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Quantification of the new triketone herbicides, sulcotrione and mesotrione, and other important herbicides and metabolites, at the ng/l level in surface waters using liquid chromatography–tandem mass spectrometry

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Abstract

The LC/ESI/MSMS method allows the trace quantification (ng/l) of the new triketone herbicides, i.e. sulcotrione and mesotrione, and important herbicides and metabolites, in natural waters. Solid phase extraction (SPE) for sample enrichment is performed with OASIS® (recoveries 94–112% for parent herbicides). Neutral and acidic compounds were analyzed separately with ESI in positive and negative mode, respectively. Quantification limits varied between 0.5 and 10 ng/l. The acidic herbicides detection was improved by a neutralizing post-column addition solution. The influence of ion suppression on quantification is discussed in detail. It is shown that we could overcome this problem and achieve reliable quantification using isotope labeled internal standards (ILIS) for every single analyte. The methods performance is illustrated with samples from a lake depth profile.

Keywords: Water analysis; Sulcotrione; Mesotrine; Pesticides

1. Introduction

For monitoring purposes and for pesticide environmental fate studies, accurate and cost-effective analytical methods are important. In order to simultaneously quantify the many existing pesticides, which usually present a variety of physical–chemical properties, liquid chromatography coupled to tandem mass spectrometry (LC/MSMS) is the current state of the art analytical technique [1–7]. It has clear advantages over traditional detection methods like, e.g., LC-UV, whose main drawbacks are the lack of selectivity and sensitivity. Compared to GC/MS, the advantage is the broader number of substances that can be analyzed [8]. Sample enrichment is a pre-requisite for reaching low detection limits of a few ng/l, which is achieved using solid phase extraction (SPE) in the case of aqueous samples [9].

LC/MSMS is highly selective technique and allows the accurate and precise determination of substances through the detection of fragments from previously selected precursor ions. However, the ionization process, which is crucial for quantitative measurements, is affected by sample matrix [10–12]. Ion suppression is attributed to the competition that occurs between matrix components (e.g. humic acids and ions) and analytes for ionization or access to the droplet surface for the gas phase emission. This phenomenon is known and different procedures for eliminating or mitigating this effects, e.g. the use of pre-column procedures for matrix elimination [13], matrix matched calibration [14,15], and quantification using standard addition have been reported [4,16]. These methods are only partly effective, except the standard addition, which is, however, very time consuming.

Despite being ion suppression a well know topic, we have observed that matrix ion suppression is seldom emphasized in the literature when it comes to the trace level quantification of pollutants in water. Therefore, one important aspect of this manuscript is to illustrate the potential errors in quantification if ion suppression is not considered. In our opinion, the use of isotope labeled internal standards (ILIS)

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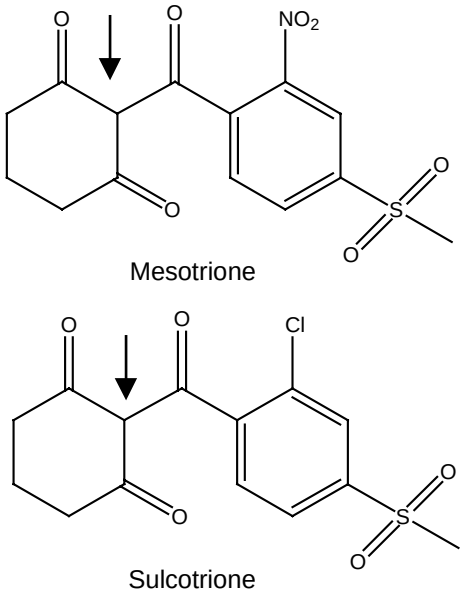

Fig. 1. Triketone herbicides (the arrows indicate the acidic proton in the molecules).

is the only cost effective way to overcome this problem and to conduct reliable quantitative LC/MSMS analysis. Ultimately, the use of ILIS is not applicable to any target analyte since availability is the limiting factor. However, in order to obtain the same data quality using, for instance, the standard addition procedure, more equipment use time would be required, which causes even greater increase in analysis costs and working time.

Important crops like corn and wheat heavily depend on the use of pesticides such as triazines, phenylureas, chloracetanilides, phenoxyacetic acids and organophosphates. Further, new herbicides, e.g. sulcotrione and mesotrione, are being introduced in corn production for which new analytical methods have to be developed (Fig. 1). They belong to the triketone class and present acidic properties (pK_a of around 3) which are determinant for their environmental behavior as well as for the analytical method development itself. Generally, several processes are responsible for the off-site transport of pesticides to surface waters; surface runoff, spray drift and leaching are examples [17,18]. Indeed, natural waters are frequently exposed to exceeding pulse concentrations of pesticides, which makes the quality status of aquatic ecosystems and water resources a permanent issue.

Here, we describe a simple, sensitive and robust LC/ESI/MSMS analytical method for aqueous samples where the triketones, sulcotrione and mesotrione, relatively new corn herbicides, are determined, for which, to our knowledge, no trace LC/MSMS analytical method is available to date. Additional worldwide important herbicides belonging to a variety of classes like triazines, phenylureas, phenoxyacetic and phenoxypropionic acids, chloroacetanilides, amides, and some metabolites are simultaneously quantified. The method uses a one step off-line SPE procedure for the enrichment of all compounds and post-column addition for improved detection in the −ESI [3,19] (Table 1). ILIS were used to overcome the negative effects of ion suppression on the quantitative results. We also illustrate the errors on quantification by presenting the results obtained when other internal standards instead of ILIS were used for quantification, or when one single ILIS is selected for a group of herbicides.

2. Experimental

2.1. Chemicals

Atrazine, desethylatrazine, isoproturon, metolachlor, MCPA and mecoprop standards were obtained by Riedel-de-Haën (Seelze, Germany). Simazine, terbutylazine, diazinon, tebutam, diuron and 2,4-D were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Sulcotrione and mesotrione were kindly supplied by Zeneca (Berkshire, UK). Dimethenamide was obtained from Sandoz Agro (Basel, Switzerland). The isotope labeled internal standards, [$^{13}C_6$]-metolachlor, [D_6]-MCPA, [D_3]-mecoprop, [D_5]-atrazine, [D_5]-simazine, [D_5]-terbutylazine, [D_6]-diuron, [D_6]-isoproturon and [$^{13}C_6$]-2,4-D were obtained from Dr. Ehrenstorfer (Augsburg, Germany). [D_3]-Dimethenamide and acetochlor oxanilic acid (OXA), were kindly supplied by Monsanto Europe (Louvain-la-Neuve, Belgium). [D_4]-Tebutam, [D_3]-sulcotrione and [D_3]-mesotrione were supplied at request by Solvias AG (Basel, Switzerland). [D_{10}]-Diazinon was supplied by Cambridge Isotope laboratories (Innerberg, Switzerland). Dimethenamide ethanesulfonic acid (ESA) and OXA were obtained from BASF AG (Limburgerhof, Germany). [$^{15}N_3$]-Desethylatrazine, metolachlor ESA and metolachlor OXA were supplied by Novartis (Basel, Switzerland). Acetochlor ESA was synthesized according to Aga et al. [20].

HPLC grade methanol and water were used (Scharlau, Barcelona, Spain). All other chemicals were purchased from Merck (Darmstadt, Germany). Nitrogen and argon gases for the LC/MSMS were supplied by Carbagas (Rümlang, Switzerland).

For all analytes, individual stock solutions were prepared in methanol with concentrations of 1000 ng/μl. Mixture solutions were prepared in concentrations of 0.1, 1 and 10 ng/μl. They were used as spike solutions for sample fortification and for the calibration curves. The internal standards solution, prepared in methanol, contained from 25 to 75 ng/10 μl of each substance.

2.2. Sampling and sample preparation

Surface water samples were collected in Lake *Greifensee* and from a small creek located in an agricultural catchment. Wastewater Treatment Plant (WWTP) effluent samples were collected in Dübendorf (Switzerland). Raw samples were

Table 1
Pesticides analyzed and respective precursor ions (protonated and deprotonated molecular ions), main product ions, and secondary product ions

Analyte	Precursor ion (*m*/*z*)	Main product ion		Secondary product ion	
		m/*z*	V	*m*/*z*	V
Atrazine	216.1[b]	174	22	104	34
[D_5]-Atrazine	221.0[b]	179	22	101	30
Desethylatrazine	188.1[b]	146	22	104	32
[$^{15}N_3$]-Desethylatrazine	193.1[b,a]	151[a]	22	109[a]	32
Diazinon[c]	305.1[b]	169	24	153	26
[D_{10}]-Diazinon	315.1[b]	170	24	154	26
Dimethenamide	276.1[b]	168	28	244	20
[D_3]-Dimethenamide	281.1[b,a]	249[a]	20	114[a]	38
Diuron	233.1[b]	72	22	46	22
[D_6]-Diuron	239.1[b]	78	22	52	22
Metolachlor	284.1[b]	252	20	176	30
[$^{13}C_6$]-Metolachlor	290.1[b]	258	20	182	30
Isoproturon	207.1[b]	72	24	46	22
[D_6]-Isoproturon	213.1[b]	78	24	52	22
Simazine	202.0[b]	132	24	124	22
[D_5]-Simazine	207.0[b]	137	24	129	22
Tebutam	234.1[b]	142	20	91	34
[D_4]-Tebutam	238.1[b]	196	20	146	20
Terbutylazine	230.0[b]	174	22	132	32
[D_5]-Terbutylazine	235.0[b]	179	22	137	32
2,4-D	218.9[d]	161	20	124	34
[$^{13}C_6$]-2,4-D	224.9[d]	167	20	130	34
Acetochlor OXA[e]	264.1[d]	146	16	144	36
Acetochlor ESA[e]	314.1[d]	121	26	80	40
Dimethenamide OXA[e]	270.1[d]	198	12	166	22
Dimethenamide ESA[e]	319.9[d]	121	30	77	38
MCPA	199.0[d], 201.0[d,a]	141	20	143[a]	20
[D_6]-MCPA	205.0[d], 207.0[d,a]	147	20	149[a]	20
Mecoprop	213.0[d], 215.0[d,a]	141	22	143[a]	22
[D_3]-Mecoprop	216.0[d], 218.0[d,a]	146[a]	22	144	22
Mesotrione	338.0[d]	291	19	170	46
[D_3]-Mesotrione	341.0[d]	212	38	294	19
Metolachlor OXA[e]	278.1[d]	206	18	158	32
Metolachlor ESA[e]	328.1[d]	80	38	135	36
Sulcotrione	327.0[d]	291	16	212	38
[D_3]-Sulcotrione	331.9[d,a]	212[a]	38	294[a]	16

[a] *m*/*z* considering the natural isotope ^{37}Cl.
[b] Neutral analyte, +ESI, $[M + H]^+$.
[c] Insecticide.
[d] Acidic analyte, −ESI, $[M - H]^-$.
[e] Metabolite.

transferred to 1 l glass bottles and stored in the dark at 4 °C until analysis. Samples were filtered at room temperature in the laboratory with a high-pressure filtration equipment MD142-5-3 (Schleicher & Schuell, Germany) using cellulose nitrate membrane filters NC45, 0.45 μm pore size, diameter 50 mm (Schleicher & Schuell, Germany). After filtration an acetate buffer (5 M) was added (ca. 1%, v/v) to yield a pH of 4. As routine procedure, the water samples analyzed had the 10 μl aliquots of ILIS added to the 1 l vessels before extraction.

2.3. Solid-phase extraction (SPE)

OASIS® *HLB* sorbent cartridges (60 mg) (Waters, Etten-Leur, The Netherlands), were used on a 12-fold vacuum extraction box (Supelco, Bellfonte, PA, USA). Cartridges were conditioned with 2 ml of MeOH followed by 2 ml of water. The 1 l samples were extracted at a flow rate of 15 ml/min. The cartridges were washed with 1 ml of a MeOH–water mixture (5:95, v/v). The excess of water was removed by opening the valves and letting air to pass through them for approximately 3 min. The analytes were eluted with 5 ml of MeOH without the use of vacuum and the aliquots collected in conical bottom glass vessels (Supelco, Bellfonte, PA, USA). The 5 ml MeOH aliquots were reduced to approximately 50 μl with the aid of a gentle nitrogen gas stream at 50 °C. The extracts (50 μl) were then transferred to 1.5 ml amber glass vials and the volume was reconstituted to approximately 500 μl of MeOH–water (50:50, v/v), to obtain the initial mobile phase conditions

for the injection into the LC/MSMS. Chromatographic runs in the +ESI mode and −ESI mode were made separately.

2.4. Recovery studies

The absolute cartridge recovery yields were tested in lake water, creek water and WWTP effluent. Absolute cartridge recoveries were determined using one liter of sample matrix spiked at five concentration levels: 10, 25, 50, 100 and 150 ng/l and 1 l of unfortified matrix to check for any original background concentration of the analytes of interest. A 10 μl aliquot of the internal standard solution was added into the 5 ml MeOH eluate in the conical bottom glass vessels, after the cartridge elution and before solvent evaporation. The data set was processed as follows: plotting the ratios between *analyte peak area* and *internal standard peak area* against corresponding concentration levels and performing a linear regression, yielding calibration curves with five concentration levels for each analyte in each matrix. Standard solutions with the same concentration levels were used to prepare analogous calibration curves (defined as external calibration curves). The absolute cartridge recovery of each analyte resulted from calculating the ratio between the slope of the calibration curve obtained from each matrix extract and the slope of the external calibration.

For sample quantification two types of calibrations curves were used: the external calibration, and calibration curves from standards extracted from spiked nanopure water (defined as extracted calibration curves). Blanks were prepared by extracting of one liter of nanopure water, in order to control for carry-over.

2.5. Ion suppression evaluation

One liter of each matrix (lake water, creek water and WWTP effluents) was extracted. The reconstituted matrix extract (500 μl) had 10 μl aliquot of internal standard solution added and was, subsequently, divided in four parts (ca. 125 μl each). Standard addition to the extracts was made to yield concentrations equivalent to one liter samples containing 0, 20, 40 and 60 ng/l of each analyte after enrichment. The four extracts were analyzed, and curves were obtained by plotting *analyte peak areas* against corresponding concentration levels for all analytes in all matrices. Linear regression was performed for each curve and the slopes were related to the respective slopes of the analogue curves obtained by plotting the *analyte peak areas* from *standard solutions* against the same concentration levels, which was adopted as reference. The ion suppression was quantified as the ratio between the slope of the curve in matrix extracts and the slope of the curve obtained using standard solutions.

2.6. Mass spectrometry

Target substances were detected with a TSQ Quantum 2000 bench-top triple quadrupole MS from Thermo Finnigan (San Jose, CA) provided with an ESI probe. High purity nitrogen was used as sheath and auxiliary gas and high purity argon was used as collision gas. The MS was calibrated using a 1,3,6-polytyrosine solution. Optimization of ionization and fragmentation conditions for the analytes were obtained by the infusion of individual analyte methanolic solutions (10 mg/l) at a flow rate of 50 μl/min, connected by a "tee-fitting" to a 50:50 (v/v) MeOH–water flow of 150 μl/min. Predominant precursor ion masses *(m/z)* were selected. Selected reaction monitoring (SRM) was used for the identification of main fragments and instrument parameters like collision energies and tube lens offset were optimized. Instrument parameters for the +ESI mode were: +3500 V spray voltage, capillary temperature 300 °C, sheath gas pressure 40 bar, auxiliary gas pressure 5 bar, scan time 0.10–0.40 s, scan width 1.0 *m/z*, peak width 0.70 *m/z*. The parameters for −ESI mode were: −2500 V spray voltage, capillary temperature 350 °C, sheath gas pressure 40 bar, auxiliary gas pressure 5 bar, scan time 0.10 s, scan width 1.0 *m/z*, peak width 0.70 *m/z*. Throughout the chromatographic run, the detection was divided in time windows where 13–20 traces were acquired.

2.7. Liquid chromatography

The HPLC system consisted of a Rheos 2000 pump equipped with a solvent degaser (Flux Instruments AG, Switzerland), a HTS Pal autosampler (CTC Analytics, Zwingen, Switzerland) and a column oven Jones 7956 (Omnilab AG, Mettmenstetten, Switzerland). The analytical columns used for analyte separation were: (1) Macherey-Nagel (Düren, Germany) Nucleodur C_{18} Gravity, 125 mm × 2 mm i.d., 3 μm, with a guard column of the same material (10 mm × 2 mm), for the neutral analytes; (2) GromSil (Rottenburg-Hailfingen Germany) polymer coated C_{18}, 150 mm × 2 mm i.d., 3 μm, with a guard column of the same material (10 mm × 2 mm), for the acidic analytes.

The optimized conditions for the analysis of the *neutral* herbicides ($[M + H]^+$) (Table 1) were as follows. The mobile phase was composed of water acidified with 0.1% formic acid (solvent A), and MeOH acidified with 0.1% formic acid (solvent B). The LC gradient for the separation of neutral herbicides was: isocratic from 0 to 10 min (50% A: 50% B); from 10 to 18 min, a linear increase of B from 50 to 80%; isocratic 80% B from 18 to 22 min. Initial conditions were re-established in 3 min and column re-equilibration was performed in 5 min. The flow rate was 0.2 ml/min and the column temperature was 35 °C.

The optimized conditions for the analysis of the *acidic* herbicides ($[M - H]^-$) (Table 1) were as follows. The mobile phase was composed of water acidified with 0.6% formic acid (solvent A), and MeOH acidified with 0.6% formic acid (solvent B). The LC gradient for the separation of acidic herbicides was: linear increase of B from 40 to 95% in 20 min; isocratic 95% B from 20 to 22 min. Initial conditions were re-established in 3 min and re-equilibration time was 5 min.

The flow rate was 0.15 ml/min and the column temperature was 60 °C. Post-column addition of neutralization solution, tris(hydroxymethyl) aminomethane (Tris base) (1 mM) in MeOH–water mixture (1:1, v/v) was made with the use of a "tee-fitting" using a Gynkotek HPLC pump, Model M 480 (Germering, Germany) operated at 0.05 ml/min flow.

3. Results and discussion

3.1. MS parameters

For all the analytes, deprotonated ($[M - H]^-$) and protonated ($[M + H]^+$) molecular ions were the major precursor ions formed. Specific and intense product ions of each target analyte were used for the quantification, and a secondary product ion was monitored for confirmatory purposes (see Table 1).

Some analytes did not present detectable secondary product ions, like MCPA, Mecoprop and [D_6]-MCPA. Once they contain one chlorine atom, this enabled the acquisition of *m*/*z* accounting for their natural ^{37}Cl isotopes ($m/z + 2$) for confirmation purposes. The ratio between the analyte precursor ion mass ($m/z_{analyte}$) and the precursor ion mass including the natural ^{37}Cl isotope ($m/z_{analyte} + 2$) is 3:1. Moreover, for ILIS containing one chlorine atom and possessing only three units masses difference from the target analyte, e.g. [D_3]-mecoprop, [D_3]- sulcotrione, [$^{15}N_3$]-desethylatrazine and [D_3]-dimethenamide, their *m*/*z* accounting for the natural ^{37}Cl isotope were used for quantification. This procedure was taken to eliminate the interference from the natural isotopes ^{13}C and ^{37}Cl of the corresponding target analyte.

3.2. LC conditions

All herbicides and their metabolites were simultaneously enriched (see Section 2.3). To obtain the best sensitivity, however, the extracts were analyzed for neutral and acidic herbicides separately.

3.2.1. Neutral analytes

The mobile phase consisted of water and MeOH acidified with formic acid to favor the protonation of the analytes in +ESI. Methanol and formic acid are good mobile phase components for LC/MSMS due to low surface tension that favors the electrospray droplet formation [21]. Even though full chromatographic resolution of analytes is not a pre-requisite for selective acquisition and quantification when using highly specific MSMS detection [22], baseline resolution of substances of interest can be helpful for achieving low detection limits because, at each time interval, the detector scans fewer *m*/*z* per time window. Therefore, we attempted to obtain good baseline resolution. In our case, baseline separation was achieved for most of the compounds, as can be seen in Fig. 2a.

3.2.2. Acidic analytes

Most probably the polymer coated silanol groups of the chosen column were responsible for overcoming peak tailing of the triketones, through preventing strong interactions between analytes and the stationary phase. Acetochlor OXA required an elevated temperature of 60 °C for the chromatography, since this substance is a diastereosomer. At room temperatures the analyte would be split in two peaks [23]. By maintaining the column at 60 °C, the rotational barrier is overcome resulting in the elution of a single peak for acetochlor OXA.

The use of TFA in the mobile phase, despite producing excellent peak shapes for the acidic analytes, as observed with UV detection, could not be adopted because of the extreme suppression of the $[M - H]^-$ deprotonated precursor ion. For this reason, formic acid was used instead. Fig. 2b shows the chromatogram obtained for the acidic herbicides detected in −ESI mode.

Some studies have demonstrated that the sensitivity of −ESI can easily be influenced by liquid chromatographic conditions and matrix interference, worsening the method detection limits [3,10,12,19]. In order to maximize detector response, as one of the last steps of the method development, different solutions were tested for post-column neutralization: ammonium acetate (100 mM), ammonium hydroxide (100 mM), tributylamine (100 mM) and Tris base (1 mM). Best results, evaluated in terms of peak areas (data not shown), were obtained with the Tris base solution, improving the sensitivity for the analytes by a factor of 13–22. Using a 1 mM solution, the problem of clogging the ESI source was prevented, while still producing the desired neutralization effects.

3.3. Sample preparation and quantification of recoveries

The SPE procedure described by Ollers et al. [24] using OASIS® HLB sorbent was adopted, except that final cartridge elution was made with methanol instead of ethylacetate–acetone to be compatible with the chromatographic eluents. It is possible to distinguish two qualities of absolute cartridge recoveries (Table 2). Very high absolute cartridge recoveries in lake water, creek water and WWTP effluent were achieved for the analytes that were quantified with the use of ILIS (94–112%). Whereas the absolute cartridge recoveries of the herbicide degradation products, OXA and ESA metabolites of metolachlor and dimethenamide, where no ILIS were used, were much lower. For these analytes acetochlor OXA and ESA were used. In these particular cases it is desirable to know if the reduced analyte recovery observed is related to ion suppression and the different ionization efficiencies for analyte and internal standard, or really actual losses during the enrichment step. Therefore, in order to know the correct absolute cartridge recovery for this group of metabolites, their respective ion suppression was compensated for (see discussion below in Section 3.4). This

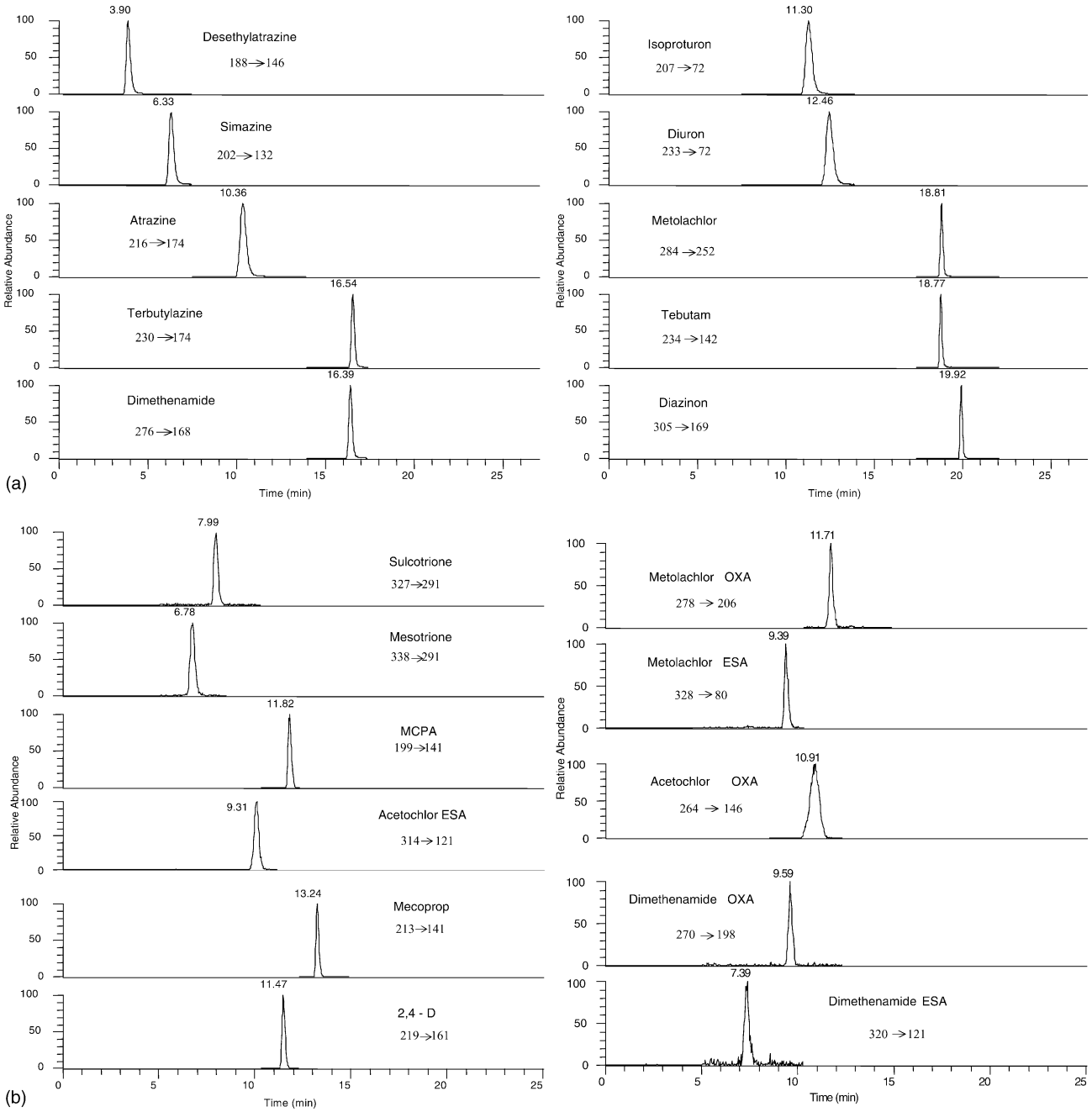

Fig. 2. Chromatogram of pesticides acquired in (a) +ESI and (b) −ESI in extracted Lake water (1 l) spiked at the 50 ng/l level. Refer to the winter lake profile (Fig. 4) for original background concentrations in addition to the spiked amount.

yielded the real absolute cartridge recoveries, e.g. for metolachlor ESA and OXA, of 67 to 99%. For dimethenamide ESA and OXA, recoveries were between 16 and 50%. Being these compounds the ones that suffer less from ion suppression, we attribute these low cartridge recoveries to the extraction material used, which was probably not as effective in extracting these substances as it showed to be for the other analytes. In addition, evidences of breakthrough were observed for the herbicide metabolites (data not shown).

3.4. Quantification of ion suppression

An important but not often considered problem in quantitative LC–MS/MS is the matrix interference, which affects sensitivity and, even worse, leads to large quantification errors.

Table 3 shows the matrix ion suppression effects, obtained with the experimental conditions presented in Section 2.5. Ion suppression occurred for practically all analytes and in all matrices investigated, reaching up to 71%. For

Table 2
Absolute cartridge recoveries obtained in different matrices

Substance	Absolute recovery (%)		
	Lake water	Creek water	WWTP
Neutral analytes			
Atrazine[a]	105	109	104
Desethylatrazine[a]	102	105	108
Diazinon[a]	94	102	94
Dimethenamide[a]	106	107	103
Diuron[a]	105	105	104
Isoproturon[a]	106	106	106
Metolachlor[a]	102	101	100
Simazine[a]	106	108	105
Terbutylazine[a]	99	100	101
Tebutam[a]	99	100	94
Acidic analytes			
2,4-D[a]	112	108	106
Dimethenamide ESA[b]	42 (33)	50 (41)	30 (19)
Dimethenamide OXA[c]	28 (23)	29 (25)	16 (14)
MCPA[a]	105	108	111
Mecoprop[a]	107	105	105
Mesotrione[a]	100	98	109
Metolachlor ESA[b]	91 (76)	99 (80)	67 (46)
Metolachlor OXA[c]	97 (90)	85 (83)	70 (60)
Sulcotrione[a]	112	106	105

[a] Use of isotope labeled internal standard.
[b] Acetochlor ESA as internal standard.
[c] Acetochlor OXA as internal standard (acetochlor is a herbicide not allowed in Switzerland); in brackets: absolute recoveries without the compensation of ion suppression effects.

Table 3
Ion suppression for the analytes of interest in different matrices

Substance	Matrix		
	Lake water (%)	Creek water (%)	WWTP (%)
Neutral analytes			
Atrazine	36	30	53
Desethylatrazine	43	39	62
Diazinon	47	27	65
Dimethenamide	22	15	52
Diuron	26	17	45
Isoproturon	25	15	45
Metolachlor	22	15	46
Simazine	42	36	59
Terbutylazine	27	20	50
Tebutam	30	25	55
Acidic analytes			
2,4-D	43	28	57
Dimethenamide ESA	17	+2	44
Dimethenamide OXA	14	0	23
MCPA	46	30	54
Mecoprop	28	8	16
Mesotrione	53	41	60
Metolachlor ESA	12	8	33
Metolachlor OXA	2	+10	28
Sulcotrione	59	42	71

dimethenamide ESA and metolachlor OXA, the positive values correspond to a signal enhancement caused by the creek water matrix. As expected, ion suppression was the highest in WWTP effluent samples followed by the creek and lake water samples, where it occurred in the same extent. Interestingly, it was observed that ion suppression for the acidic analytes, measured in −ESI, was not distinguishably higher than ion suppression of the neutral analytes, measured in the +ESI, even though −ESI has been reported to suffer the most from interferences [10]. Furthermore, it was shown that ion suppression caused by creek water and lake water for the herbicide metabolites metolachlor and dimethenamide OXA and ESA, were slightly lower than for the other analytes.

3.5. Overcoming quantification problems with the use of ILIS

The data set used for evaluating ion suppression (see Section 2.5 for details) was also the basis for constructing calibration curves in matrices using the ratio *analyte peak area*/*internal standard peak area* plotted against concentration levels. This leads to curve slopes identical to those of the analogue calibration curves obtained using standard solutions. This is because the ILIS have the same retention time, ionization efficiency and ion suppression as the target analytes. Therefore, the ratio *analyte peak area*/*internal standard peak area* remains constant whether the matrix is present or not, or throughout the batches, hence, calibration curves in standard solution and extracts are identical and we are able to, safely, use calibration curves obtained from standard solutions to quantify samples that contain any of the matrices we investigated. Clearly, ILIS compensate matrix effects and allow accurate and precise quantification. However, in the cases of the herbicide metabolites dimethenamide and metolachlor OXA and ESA, where non-ILIS were adopted (acetochlor ESA and OXA were used as internal standards), we observed that the slopes of the calibration curves from standard solutions and, for instance, creek water matrix differed up to 25%. The reason for the differences in the slopes of calibration curves in matrix and standard solutions is that these analogue internal standards did not fully compensate the matrix effects because, based on minor differences in molecular structure, they do not co-elute with target analytes and behave differently during the ionization process. With this observation, we could conclude that, for the herbicide metabolites where non-ILIS are used, the quantification in this matrix could contain variations of up to 25%.

In Fig. 3, we illustrate the quantification uncertainty resulting when one single ILIS is adopted for the quantification of a group of analytes. This is a procedure found in the literature for the quantification of acidic herbicides in water using LC/MSMS [25]. Here we describe the quantification uncertainties obtained when using [D_3]-mecoprop as ILIS for quantifying the acidic analytes. They are based on the different ion suppression and recovery values that

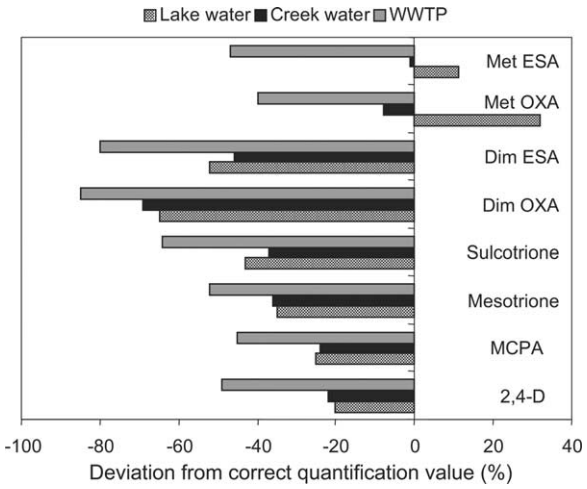

Fig. 3. Errors in quantification for acidic analytes in different matrices when using [D_3]-mecoprop instead of the correspondent isotope labeled internal standard.

each acidic analyte has related to this internal standard (data from Tables 2 and 3). The predicted deviations from the correct quantification values (when the corespondent ILIS is used) are from −85 to +32% (see Fig. 3). For the acidic analytes which have recoveries of 100%, the uncertainties are attributable to differences in ion suppression between [D_3]-mecoprop and the analyte, whereas the recoveries are compensated. However, for the analytes which do not have 100% recoveries, the metabolites, the uncertainties originate from recovery differences between the [D_3]-mecoprop and the analyte plus the differences in ion suppression that they posses. When the same estimation is made for the neutral analytes, desethylatrazine, simazine, terbutylazine, diuron and isoproturon using [D_5]-atrazine, the errors would range between −19 and +21% from the correct ones (data not shown). Due to the variability of matrix influence on ion suppression and recovery, the observed errors cannot be extrapolated to different samples and, as a result, the use of correction factors is not possible. This illustrates once more the need of using corresponding ILIS for LC/MSMS quantitative trace analysis.

3.6. Method detection limits and precision

The method detection limits (MDL), 3:1 signal to noise ratio, obtained in one liter spiked and extracted nanopure water and creek water, for neutral substances were between 0.15 and 0.6 ng/l and the method quantification limits (MQL), 10:1 signal to noise ratio, were between 0.5 and 2 ng/l. Among neutral analytes, best sensitivities were observed for diazinon and tebutam, and diuron presented the lowest one. The MDL for the acidic analytes in creek water were more variable, between 0.3 and 3 ng/l, and higher compared to the MDL in nanopure water

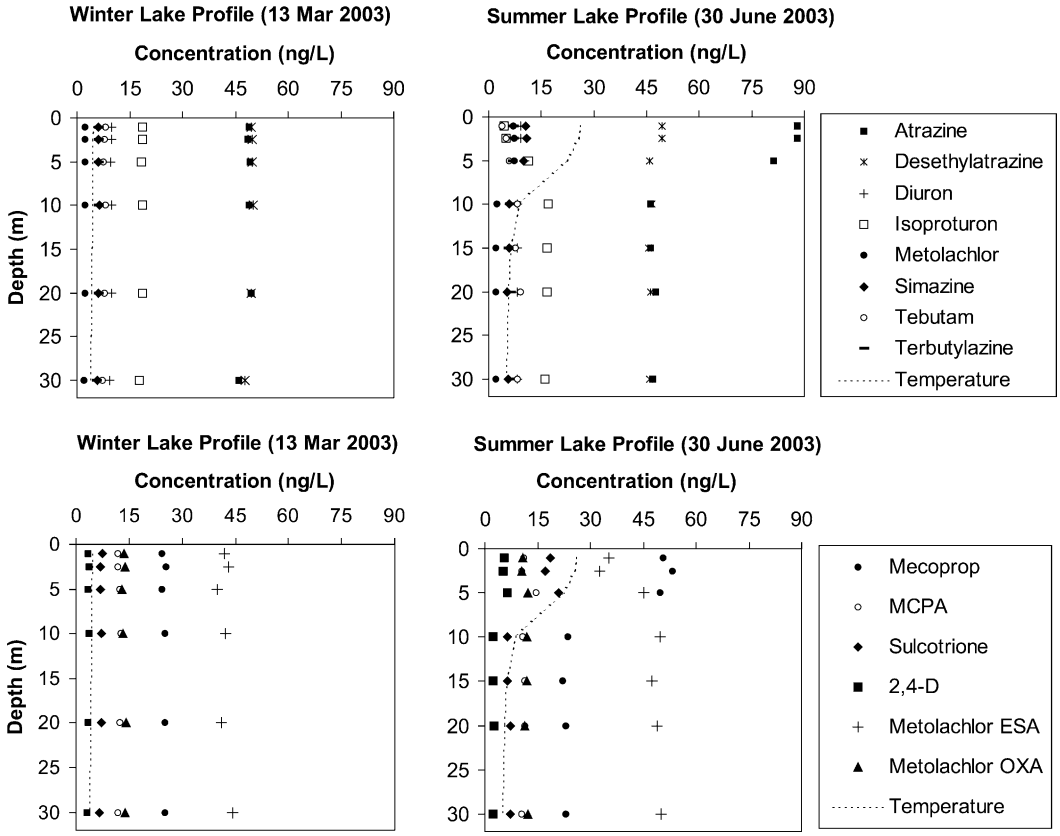

Fig. 4. Herbicide concentration levels measured in lake water (*Greifensee*) at different depths and seasons (summer and winter).

extracts (between 0.15 and 1.5 ng/l). The higher MDL for these analytes in creek water can only result from poorer ionization efficiency in −ESI in the presence of matrix, since all analytes studied showed similar ion suppression magnitudes. The MQL for the acidic substances varied between 0.5 and 10 ng/l. Best sensitivities were observed for 2,4-D, MCPA, mecoprop and mesotrione, whereas dimenthenamide OXA and ESA presented the lowest ones.

Furthermore, the reproducibility of the analytical procedure (SPE and LC/MSMS analysis) was evaluated by extracting and analyzing unfortified replicates ($n = 3$) of lake water and verifying the relative standard deviations (R.S.D.) of the analyte concentrations. The relative standard deviations observed were very small, i.e. between 0.15 and 5.50% for all substances except metolachlor ESA and OXA where the R.S.D. ranged from 10 to 17%. The reproducibility results were not assessed for mesotrione, dimethenamide OXA and ESA that were not present in the unfortified lake samples. For quality assurance the ratio between the analyte precursor ion mass (m/z) and the precursor ion mass including the ^{37}Cl natural isotope ($m/z + 2$) was used. Moreover, enriching and processing a number of one to three previously analyzed samples at each new analysis batch and comparing the results checked the method repeatability. In this case, variations under 5% between two measurements were obtained. As part of the quality control, extracted and external calibrations curves were prepared at each batch of samples to be quantified.

3.7. Application

The applicability of the analytical method developed is illustrated with determinations in natural waters. Fig. 4 shows vertical concentration profiles measured for Lake *Greifensee* (Switzerland) in summer and winter. In summer the lake is stratified presenting two distinct water layers. Each layer can be regarded as completely mixed. Therefore, the concentrations measured in the lake water column are expected to be homogeneous within the layer but differing between the epilimnion (upper layer) and hipolimnion (lower layer). In winter, when the lake water column is completely mixed, an homogeneous concentration profile is expected and, therefore, these profiles can be used as a means of method performance verification. The winter lake profiles corroborate nicely with the expected results and the relative standard deviations are within the reproducibility values described above.

Concerning the new herbicides, sulcotrione and mesotrione, only the former was detected in the analyzed samples to date. However, the interest on mesotrione consists in the lately increase in its use in corn crops. Additionally, the method is a valid analytical tool for monitoring and in the research on pesticide fate and transport, where conditions like ruggedness and reliability, indispensable for a long-term application, are fulfilled.

4. Conclusions

The analytical method described here shows the rapid and simple application of LC/ESI/MSMS to the simultaneous analysis of relevant classes of herbicides in natural waters, specially the new herbicides from the triketone class, sulcotrione and mesotrione, for which up to date no LC/MSMS method was described in the literature. It was demonstrated that adopting one sample preparation procedure, the structurally different chemicals could be simultaneously enriched. LC/MSMS is a powerful technique, however, we showed that matrix ion suppression effects are crucial and should always be considered in LC/MSMS quantitative trace analysis. Despite the fact that ion suppression was sometimes high, it was still possible to obtain sensitivity that fulfilled our initial goals. It was also illustrated, that the use ILIS for each compound is a prerequisite for achieving reliable quantification and high precision (R.S.D. $\leq$ 6%).

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