez et al., 2003). This involves converting the carboxylic group present on these drugs to the methyl ester derivative using diazomethane (Rodríguez et al., 2003; Öllers et al., 2001; Ternes, 2001; Poole, 1991). The yield of the reaction is usually high, however, because of high toxicity and low stability of diazomethane, alternatives have been proposed. Koutsouba et al. (2003) and Sacher et al. (2001) derivatise the carboxylic group using pentafluorobenzyl bromide with triethylamine as a catalyst. The most widely used alternatives to diazomethane are alkylsilyl reagents (Poole, 1991), namely *N*-methyl-*N*-(tert.-butyldimethysilyl) trifluoroacetamide (Rodríguez et al., 2003) or *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) (Heath, 1998).

An analytical procedure for the determination of NSAIDs in water, based on solid phase extraction (SPE) with a new, patent-pending sample preparation sorbent Strata™ X, followed by derivatisation with MSTFA and GC-MSD analysis was developed and tested on synthetic and authentic well, tap and river water samples.

2. Experimental

2.1. Chemicals

Sigma-Aldrich Company Ltd (Gillingham, GB) supplied all the drugs under investigation (ibuprofen, diclofenac, naproxen and ketoprofen) and the derivatisation agent MSTFA (*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide). Mecoprop (2-(4-chloro-2-methylphenoxy) propanoic acid) was used as an internal standard and was obtained from Labor. Dr. Ehrenstorfer-Schäfers (Ausburg, Germany). Methanol (MeOH), toluene and 37% hydrochloric acid (HCl) were of analytical grade and were provided by Merck (Darmstadt, Germany).

2.2. Synthetic water

Water solution of pharmaceutical compounds (1 mg of each studied compound in 500 mL of distilled water) was spiked with 1 mg of the internal standard mecoprop. The spiked solution was diluted to concentrations from 0.2 mg L^{-1} to 0.02 mg L^{-1}.

2.3. Environmental samples

Water samples were collected in June–July and September 2004. In total, well water samples from two sites, eleven tap water and river water samples from nine locations were collected. River water samples (1L) were collected at a depth of 0.25 to 1.0 m from the riverbank. Each 1 L of sample was filtered (0.45-μm filter, Sartorius, Goettingen, Germany), acidified to pH 2.6 to enhance trapping of the acidic compounds on the solid-phase extraction (SPE) sorbent and stored at 4 °C prior to solid-phase extraction SPE.

2.4. Solid phase extraction

Commercially available 3 mL SPE cartridges with 60 mg of Strata™ X (surface modified styrene-divinylbenzene polymer) sorbent (Phenomenex®, Torrance, ZDA), were used. SPE was performed using 12-fold vacuum extraction

box (Supelco, Bellefonte, USA). The SPE cartridges were first conditioned with 1.5 mL MeOH and 1.5 mL matrix-solvent (aq. HCl with pH 2.6). Extraction volumes were 500 mL in case of synthetic water samples and 1000 mL for the actual water samples. Extraction was performed under vacuum at a flow rate of 1–2 mL min^{-1}. After the enrichment step, the cartridge was dried for 1 min in vacuum (approx. −16 mm Hg). The analytes were eluted with three fractions of 0.5-mL elution solvent (MeOH) and the eluant was collected in 1.5-mL glass vial and dried under a stream of nitrogen. The residues were dissolved in 0.5 mL (synthetic water) and 0.1 mL (water samples) of toluene and derivatised by adding 70 μL (synthetic water) or 30 μL (water samples) of MSTFA. The samples were reacted in the dark on a shaker (Veb MLW Labortechnic, Ilmenau, Germany) for 12 h.

2.5. Gas chromatography–mass spectrometry

Derivatised drugs were determined by GC-MSD on an instrument HP 6890 (Hewlett-Packard, Waldbron, Germany) fitted with a 30 m×0.25 mm×0.25 μm Hewlett-Packard HP-5 MS capillary column. Carrier gas was helium, with a flow rate held at constant velocity of 37 cm s^{-1}. Injection was performed in the splitless mode at an injection temperature of 250 °C. Injection volume was 1 μL. The GC oven was programmed as follows: 2 min at 100 °C, first ramp at 4 °C min^{-1} to 180 °C, second ramp at 10 °C min^{-1} to 230 °C (held for 20 min) and then, ramped at 20 °C min^{-1} to 270 °C and held at this temperature for 7 min. Mass spectra were obtained in the electron impact mode (70 eV), detection was in mass range 50–500 m.u. (full-scan) with the transfer line temperature set at 280 °C.

3. Results and discussion

3.1. SPE

Breakthrough of the selected SPE sorbent was investigated using a synthetic wastewater containing approx. 0.1 mg of each of the test compounds and 500 mL of the samples were passed through two cartridges connected sequentially. After the enrichment step, the cartridges were then analysed separately. Since the analytes were not detected in the eluant from the second cartridge, it was proven that the applied cartridge dimension (60 mg/3 mL) was adequate for the quantitative adsorption of the investigated drugs.

The recommended wash and elution volume (Phenomenex® users guide) for the 60 mg/3 mL Strata™ X cartridge is at least 1.2 mL. Therefore, the analytes were eluted from the cartridge with 3 fractions of 0.5 mL MeOH and the elution was performed twice (2×1.5 mL) using the same cartridge. Since there was no trace of analytes in the second portion of eluant we conclude that 1.5 mL of the elution solvent was a sufficient volume for the quantitative elution of the tested analytes.

3.2. GC-MS

The trimethylsilyl (TMS) derivatives of target compounds, ibuprofen, mecoprop (internal standard), naproxen, ketoprofen and diclofenac (Fig. 1), were determined by capillary gas chromatography with mass spectrometric detection in EI mode of operation.

Typical total ion chromatogram of selected pharmaceuticals and internal standard is shown in Fig. 2.

The chemical structure of all investigated compounds after derivatisation (TMS-esters) was proved with mass spectrometric detection. Table 1 shows the retention times (t_R) of the derivatised compounds, molecular weight of pure compounds and their derivatised compounds, mass-to-charge ratio of typical fragments and their intensity.

Fig. 3 is an example mass spectrum of trimethylsilyl ester of ibuprofen. Its molecular ion is seen at m/z=278. Fragment ion at m/z=263 represents the fragmentation of methyl group from molecular ion ($[M_{TMS}–CH_3]^+$), while m/z=205 shows trimethylsilyl group fragmentation ($[M_{TMS}–SiMe_3]^+$). Ion at m/z=160 is developed with sequential fragmentation of trimethylsilyl and carboxyl groups. The

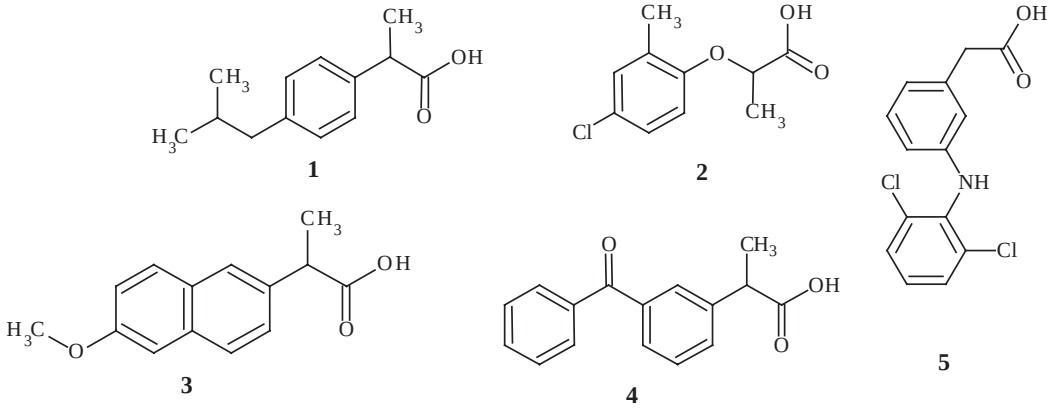

Fig. 1. Chemical structures of investigated compounds: ibuprofen (1), mecoprop (internal standard [2]), naproxen (3), ketoprofen (4) and diclofenac (5).

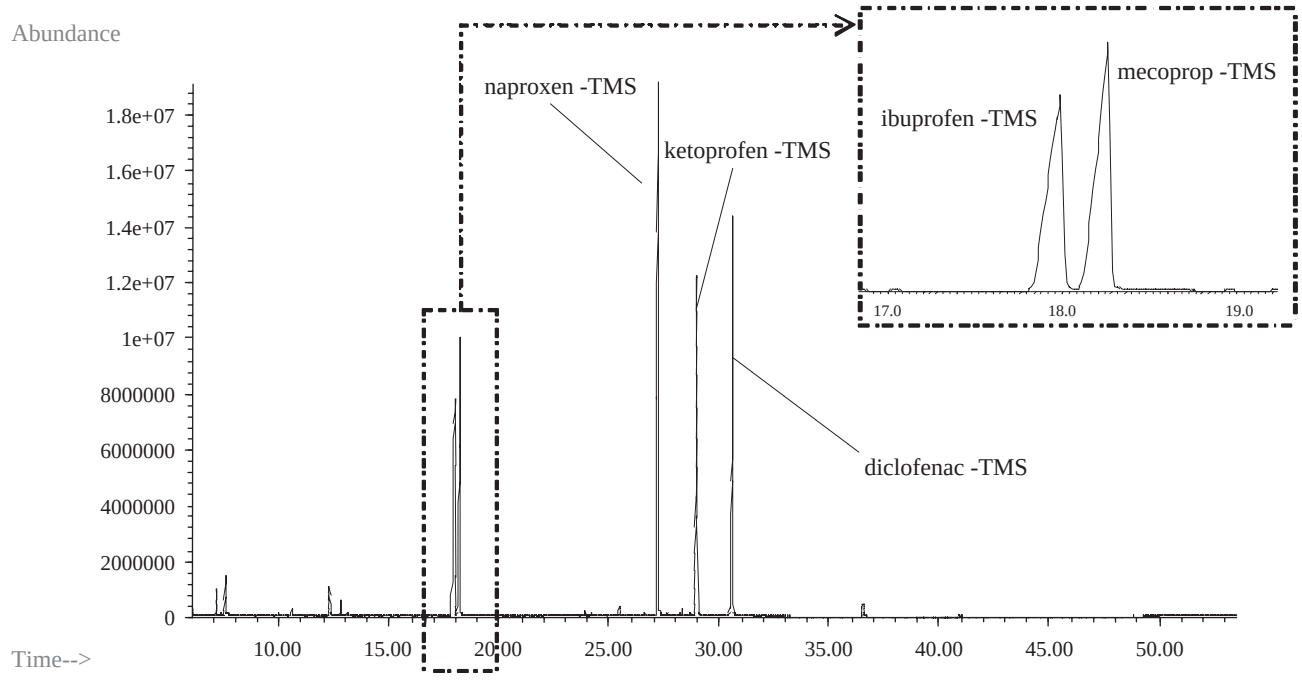

Fig. 2. Total ion chromatogram of the selected compounds after derivatisation.

base ion is present at m/z=73 and is typical for MSTFA derivatisation (Heath, 1998).

3.3. Extraction efficiency

Extraction efficiency ($\%_{EXTR}$) was calculated for each compound (ibuprofen, ketoprofen, diclofenac, naproxen and internal standard mecoprop) as a ratio of a mean of peak areas of extracted and derivatised compound ($A_{SPE+DER}$) to a mean of peak areas of derivatised compound without previous SPE (A_{DER}) performed (Eq. (1)). 2–6 parallel samples were used for this purpose (Table 2).

$$\%_{EXTR} = A_{SPE+DER}/A_{DER} \quad (1)$$

Water solutions of pharmaceuticals at two concentrations (0.02 mg L^{-1} and 0.20 mg L^{-1}) were spiked with internal standard and processed as described in the Experimental section. Toluene solutions of pharmaceuticals with the internal standard at two concentrations (0.02 mg mL^{-1} and 0.20 mg mL^{-1}) were derivatised as described herein. Extraction efficiencies ($\%_{EXTR.}$) for all pharmaceuticals and internal standard are given in Table 2 and range from 91 to 103% at the higher concentration and from 84 to 104% at the lower concentration for all compounds except diclofenac. An extraction efficiency of 157% and high deviation ($\delta/2$) was observed for diclofenac, which was probably due to disintegration of its TMS ester during GC-MSD analysis.

Table 1
Retention time (t_R) of derivatised compounds, molecular weight (M_X), molecular weight of TMS ester ($M_{X\text{-TMS}}$), mass-to-charge ratio (m/z) of typical fragments and their relative intensity

Analyte–TMS	Ret. time t_R/min	M_X	$M_{X\text{-TMS}}$	m/z	Fragment structure and their relative intensity
Ibuprofen-TMS	17.97	206	278	73	-Si$(CH_3)_3$,100 %
				160	30 %
				278	$[M_{IP\text{-}TMS}]^+$,10 %
				263	$[M_{IP\text{-}TMS}\text{-}CH_3]^+$,25 %
Naproxen-TMS	27.21	230	302	185	100%
				73	-Si$(CH_3)_3$,55 %
				302	$[M_{NP\text{-}TMS}]^+$,45 %
				287	$[M_{NP\text{-}TMS}\text{-}CH_3]^+$,25 %
Ketoprofen-TMS	28.99	254	326	282	100 %
				73	-Si$(CH_3)_3$,80 %
				311	$[M_{KP\text{-}TMS}\text{-}CH_3]^+$,20 %
Diclofenac-TMS	30.54	296	368	214	100%
				73	-Si$(CH_3)_3$,35 %
				367	$[M_{DF\text{-}TMS}\text{-}H]^+$,25 %
				352	$[M_{DF\text{-}TMS}\text{-}H\text{-}CH_3]^+$, 10 %
Mecoprop-TMS (internal standard)	18.23	214	286	73	-Si$(CH_3)_3$,100 %
				286	$[M_{MP\text{-}TMS}]^+$,50 %
				271	$[M_{MP\text{-}TMS}\text{-}CH_3]^+$,5 %

3.4. Derivatisation efficiency

Since derivatised standards of selected compounds were commercially unavailable, it was not possible to calculate the derivatisation efficiency. However, since after derivatisation no underivatised compounds were found to be present when analysed by GC-MS, derivatisation was assumed complete.

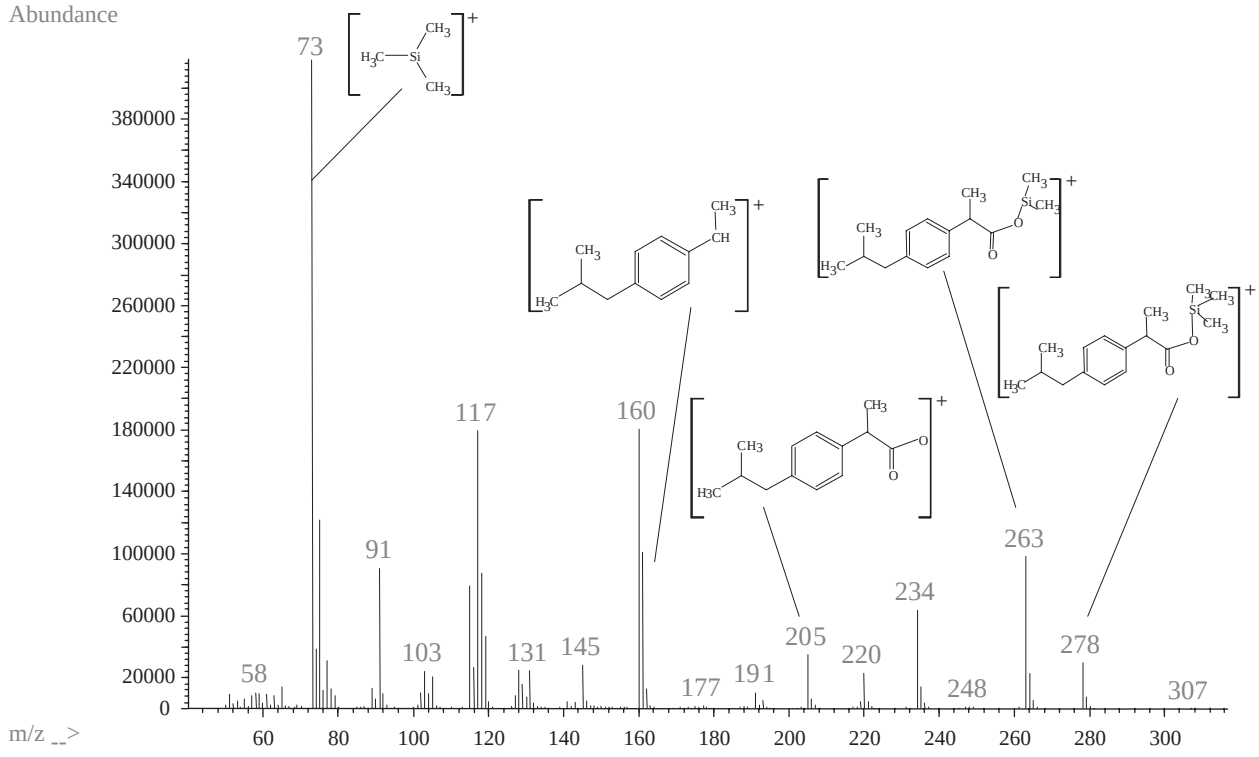

Fig. 3. Mass spectrum of ibuprofen-TMS.

3.5. Linearity

The linearity of the analytical method was tested using pre-derivatised standard mixtures containing mecoprop as internal standard (0.20 mg mL^{-1}), and the test compounds in concentrations between 0.02 and 0.25 mg mL^{-1}. With the exception of diclofenac (r^2=0.990) correlation coefficients higher than 0.996 were obtained for all the compounds under investigation. The TMS ester of diclofenac was proved to be unstabile under the applied conditions. At the same time as lower diclofenac-TMS quantities, an additional peak appears in total ion chromatogram at t_R 28.7 min. From analogy between the mass spectra of diclofenac-TMS and the mass spectra of the unknown peak we conclude that the latter originates from the breakdown of diclofenac-TMS.

Table 2
Extraction efficiencies ($\%_{EXTR}$) for the investigated drugs and internal standard

Compound	$c_{\text{before DER}}$/ mg mL^{-1}	$n_{SPE+DER}$	n_{DER}	$\%_{EXTR}$	$\pm\delta/2$
Ibuprofen	0.0331	4	2	84.2	0.671
	0.296	6	6	91.9	0.336
Mecoprop	0.0214	4	2	91.5	0.399
	0.191	6	6	96.1	0.157
Naproxen	0.0188	4	2	104.1	0.129
	0.114	2	4	102.1	0.057
Ketoprofen	0.0320	4	2	99.3	0.017
	0.286	6	6	103.4	0.158
Diclofenac	0.0254	4	2	103.7	0.088
	0.227	6	6	157.0	22.053

Abbreviations: $c_{\text{before DER}}$, concentration of the selected compound in toluene prior to derivatisation; $n_{SPE+DER}$, number of extracted and derivatised samples; n_{DER}, number of derivatised samples; $\pm\delta/2$, deviation from $\%_{EXTR}$ calculated as:

$$\delta/2 = 1/2\left[\left(\text{mean}A_{SPE+DER} + s_p/\text{mean}A_{DER} + s_p\right) - \left(\text{mean}A_{SPE+DER} - s_p/\text{mean}A_{DER} - s_p\right)\right]$$

$$s_p^2 = \left(\left[\left(n_{SPE+DER} - 1\right)s_1^2 + \left(n_{DER} - 1\right)s_2^2\right]/\left[n_{SPE+DER} + n_{DER} - 2\right]\right);$$

s_p^2—pooled variance; mean$A_{SPE+DER}$: mean peak area of extracted and derivatised sample with standard deviation s_1; meanA_{DER}: mean peak area of derivatised sample with standard deviation s_2.

3.6. Detection limits

Instrumental limits of detection (ILDs) were determined by selecting the lowest concentration of the spiked sample that produces a chromatographic peak having an area under curve equal to three times the standard deviation of the baseline noise of the blank sample (Knoll, 1985). The ILDs in full-scan acquisition mode are in μg L^{-1} (Table 3) but can

Table 3
ILDs of TMS derivatives of target compounds in toluene obtained with full-scan acquisition mode and MLDs of the investigated drugs in distilled, deionised water

Pharmaceutical compound	Ibuprofen	Naproxen	Ketoprofen	Diclofenac
ILD/μg L^{-1}	20.3*	56.1*	20.9*	31.5*
MLD/ng L^{-1}	1.96	5.55	2.12	3.06

* TMS-esters of pharmaceutical compounds.

be reduced further by using selected ion monitoring (SIM) mode.

Method limits of detection (MLDs) were calculated from ILDs taking into account the concentration factors from SPE procedure. For this calculation, a 100% extraction efficiency was presumed and the results are shown in Table 3.

Comparison of the ILD and MLD (Table 3) shows that approx. 1000 times concentration of the compounds in a sample is achieved. However, the concentration factor and therefor, to a certain extent, MLDs can be further improved by increasing the amount of water extracted, by reducing the volume of toluene used for dissolution of the dry extract or by reducing the derivatising agent volume. The proposed suggestions are under investigation.

The minimal volume of surface water needed for isolation using our analytical procedure was calculated on the basis of ILD (Table 3). For calculation purposes we assumed that the Slovene environment is polluted with pharmaceutical residues in the same range as the rest of Central Europe, including Germany (Kümmerer, 2001b) where the data were taken from. Calculations were made for two model compounds: ibuprofen and diclofenac according to Eq. (2):

$$V_x = 1/c_x(V_K{*}\mathrm{MLD}{*}M_x/M_{X-\mathrm{TMS}}) \quad (2)$$

where V_x represents the volume of water sample, c_x is the lowest concentration of the compound determined in literature (Kümmerer, 2001b), V_K is the volume of the solution before GC-MSD analysis (0,13 mL), MLD is method limit of detection, M_x is molecular mass of compound and $M_{X\text{-TMS}}$ is molecular mass of trimethylsilylised molecule.

The results of our calculations showed that the minimal volume for detecting ibuprofen is expected to be from 7 to 39 mL of surface water sample. In case of diclofenac the minimal volumes were from 7 to 659 mL of surface water sample. These results show that the extraction volume (1000 mL) of the surface water sample should allow detection of selected NSAIDs in the low ng L^{-1} range.

3.7. Determination of the selected pharmaceuticals in environmental samples

Nine tap, two well and ten river water samples from Slovenia were examined using the procedure described in the methodology. Some of river samples were sampled twice (June–July 2004 and September 2004). There were no traces of the pharmaceuticals under study in the tap and well water samples, while 11 out of 16 river samples contained naproxen (17–80 ng L^{-1}) and diclofenac (9–49 ng L^{-1}), and one sample showed a concentration approximately four to five times higher than the rest of tested compounds: naproxen: 313 ng L^{-1} and diclofenac: 282 ng L^{-1}. This sample was taken in a river downstream of a pharmaceutical factory. According to fragmentation pattern, ketoprofen was detected in 8 and ibuprofen in 1 out of 16 of the river samples, but because interferences were present with the same retention time, their quantification could not be determined in SCAN mode. However, these limitations will be addressed by modifying the chromatographic separation and using SIM. Sampling of river waters that showed higher concentrations of selected contaminants was repeated at the same sampling sites 2 months after the initial sampling (September 2004). From the results (Table 4) it is seen that the concentrations of the tested NSAIDs were lower in the September samples. The sample that contained the highest amount of naproxen and diclofenac sampled in June (Krka river sample downstream) where approximately five times lower in case of autumn sampling. The reason might be the nature of sampling (not continuous sampling) and/or the time of sampling, i.e. second sampling was performed when river flow was higher resulting in greater dilution. In the future sampling will be repeated using a greater number of sampling points and continuous sampling. Also, sediment sampling will be taken into account.

Table 4
Determined concentrations of the selected NSAIDs

Sample	Date of sampling	Concentration (ng L^{-1})		
		Naproxen	Diclofenac	Ketoprofen
KRKA 1	Jul–04	*	–	–
	Sep–04	–	–	*
KRKA 2	Jul–04	313	282	*
	Sep–04	60	49	*
LJUBLJANICA 1	Jul–04	–	–	–
	Sep–04	<MLD	–	–
LJUBLJANICA 2	Jul–04	73	*	–
	Sep–04	<MLD	–	*
SAVA	Jul–04	80	9	*
	Sep–04	*	*	–
MURA	Jul–04	49	41	*
DRAVA 1	Jul–04	46	26	*
	Sep–04	<MLD	–	–
DRAVA 2	Jul–04	24	32	*
	Sep–04	42	–	–
PŠATA	Jul–04	17	*	–

* Compound was detected, but quantification could not be determined in SCAN mode.

4. Conclusions

In the presented work an analytical procedure for determination of pharmaceutical residues in water samples was developed. The qualitative determination of the selected compounds (naproxen, ketoprofen, ibuprofen, diclofenac) included development and optimisation of following analytical steps: SPE, derivatisation and GC-MSD analysis. Under optimal working conditions (flow, solvent volume, cartridge dimension, derivatisation conditions) isolation of selected compounds from water samples with efficiencies >84% was achieved. For all four tested compounds, ILDs were determined (20–56 μg L^{-1}) and MLDs for optimised method were calculated (2–6 ng L^{-1}).

Finally, the contamination of NSAIDs in representative samples of Slovene waters (two well, eleven tap and nine river water samples) was determined. According to our results the contamination of Slovene waters is comparable with the published literature for Central Europe (Kümmerer, 2001b). However, to determine the extent of pharmaceutical residue contamination in natural Slovene waters, we intend a more comprehensive study taking into account a greater number of sampling points, continuous water sampling and sediment samples.

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References

Christensen FM. Pharmaceuticals in the environment—a human risk? Regul Toxicol Pharmacol 1998;28:212–21.

Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere 1998;36(2):357–93.

Heath, E. Mikrobiološka razgradnja nekaterih poliaromatskih ogljikovodikov in identifikacija njihovih produktov. Doktorska disertacija. Ljubljana: Fakulteta za kemijo in kemijsko;1998:26-36.

Knoll JE. Estimation of the limit of detection in chromatography. J Chromatogr Sci 1985;23(9):422–5.

Koutsouba V, Heberer T, Fuhrmann B, Schmidt-Baumler K, Tsipi D, Hiskia A. Determination of polar pharmaceuticals in sewage water by gas chromatography–mass spectrometry. Chemosphere 2003;51(2):69–75.

Kümmerer K. Pharmaceuticals in the environment. Sources, fate, effects and risks. 1st ed. Berlin: Springer–Verlag; 2001a. p. 1–7.

Kümmerer K. Pharmaceuticals in the environment. Sources, fate, effects and risks. 1st ed. Berlin: Springer–Verlag; 2001b, p. 82.

Öllers S, Singer HP, Fässler P, Müller SR. Simultaneous quantification of neutral and acidic pharmaceuticals and pesticides at low-ng/L level in surface and waste water. J Chromatogr Sci, A 2001;911(2):225–34.

Oražem A, Pečar-Čad S, Ambulantno predpisovanje zdravil v Sloveniji po ATC klasifikaciji v letu 1999, vol. 39 (7). Ljubljana: Inštitut za varovanje zdravja Republike Slovenije; 2000.

Oražem A, Pečar-Čad S, Ambulantno predpisovanje zdravil v Sloveniji po ATC klasifikaciji v letu 2000, vol. 40 (7). Ljubljana: Inštitut za varovanje zdravja Republike Slovenije; 2001. p. 90–1.

Oražem A, Pečar-Čad S, Ambulantno predpisovanje zdravil v Sloveniji po ATC klasifikaciji v letu 2001. Inštitut za varovanje zdravja Republike Slovenije, Ljubljana 2002;41(7):91–2.

Phenomenex®, Solid phase extraction; Reference manual & Users Guide.

Poole CF. In: Poole SK, editor. Chromatography today. Amsterdam: Elsevier Science Publishers; 1991. p. 735–946.

Rodríguez I, Quintana JB, Carpinteiro J, Carro AM, Lorenzo RA, Cela R. Determination of acidic drugs in sewage water by gas chromatography–mass spectrometry as tert-butyldimethylsilyl derivatives. J Chromatogr Sci, A 2003;985(1–2):265–74.

Sacher F, Lange FT, Brauch HJ, Blankenhorn I. Pharmaceuticals in groundwaters; analytical methods and results of a monitoring program in Baden-Württemberg, Germany. J Chromatogr Sci, A 2001;938(1–2):199–210.

Ternes TA. Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. TrAC, Trends Anal Chem 2001;20(8):419–34.

Zuccato E, Calamari D, Natangelo M, Fanelli R. Presence of therapeutic drugs in the environment. Lancet 2000;355(9217):1789–90.