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High precision procedure for determination of selected herbicides and their degradation products in drinking water by solid-phase extraction and gas chromatography–mass spectrometry

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Abstract

For target monitoring of selected herbicides in groundwater transport studies, a precise and accurate method for the determination of atrazine (ATR), desethylatrazine (DEAT) and 2,6-dichlorobenzamide (BAM) was developed. The method is based on solid-phase extraction and GC–MS analysis. Deuterated standards are used as surrogates for calibration by the overall procedure. For legal requirements the method described was validated and is regularly subject to external quality control. Typical limits of detection are 2 ng/l. Uncertainty contributions were evaluated using the GUM workbench modelling software. At the concentration level of interest (100 ng/l), an expanded uncertainty of no more than 10% was estimated. Accurate data on the distribution of ATR, DEAT and BAM in affected well fields enabled operational changes to be implemented to control the drinking water supply according to legal requirements.

Keywords: GC–MS; Pesticides; Deuterated standards; Surrogates; Groundwater; 2,6-Dichlorobenzamide

1. Introduction

EU drinking water directive 98/83/EC allows a maximum concentration (MCL) of 0.10 μg/l of pesticides and their degradation products in drinking water. Regular quantitative determination of compounds that are likely to be present in the aquifer is required. An accurate and precise method for determination of the relevant compounds is therefore necessary [1–6].

Pesticides and other organic contaminants have been of scientific and public concern in the last two decades. The analytical requirements are mainly dictated by low concentrations in different water samples. Among various chromatographic methods published in the past, hyphenated GC–MS and LC–MS techniques are most widely used nowadays. In very recent papers [1,2] the most important problems, including separation problems and LC–MS/MS techniques, are discussed.

In our work, US Environmental Protection Agency method 526.1 – solid-phase extraction and GC–MS, was modified with an extended calibration by the overall procedure, using deuterated standard compounds (I.S.) [7–16].

Qualitative procedures for selection of relevant compounds (Tables 1 and 2) were described elsewhere [2,17]. Additional criteria for analyte selection and/or modification of the analyte list are the results of surface water and shallow groundwater monitoring [5]. Qualitative analysis indicated the presence of the previously mentioned herbicides and their degradation products and additionally several other compounds. Traces of degradation products of the herbicide metolachlor, most probably metolachlor ESA (*m*/*z* 162, 282) and dechlorinated metholachlor (*m*/*z* 162, 204) were found.

A high concentration of nitrate and the presence of the drug carbamazepine indicate possible pollution by sewage. In samples where high concentrations of nitrate without the selected analytes were found, the presence of other herbicides and their degradation products is strongly indicated. Our current research proved that only listed compounds were present [6].

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Table 1
Retention times and method performance for target compounds in METH2, 1st SIM run

Compound (CAS NO)	t_r (min)	SIM, *m/z* (QVN/QVL1, QVL2)	LOD (ng/l)	LOQ (ng/l)	Working range (ng/l)
Desethylatrazine D6 – I.S.	12.09	175/173, 193	–	–	200
Desethylatrazine (6190-65-4)	12.16	172/173, 187	2.0	6.7	6.7–600
Desethylterbuthylazine (30125-63-4)	12.47	186/145, 201	2.0	6.7	6.7–600
Hexachlorobenzene – CS	13.13	284/142, 249	–	–	400
Atrazine D5 – I.S.	13.66	205/178, 220	–	–	200
Atrazine (1912-24-9)	13.73	200/215, 173	2.0	6.7	6.7–600
Terbuthylazine D5 – I.S.	14.16	219/234,178	–	–	200
Terbuthylazine (5915-41-3)	14.23	214/229,173	1.0	3.3	3.3–600
Ametryn (834-12-8)	17.04	227/170, 212	5.0	16.7	16.7–600
Prometryn D5 – I.S.	17.08	247/232, 185	–	–	200
Terbutryn D5 – I.S.	17.65	246/175, 190	–	–	200
Terbutryn (886-50-0)	17.75	241/185, 226	5.0	16.7	16.7–600
Metolachlor D6 – I.S.	18.30	166/242, 246	–	–	200
Metolachlor (51218-45-2)	18.41	162/238, 240	2.0	6.7	6.7–600
Carbamazepin D10 – I.S.	28.62	203/246, 178	–	–	200
Carbamazepin (298-46-4)	28.81	193/236, 168	10.0	33.3	33.3–600

QVN, quantitation ion; QVL1 and QVL2, confirmation ions.

An important steep in verification of a procedure is the possibility to predict final concentrations of analytes in tap water from the mass balance of listed compound in water from the pumping wells.

A procedure will be demonstrated as a powerful tool for trend analysis and for groundwater transport studies by target monitoring of selected herbicides and their degradation products [11,12].

This procedure allows efficient groundwater monitoring and is an important decision making tool for drinking water management.

2. Experimental

2.1. Materials

GC–MS: 17A/QP 5050A with AOC 20i auto sampler, Shimadzu Corporation, Kyoto, Japan. Silanized injection liners, SGE International Pty Ltd., Ringwood, Australia. DB 5MS column, 30 m × 0.25 mm i.d., d.f. 0.25 μm, Agilent (J&W Scientific), Folsom, USA. Personal computer with CLASS 5000 software and NIST 21, NIST 107 and PMW TOX 2 spectral libraries. One litre brown Duran sampling bottles, Schott AG, Mainz, Germany.Alltech SPE vacuum unit for 12 samples, Alltech Associates, Deerfield, USA. SPE cartridges EN 200 mg, Merck KGaA, Darmstadt, Germany and Chromabond RP 200 mg, Macherey-Nagel GmbH & Co., KG, Düren, Germany. Gases: helium, 99.9999%; nitrogen 99.999% purity, Messer Slovenia d.o.o., Ruše, Slovenia. Acetone, methanol, ethylacetate, and dichloromethane (DCM) for GC–MS analysis, Rathburn Chemicals Ltd., Walkerburn, UK. Spring water from non-affected area, ultra pure water (upw) – Easypure LF, Barnstead/Thermolyne International, Dubuque, USA. Hexachlorobenzene (HCB), solid, analytical-reagent grade, Fluka, Buchs, Switzerland. Pure analyte standards and standard solutions of deuterated analytes were from Dr. Ehrenstorfer, Augsburg, Germany.

2.2. Methods

2.2.1. Preparations of standard solutions (Fig. 1)

Solid target analytes and standard solutions of deuterated analytes were used. The spiking solutions were prepared by serial dilutions in acetone. Final dilutions were made by upw.

Table 2
Retention times and method performance for target compounds in METH2, 2nd SIM run

Compound (CAS NO)	t_r (min)	SIM *m/z* (QVN/QVL1, QVL2)	LOD (ng/l)	LOQ (ng/l)	Working range (ng/l)
Desisopropylatrazine D5 – I.S.	11.90	178/160, 180	–	–	200
Desisopropylatrazine (1007-28-9)[a]	11.92	158/173, 175	10.0	33.3	33.3–600
Desethylatrazin D6 – I.S.	12.08	175/173, 193	–	–	200
2,6-Dichlorobenzamide (2008-58-4)	12.28	173/189, 175	2.0	6.7	6.7–600
Simazine D10 or D5 – I.S.	13.42	211/179, 193 or 206/174, 188	–	–	200
Simazine (122-34-9)	13.56	201/200, 186	2.0	6.7	6.7–600
Propazine D6 – I.S.	13.77	235/193, 220	–	–	200
Propazine (139-40-2)	13.83	214/229, 186	2.0	6.7	6.7–600
Prometryn D5 – I.S.	17.07	247/190, 232	–	–	200
Prometryn (7287-19-6)	17.16	241/184, 226	2.0	6.7	6.7–600

QVN, quantitation ion; QVL1 and QVL2, confirmation ions.
[a] Because of occasional interference on the desisopropylatrazine 173 ion, ion 158 was selected.

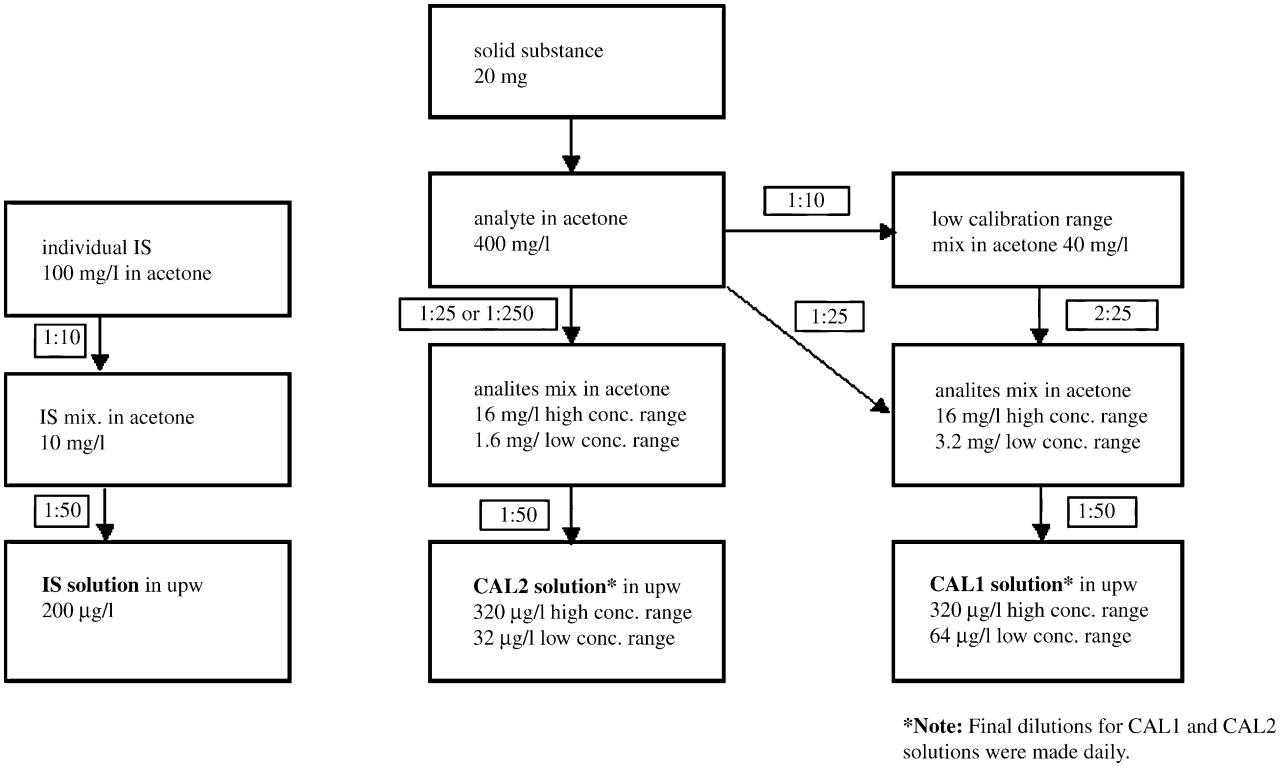

Fig. 1. Flow chart for the preparation of the standard solutions.

2.2.2. SPE procedure (Fig. 2)

The SPE cartridges were washed with 10 ml of acetone, conditioned with 10 ml of methanol, followed by 10 ml of spring water. 1.15 l of the water sample, the standard (calibration) solutions and the control sample were extracted using SPE cartridges at a sample flow rate of 3–5 ml/min. The cartridges were dried for 2 min and stored in a refrigerator at +4 °C for no longer than 3 days. The SPE cartridges were eluted with 10 ml of DCM. Traces of water were removed with anhydrous sodium sulphate. The eluate was dried with nitrogen and redissolved in 1 ml of 400 µg/l HCB solution in acetone or acetone/DCM. HCB is an easily degradable compound with low noise *m*/*z* 284, 142. It was used to check the GC–MS performance for every sample run.

2.2.3. GC–MS analysis (Fig. 2)

One microlitre of the sample solution was injected by the splitless method into the GC–MS (e.i.). A temperature programme from 50 °C (1 min) to 270 °C, with a total time of 45 min and initial fast heating was used. The injector temperature was 280 °C and the detector temperature was 300 °C, 1.7 kV (METH2 in the flow chart, Fig. 2). A daily control run was performed before each sample analysis (Fig. 2). For signal-to-noise (S/N) calculation, 1 µL of the HCB solution in DCM was injected by the splitless method. A temperature programme from 80 °C to 220 °C, with initial fast heating was used. The scan mode was used between *m*/*z* 40 and 350. Temperatures of the injector and the detector were both maintained at 250 °C (METH1, Fig. 2). The same GC–MS programme (METH2, Fig. 2) and the same injection solvent were used for the control run with endrin and *p,p*-DDT, and for sample analysis.

2.3. Preparation of control samples and method validation

2.3.1. Preparation of control samples

For the preparation of control samples in the range 0–600 ng/l, 0–2.0 ml of CAL2 was used (Fig. 2). 40% of the control samples were prepared between the LOD and the lower calibration limit. 20% of the control samples were blanks, with or without the addition of I.S. Twenty percent of the control samples were within the calibration range and 20% were above the upper calibration limit.

2.3.2. Method validation

A calibration curve by the overall procedure, with the area ratios ($A/A_{I.S.}$) versus mass ratios ($m/m_{I.S.}$), was calculated by linear regression within the calibration range (Table 3). The calibration range was determined by analysis of the results from real samples.

For extrapolation towards the LOD and above the upper calibration limit, the response factor calculation was used (Table 3). Extrapolation accuracy was checked with control samples (Tables 3–7).

Absolute recoveries [15] were from 70% (carbamzepine) to 92% (2,6-dichlorobenzamide) in the range from the LOQ to 600 ng/L.

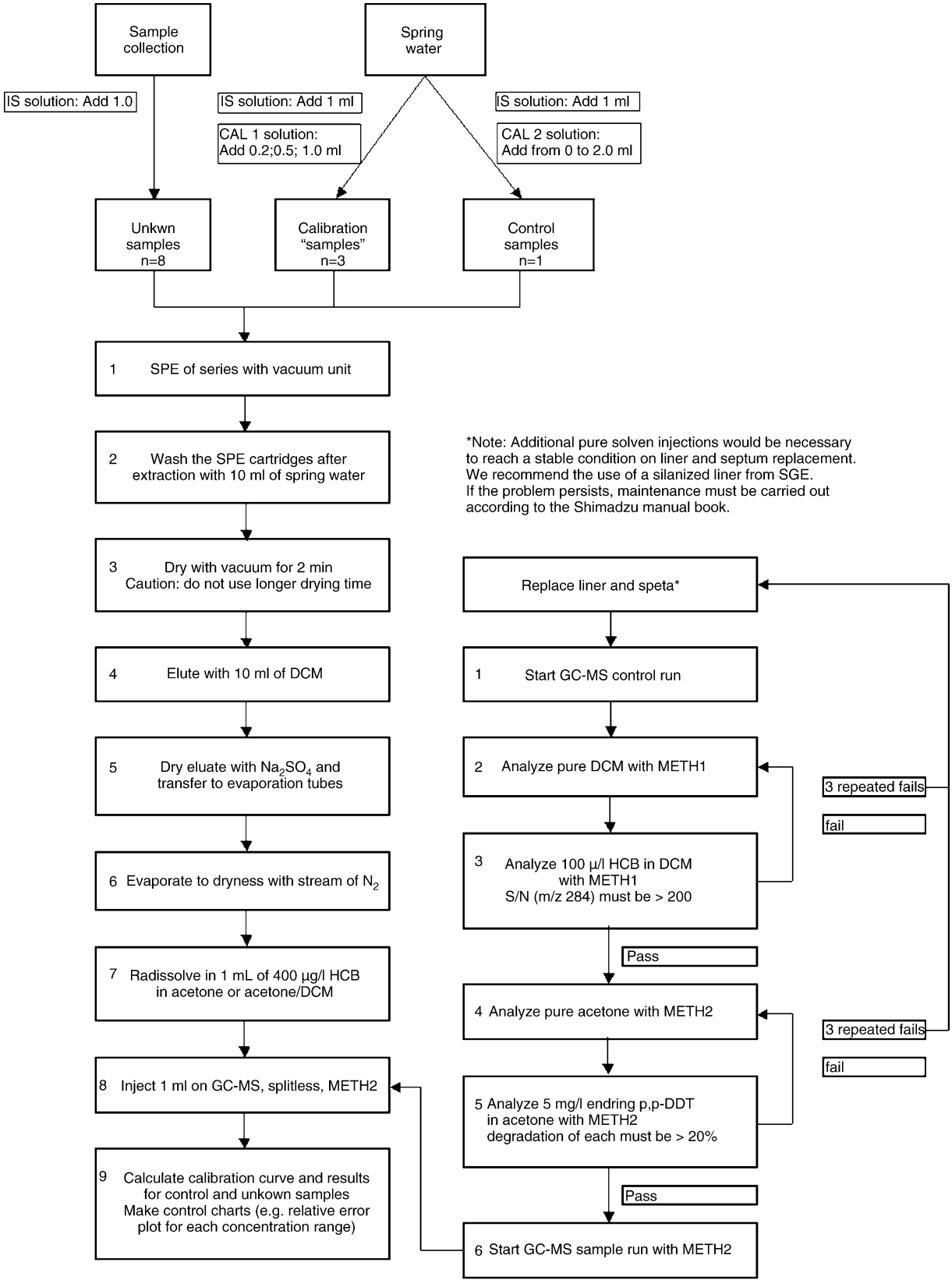

Fig. 2. Flow chart for the SPE and GC–MS procedure.

Table 3
The example for generation of daily calibration curve and control of calibration for DEAT, b (slope) = 1.258, a (intercept) = −0.0314

Calibration

	m_{DEAT} (ng)	$m_{DEAT}/m_{I.S.}$	$A_{DEAT}/A_{I.S.}$
1	62,4	0.312	0.3629
2	156	0.780	0.9472
3	312	1.560	1.9325

Control[a]

	$A_{DEAT}/A_{I.S}$	γ (ng/L)	μ (ng/l)
1	0.1798	26.9	27,1
2	0.1989	29.7	27,1
3	0.1778	26.6	27,1
4	0.1827	27.3	27,1

γ, Mass concentration; μ, ng/l, added DEAT s.c. "true value". Results within the calibration level: $\gamma_{DEAT} = ((A/A_{I.S.}) - a) \times m_{I.S.}/(V_{sample} \times b)$. Results for control were below the lower calibration limit and response factor (RRF) calculation was used as: RRF (64 ng) = $(A_{DEAT}/A_{I.S.})/(m_{DEAT}/m_{I.S.}) = 1.163$; $\gamma_{DEAT} = (A/A_{I.S.}) \times m_{I.S.}/(V_{sample} \times RRF)$; $V_{sample} = 1.15$ l; $m_{I.S.} = 200$ ng.
[a] For calibration and control two independently prepared spiking solutions were used (Figs. 1 and 2).

Table 4
Study of the repeatability of the whole procedure at sub-LOD and sub-LOQ levels for terbuthylazine

n	γ (ng/l)	RSD (%)	U (ng/l)	μ (ng/l)	U (%)	E_r (%)
4	0.55	19.2	0.38	0.72	53.2	−24.5
4	3.0	8.5	0.63	2.7	23.1	10.3

U (ng/l), reported uncertainty; E_r (%), relative error.

Analysis of the results for metolachlor (MET) showed that most of the samples had concentrations at or below the LOQ and none exceeded 100 ng/l. Therefore, only calibration at low concentration level (12–60 ng/l) was used and control samples were mostly at the LOQ level (Table 6) [18]. Even below the reported LOQ for MET (6.7 ng/l), some values are reported with 90% confidence level, as it has been demonstrated in Tables 5 and 6.

The next example was desethylatrazine (DEAT), with prevailing concentrations between 50 and 300 ng/l in real samples. Calibration was performed at high concentration level only (60–300 ng/l) and control samples were mostly at the 30 ng/l level (Table 7).

Table 6
An example of validation of the calibration procedure at low concentration level for metolachlor – MET

γ (ng/l)	U (ng/l)	U (%)	μ (ng/l)	E_r (%)
6.2	1.3	20.8	5.5	14.1
6.3	1.3	20.7	5.5	14.6
10.8	1.8	16.2	10.9	−0.9
26.7	3.3	12.5	27.3	−2.1
52.1	5.9	11.3	54.5	−4.5
81.2	8.8	10.8	81.8	−0.7
79.0	8.6	10.8	81.8	−3.4
111	12	10.6	109	1.6
111	12	10.6	109	1.5

Table 7
Validation of calibration procedure at high concentration level for desethylatrazine – DEAT

γ (ng/l)	U (ng/l)	U (%)	μ (ng/l)	E_r (%)
29.6	3.6	12.3	27.1	9.2
26.1	5.9	22.7	27.1	−3.6
52.7	8.6	16.3	54.3	−3.0
136	17	12.0	136	0.1
286	32	11.0	271	5.3
415	45	11.0	407	2.0
411	44	11.0	407	0.9
556	59	11.0	543	2.5
539	57	11.0	543	−0.8

The best validation and verification of the procedure is a mass balance calculation of the concentration of analytes for tap water from defined pumping wells (Figs. 3 and 4, Table 8). In Fig. 3, the performance of the procedure in the range between 10 and 150% of the maximum concentration (MCL) (0.10 μg/l) is shown.

Several additional validation procedures were described in the latest EU documents and other sources [4,18–22]. The uncertainty budget was calculated with GUM Workbench version 1.2 modelling software (Danish Technological Institute).

Table 5
S/N calculation (n = 4) for target compounds in METH2 1st and 2nd SIM run[a]

Compound	t_r (min)	QVN ion	Reported LOD (ng/l)	μ (ng/l)	Average S/N (n = 4)	SD
Desethylatrazine	12.16	172	2.0	3.0	25.7	9.5
Desethylterbuthylazine	12.47	186	2.0	6.0	23.2	2.6
Atrazine	13.73	200	2.0	3.0	34.3	8.6
Terbuthylazine	14.23	214	1.0	6.0	31.6	4.3
Ametryn	17.04	227	5.0	6.0	16.3	1.9
Terbutryn	17.75	241	5.0	6.0	7.3	2.4
Metolachlor	18.41	162	2.0	5.5	23.0	1.7
Carbamazepine	28.8	193	10.0	27.8	27.8	5.1
Desisopropylatrazine	11.92	158	10.0	27.8	26.9	2.0
2,6-Dichlorobenzamide	12.28	173	2.0	3.2	17.2	4.5
Simazine	13.56	201	2.0	5.1	10.7	2.9
Propazine	13.83	214	2.0	6.7	21.2	2.7
Prometryn	17.16	241	2.0	6.7	26.0	5.9

[a] Matrix selection is very important for the calibration, control and validation procedure. For calibration and control samples, we selected natural spring water without the target compounds and with a similar organic matrix.

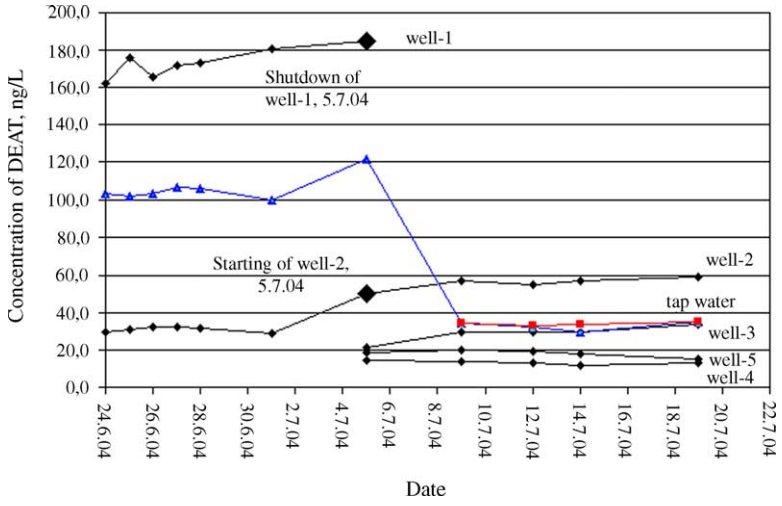

Fig. 3. Verification of the procedure with mass balance calculation for tap water from pumping wells 1–5 after the shutdown of well-1 (triangles: measured values; squares: predicted values).

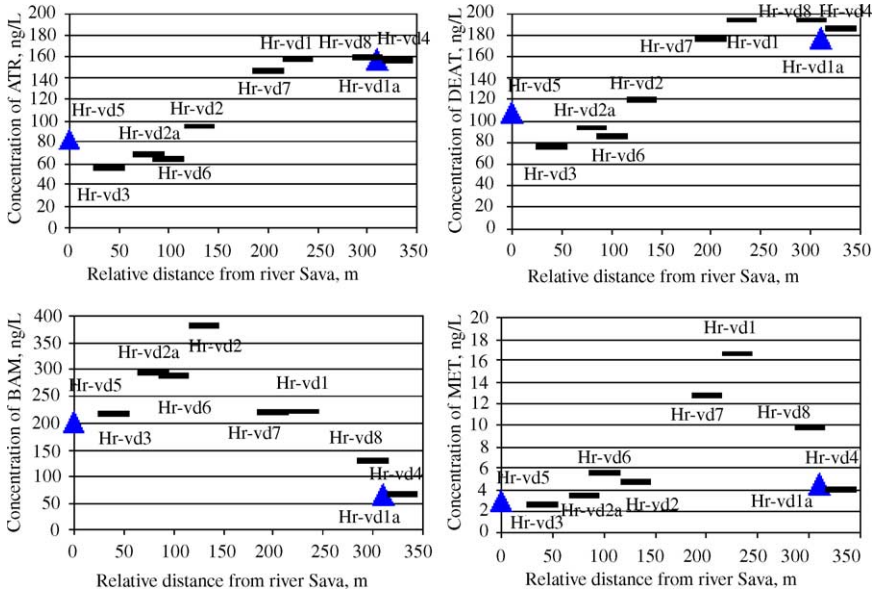

Fig. 4. Spatial distribution of ATR, DEAT, BAM and metolachlor (MET) across the affected well field in September 2003. Triangles are two operational wells.

3. Results and discussion

The best verification of the validation procedure was the control of the operation of the affected well field in the years 2003–2004, to keep the concentration of target herbicides and their degradation products in drinking water below 100 ng/l.

Widespread use of pesticides in the past caused the accumulation of the pesticides atrazine (ATR), DEAT and 2,6-dichlorobenzamide (BAM) in some parts of the aquifer of

Table 8
An example of the use of the method for drinking water management (September 2003)

Analyte	Non-affected well field Jb-vd1	Hr-vd1a	Hr-vd5	Drinking water at the tap	Predicted concentration at the tap	E_r (%)
γ (BAM) (ng/l)	<LOD = 2.0	66.6	202	68.1	67.2	−1.4
γ (ATR) (ng/l)	6.9	157	82.5	67.5	64.9	−6.2
γ (DEAT) (ng/l)	17.2	177	108	85.9	81.3	−7.0
γ (MET) (ng/l)[a]	<LOD = 2.0	(4.5)	(3.0)	(3.0)	1.9	−38.3

[a] All the results for MET are below the LOQ value of 6.7 ng/l.

Ljubljansko polje, which is the main source of drinking water for the city of Ljubljana.

Accurate data on the distribution of ATR, DEAT and BAM in the affected well field (Fig. 4) helped us in adapting the operation regime of the well field to meet government requirements. Only some wells were left in operation because of the distribution of BAM, ATR and DEAT residues across the well field. The position of the wells in the well field is represented spatially as the relative distance from the river Sava, starting with the well closest to the river Sava as 0 m (Fig. 4). Water from the river Sava lowers the concentration of contaminants in the northern part of the well field.

Two wells, Hr-vd5 at 0 m (the relative distance from river Sava), and Hr-vd1a at 330 m (relative distance from the river Sava) (Fig. 4), had the lowest concentrations of ATR, DEAT and BAM and remained in operation, while additional drinking water was supplied by other well fields. In 95% of the samples of finished drinking water the concentration of ATR, DEAT and BAM was below the maximum allowed concentration of 0.1 μg/l (Fig. 4, Table 8). Since 2002, the use of dichlobenil and atrazine containing formulations has been prohibited. The first signs of improvement were seasonal fluctuations in the concentration of MET at the ng/l level, without an increase in the concentration of ATR. The situation in the affected well field has a tendency towards significant improvement. An additional well has been put in operation recently.

4. Conclusions

For the determination of semi-volatile organic compounds, the SPE GC–MS method, using deuterated standards as surrogates for calibration by the overall procedure, is sufficiently accurate and precise. For the optimization of target monitoring, selection of the analyte list and the calibration level are important. The use of deuterated standards as surrogates for calibration by the overall procedure is very suitable when we have a limited number of analytes of interest.

Two calibration levels were used and each of them would give the appropriate result at the MCL for pesticides and their degradation products of 0.1 μg/l. At the level of 100 ng/l, an expanded uncertainty of 10% was determined. The procedure was audited according to ISO EN 17025 and inter-laboratory comparisons were consistently within the assigned range. Attention must still be paid to the rigorous control of GC–MS stability. Calibration errors from the SPE procedure and those from GC–MS analysis are compensated by the use of deuterated standards. A limited number of deuterated standards are available, which may be a disadvantage. In such a case, daily use of control samples, additional use of I.S. for the GC–MS analytical step and rigorous validation procedures are necessary.

A highly precise and accurate analytical method can improve the safety of drinking water. This is even more important when the water supplier is obliged to use water from wells contaminated by the widespread use of pesticides in the past.

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