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Study of matrix effects on the direct trace analysis of acidic pesticides in water using various liquid chromatographic modes coupled to tandem mass spectrometric detection

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Abstract

This study investigated the effects of matrix interferences on the analytical performance of a triple quadrupole mass spectrometric (MS–MS) detector coupled to various reversed-phase liquid chromatographic (LC) modes for the on-line determination of various types of acidic herbicides in water using external calibration for quantification of the analytes tested at a level of 0.4 μg/l. The LC modes included (i) a single-column configuration (LC), (ii) precolumn switching (PC-LC) and (iii) coupled-column LC (LC–LC). As regards detection, electrospray (ESI) and atmospheric pressure chemical ionization (APCI) in both positive (PI) and negative (NI) ionization modes were examined. Salinity and dissolved organic carbon (DOC) were selected as interferences to study matrix effects in this type of analysis. Therefore, Milli-Q and tap water samples both fortified with 12 mg/l DOC and spiked with sulfometuron-methyl, bentazone, bromoxynil, 2-methyl-4-chlorophenoxyacetic acid, and 2-methyl-4-chlorophenoxypropionic acid at a level of about 0.4 μg/l were analyzed with the various LC–MS approaches. Direct sample injection was performed with volumes of 0.25 ml or 2.0 ml on a column of 2.1 mm I.D. or 4.6 mm I.D. for the ESI and APCI modes, respectively. The recovery data were used to compare and evaluate the analytical performance of the various LC approaches. As regards matrix effects, the salinity provided a dramatic decrease in response for early eluting analytes (*k* value of about 1) when using the LC mode. Both PC-LC and LC–LC efficiently eliminated this problem. The high DOC content hardly effected the responses of analytes in the ESI mode, while in most cases the responses increased when using APCI-MS–MS detection. Of all the tested configurations, LC–LC–ESI-MS–MS with the column combination Discovery C_{18}/ABZ+ was the most favorable as regards elimination of matrix effects and provided reliable quantification of all compounds using external calibration at the tested low level. The major observed effects were verified with statistical evaluation of the data employing backwards ordinary least-square regression. All tested column-switching modes hyphenated to ESI- or APCI-MS–MS allowed the on-line multi-residue analysis of acidic pesticides in the reference water down to a level of 0.1 μg/l in less than 10 min, emphasizing the feasibility of such an approach in this field of analysis.

Keywords: Matrix effects; Water analysis; Pesticides

1. Introduction

Recently, we have developed an efficient methodology for the screening of acidic pesticides in

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environmental water samples [1,2]. The approach consists of a simple solid-phase extraction (SPE) of the water sample on a C_{18} cartridge and the instrumental processing of uncleaned extracts by coupled-column liquid chromatography (LC–LC) with UV detection. The use of at least one analytical column packed with restricted access material (RAM) adequately solved the chromatographic humic acid hump problem encountered in these types of analyses. The obtained improvement in UV detection (228 nm) allowed in a cost-effective way the fast screening of acidic pesticides in environmental water samples down to a level of at least 0.1 μg/l [1,2].

Unfortunately, UV detection at low wavelengths does not provide sufficient selectivity. Once a positive sample is encountered, additional confirmation is necessary in order to avoid false positives. For example, our usual approach consisted of the screening of samples with robust LC–LC–UV, followed by a confirmation of the analytes in positive samples by gas chromatography–mass spectrometry (GC–MS) following derivatization of the analytes [2,3].

Nowadays, the availability of easy-to-operate LC–MS systems offering high sensitivity and selectivity [4] makes LC–MS a fast growing technique for the trace analysis of pesticides [5–8] in environmental samples. Moreover, LC–MS equipped with the robust and efficient atmospheric pressure ionization (API) techniques, viz. electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) allows the determination of a broad spectrum of pesticides, and hence, offers high productivity.

However, MS(MS) instruments are much more expensive in comparison to current conventional LC detectors. Hence, replacement of existing methodology (see also above) by LC–MS or LC–MS–MS procedures will depend on cost reduction in time of sample pretreatment, chromatographic run time and method development time.

Another aspect to be considered is the effect of matrix on MS detection. For example, in the case of the trace analysis of pesticides in environmental water samples signal suppression caused by the presence of humic acids [9–12] has been observed.

Hence, special attention has to be paid to calibration, e.g., the use of adequate internal standards or matrix-matched standards, in order to obtain reliable quantification. Depending on the type and number of pesticide/matrix combinations, these calibration procedures can be laborious and difficult or, in case of isotope standards, very expensive.

Recent studies [13–20] demonstrate that for the LC–MS analysis of various types of pesticides in environmental samples, adequate calibration can be performed with an external standard method. In the case of neutral and or slightly basic polar pesticides, such as phenylurea and triazine herbicides, sample extracts or samples can be processed with LC–MS without any clean-up [11–17].

Unfortunately, in comparison to neutral/basic compounds, an additional relatively large amount of humic acid matrix is always co-extracted with the acidic pesticides. The information about the effects of these type of interferences on MS and/or MS–MS is conflicting. Using LC–ESI-MS–MS, significant signal suppression of various types of polar pesticides was observed in the presence of humic material [9–12]. In the LC–APCI-MS analysis of acidic pesticides in uncleaned extracts of environmental waters, the co-extracted interferences did not significantly effect the sensitivity [8]. Other methods apply off-line SPE procedures including a clean-up on selective sorbents [11,18–20] or make use of isotope analytes as internal standards [12] in order to perform reliable quantification.

The aim of this study is to investigate the performance of a triple-stage quadrupole MS–MS detector in the high-speed trace analysis of acidic pesticides in water, including the effect of matrix interferences. Analogously to our previous study [1], bromoxynil, bentazone, 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2-methyl-4-chlorophenoxypropionic acid (MCPP) and sulfometuron-methyl were selected as model compounds to represent a heterogeneous group of acidic pesticides. Drinking water fortified at a level of 12 mg/l with dissolved organic carbon (DOC) was used as reference sample to study the matrix effects on quantification.

2. Experimental

2.1. Chemicals

The acidic herbicides sulfometuron-methyl, bentazone, bromoxynil, MCPA and MCPP, 99% pure or higher, were obtained from Dr. S. Ehrenstorfer

(Augsburg, Germany). Acetonitrile and methanol, both LC grade, were obtained from ScharLab (Barcelona, Spain). Analytical-grade formic acid (content 98%) was from Fluka (Buchs, Switzerland). LC-grade water (pw) was obtained by purifying demineralized water in a Nanopure II system (Barnstead, Newton, MA, USA). Tap water (tw) was from the laboratory.

Stock standard solutions (ca. 500 mg/l) of herbicides were prepared in acetonitrile. For the LC–MS analysis, the stock solutions were diluted and mixed with LC-grade water containing 0.1% (v/v) formic acid.

An aqueous stock solution of DOC of 1.33 mg/ml was prepared from a commercial humic acid material (Fluka; lot/product No. 35069 288/53680) under defined conditions [21].

Mobile phases consisted of mixtures of acetonitrile–0.1% aqueous formic acid (pH about 2.7).

2.1.1. Precolumn

A 4.6 mm×5.8 mm I.D. column packed with Hypersil 10 μm Prelute ODS (Gilson, Villieres-le-Bel, France) was used. The precolumn was always used in the backflush mode.

2.1.2. Analytical columns

A 50 mm×4.6 mm I.D. column packed with 3 μm C_{18} Microspher (Chrompack, Bergen op Zoom, The Netherlands); a 50 mm×2.1 mm I.D. column packed with 5 μm Discovery C_{18} (Supelco, Bellefonte, PA, USA); a 50 mm×2.1 mm I.D. column packed with 5 μm Pinkerton ISRP GFF-II-S5-80 (Regis, Morton Grove, IL, USA); a 50 mm×2.1 mm I.D. column packed with 5 μm SPS-5PM-S5-100-ODS (Regis); and a 100 mm×2.1 mm I.D. column packed with 5 μm Supelco ABZ+ (Supelco) were used.

2.2. Equipment

The LC systems schematically shown in Fig. 1 consisted of a Model 233 XL autosampler, AS, from Gilson (Villiers-le-Bel, France) equipped with an auxiliary high-pressure valve, HV, for column switching, a Model 510 isocratic LC pump, P-1,

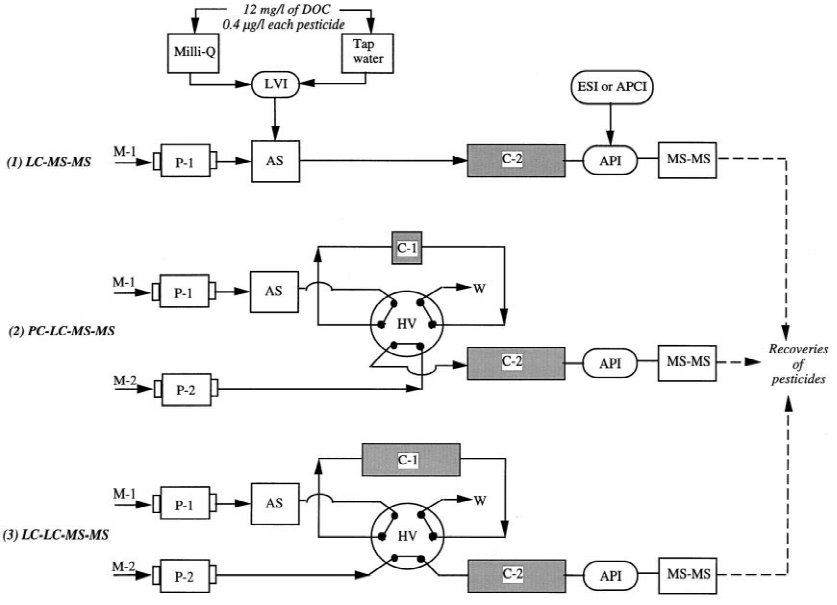

Fig. 1. Schemes of the LC–MS–MS systems applied for the on-line analysis of acidic pesticides in water. LC, use of a single analytical column; PC-LC, column switching with a precolumn and an analytical column; LC–LC, column switching with two analytical columns; LVI, large volume injection; C-1 and C-2, first and second LC column; M-1 and M-2, first and second mobile phase; AS, autosampler; P-1 and P-2, LC pumps; HV, six-way high-pressure valve; API, atmospheric pressure ionization; ESI, electrospray ionization; APCI, atmospheric pressure chemical ionization; MS–MS, triple stage quadrupole mass spectrometer; W, waste.

Table 1
Information on columns used in LC–MS–MS approaches

Dimension length×I.D. (mm)	Packing material	Code column	Used as[a]	API-MS mode
4.6×5.8	10 μm Prelute ODS	A	PC	ES and APCI
50×2.1	5 μm Discovery C_{18}	B	C-1 and C-2	ES
100×2.1	5 μm Supelco ABZ+	C	C-2	ES
50×2.1	5 μm ISRP GFF-II	D	C-1	ES
50×2.1	5 μm SPS C_{18}	E	C-1	ES
50×4.6	3 μm Microspher C_{18}	F	C-1 and C-2	APCI

[a] PC, Precolumn used in precolumn switching (PC-LC); C-1 and C-2, first and second analytical separation column used in the single-column approach (LC) or in the coupled-column (LC–LC) approach.

from Waters (Milford, MA, USA) for the delivery of M-1, and a Model 600 quaternary gradient pump, P-2, from Waters for the delivery of M-2.

The MS–MS system consisted of a Quattro LC triple-stage quadrupole instrument equipped with a dual electrospray/atmospheric pressure chemical ionization source (Micromass, Manchester, UK). Drying gas as well as nebulizing gas was nitrogen generated from pressurized air in a NG-7 nitrogen generator (Aquilo, Etten-Leur, The Netherlands). Infusion experiments were performed using a Model 11 single syringe pump (Harvard, Holliston, USA), directly connected to the interface.

For operation in the MS–MS mode, collision gas was argon 99.995% (Carburos Metalicos, Valencia, Spain) with a pressure of $5 \cdot 10^{-4}$ mbar in the collision cell. The source temperature was set to 120°C and dwell times of 0.2 s/scan were chosen.

2.3. Samples and pretreatment

Two types of reference water samples were prepared by adding to a volume of both purified Milli-Q water (pw) and tap water (tw): (i) 0.1% (v/v) of formic acid, (ii) DOC to a level 12 mg/l and (iii) herbicides at a level of 0.4 μg/l (each analyte).

Samples were directly injected into the LC–MS systems studied, without any further pretreatment.

2.4. LC–MS procedures

Information on the columns and LC–MS methods, including conditions for LC and MS detection modes, is given in Fig. 1, Tables 1 and 2, respectively. Information on the applied MS–MS conditions for the detection and quantification of analytes is given in Table 3.

Table 2
Overview of LC–MS–MS methods and conditions

LC–MS–MS method[a]		Columns		Mobile phase[b]		Volumes (ml) applied for		
Description	Code	C-1	C-2	M-1	M-2	Injection	Clean-up	Transfer
LC–ESI	1e	–	B	35%; 0.2 ml/min	–	0.25	–	–
LC–APCI	1a	–	F	35%; 1.0 ml/min	–	2.0	–	–
PC-LC-ESI	2e1	A	B	0%; 0.50 ml/min	35%; 0.2 ml/min	0.5	1.0	3.0
PC-LC-ESI	2e2	A	B	0%; 0.50 ml/min	35%; 0.2 ml/min	1.0	1.0	3.0
PC-LC-ESI	2e3	A	C	0%; 0.50 ml/min	65%; 0.2 ml/min	1.0	0.5; 1.0; 2.0	3.0
PC-LC-APCI	2a1	A	F	0%; 1.0 ml/min	35%; 1.0 ml/min	2.0	1.0	3.0
LC–LC-ESI	3e1	B	B	25%; 0.3 ml/min	45%; 0.2 ml/min	0.25	1.35	0.70
LC–LC-ESI	3e2	B	B	35%; 0.3 ml/min	45%; 0.2 ml/min	0.25	1.05	0.70
LC–LC-ESI	3e3	B	C	35%; 0.3 ml/min	65%; 0.2 ml/min	0.25	0.90	0.50
LC–LC-ESI	3e4	D	B	30%; 0.3 ml/min	50%; 0.2 ml/min	0.25	0.69	2.04
LC–LC-ESI	3e5	E	B	30%; 0.3 ml/min	50%; 0.3 ml/min	0.25	1.05	0.75
LC–LC-APCI	3a1	F	F	35%; 1.0 ml/min	45%; 1.0 ml/min	2.0	5.0	2.5

[a] See Fig. 1 for experimental set-up; ESI, electrospray ionization mode; APCI, atmospheric pressure chemical ionization mode.
[b] % (v/v) acetonitrile in water containing 0.1% formic acid.

Table 3
Source and MS–MS conditions for the determination of acidic herbicides

Electrospray source				
Capillary voltage	3.5 kV (PI)			
	3.5 kV (NI)			
Desolvation temperature	350°C			
Desolvation gasflow	700 l/h			
Nebulizer gas flow	75 l/h			
APCI source				
Corona pin voltage	2.0 kV (PI)			
	4.0 kV (NI)			
Heater temperature	400°C			
Desolvation gasflow	200 l/h			
Nebulizer gas flow	Maximum			
MS–MS conditions				
Compound	Precursor ion (m/z)	Cone voltage (V)	Collision energy (eV)	Product ion (m/z)
Function 1 (positive mode)				
Sulfometuron-methyl	365	30	20	150
Function 2 (negative mode)				
MCPA	199	25	12	141
MCPP	213	25	12	141
Bentazone	239	40	25	132
Function 3 (negative mode)				
Bromoxynil	274	40	35	79
	276	40	35	79
	276	40	35	81
	278	40	35	81

Quantification was done with a one-point external calibration method using a standard solution with a concentration of 0.4 μg/l of each herbicide in Milli-Q water (pw) and 0.1% of formic acid. Recoveries were calculated by assuming a linear shift in response between two successive calibration standards caused by the matrix during the analysis of a series of samples ($n \leq 4$).

3. Results and discussion

3.1. Design of experimental set-up

The first step is to select the relevant parameters to be investigated. In order to avoid the unnecessary use of the expensive MS–MS, the study was focused by limiting the number of experiments.

As clearly demonstrated in our previous studies [1,2], the UV detection of acidic pesticides is hampered by the co-elution of humic substances. One can expect that the presence of such an excess of matrix effects the ionization of analytes during MS detection, and hence, calibration. Another important parameter to be considered is the salinity of water which can effect the ionization of early eluting compounds.

Therefore, both purified water (pw) and tap water (tw), fortified with a relatively high DOC content of 12 mg/l were selected to represent the important matrix effects encountered in this type of analysis.

Based on previous work [8], mixtures of acetonitrile–0.1% aqueous formic acid were found to

be suitable mobile phase constituents for both efficient reversed-phase liquid chromatographic (RPLC) separation and MS detection (ionization) of the selected pesticides sulfometuron-methyl, bentazone, bromoxynil, MCPA and MCPP.

As regards detection, both ESI and APCI were applied as ionization modes in MS detection.

In comparison to UV detection [1], the use of MS–MS detection must provide a significant enhancement of sample throughput in order to be attractive for cost-effective analysis. Therefore, the methodology was focused at direct sample injection and the reduction of chromatographic run times.

The matrix effect was studied with the use of (1) no clean-up, (2) a low-efficiency clean-up, and (3) a high-efficiency clean-up. For this purpose, direct large-volume injection (LVI) was combined with three different LC modes: (1) LC (a single-column separation), (2) PC-LC (precolumn switching) and (3) LC–LC (coupled-column LC).

An overview of the various experimental set-ups investigating the matrix effect on recovery of the analytes is shown in Fig. 1. As indicated above, both ESI and APCI were studied.

3.2. Selection of MS–MS conditions

The negative electrospray full-scan mass spectra and the MS–MS spectra of bentazon, MCPA, MCPP and bromoxynil are shown in Fig. 2. In the case of sulfometuron-methyl, both positive and negative electrospray full-scan mass spectra and the MS–MS spectra were acquired, as shown in Fig. 3. All spectra were obtained from infusion, at a flow of 10 μl/min, of a solution of acetonitrile–0.1% aqueous formic acid (50:50, v/v) that contained the compound (about 5 μg/ml). As can be seen in Fig. 2, the MS spectra of all analytes show strong signals as $[M-H]^-$ revealing the characteristic pattern of the presence of one chlorine (MCPA and MCPP, Fig. 2a and b, respectively) or two bromine atoms (bromoxynil, Fig. 2d) in the analyte molecule. The MS–MS spectra show that in the case of bromoxynil the major product ions for the precursor ion selected, correspond to the bromide ion. As the natural abundance of ^{79}Br and ^{81}Br are almost equal, the selection of only one transition results in a 50% signal loss. Therefore, an individual acquisition function was used for bromoxynil, monitoring four different transitions.

On the other hand, sulfometuron-methyl can simultaneously produce protonated and deprotonated molecules in the source. As can be seen in Fig. 3a and b, the number of protonated molecules is far more higher than those of $[M-H]^-$ generated under the same experimental conditions (both spectra share the same vertical axis). Therefore, the positive ion mode was selected for this herbicide. Source conditions, as well as transitions, cone voltages and collision energies are shown in Table 3.

3.3. Selection of LC conditions

Information on the selected columns and their use in LC and MS configurations is given in Table 1. In order to provide sensitivity and chromatographic speed, short analytical C_{18} columns ($L \leq 100$ mm) were selected with I.D.s of 2.1 mm or 4.6 mm to perform ESI or APCI, respectively. Because of their favorable behavior in providing efficient separation between analytes and the interfering humic acids [1,2], a Pinkerton ISRP and a SPS restricted access medium column were also included in the testing.

For convenience of comparison, the tested columns are labeled with a capital letter code.

The selected precolumn (PC) was used in both ESI- and APCI-MS detection modes.

An overview of the applied LC–MS–MS methods including the selected LC conditions is shown in Table 2. All separations were carried out with isocratic elution for individual columns or with a step-gradient elution in the case of column switching. Flows consisted of 1 and 0.2 ml/min for the columns connected to APCI-MS and ESI-MS systems, respectively.

In the PC-LC mode, the first mobile phase (M-1) consisted of acidified water without modifier (acetonitrile). In the case of ESI, sample injection and rinsing of the precolumn were performed at elevated flow in order to speed up analysis; this approach of elevated flow on C-1 was also applied in LC–LC.

In the case of LC–LC, the eluotropic strength of the first mobile phase (M-1) was chosen to allow a clean-up of the column with at least four void

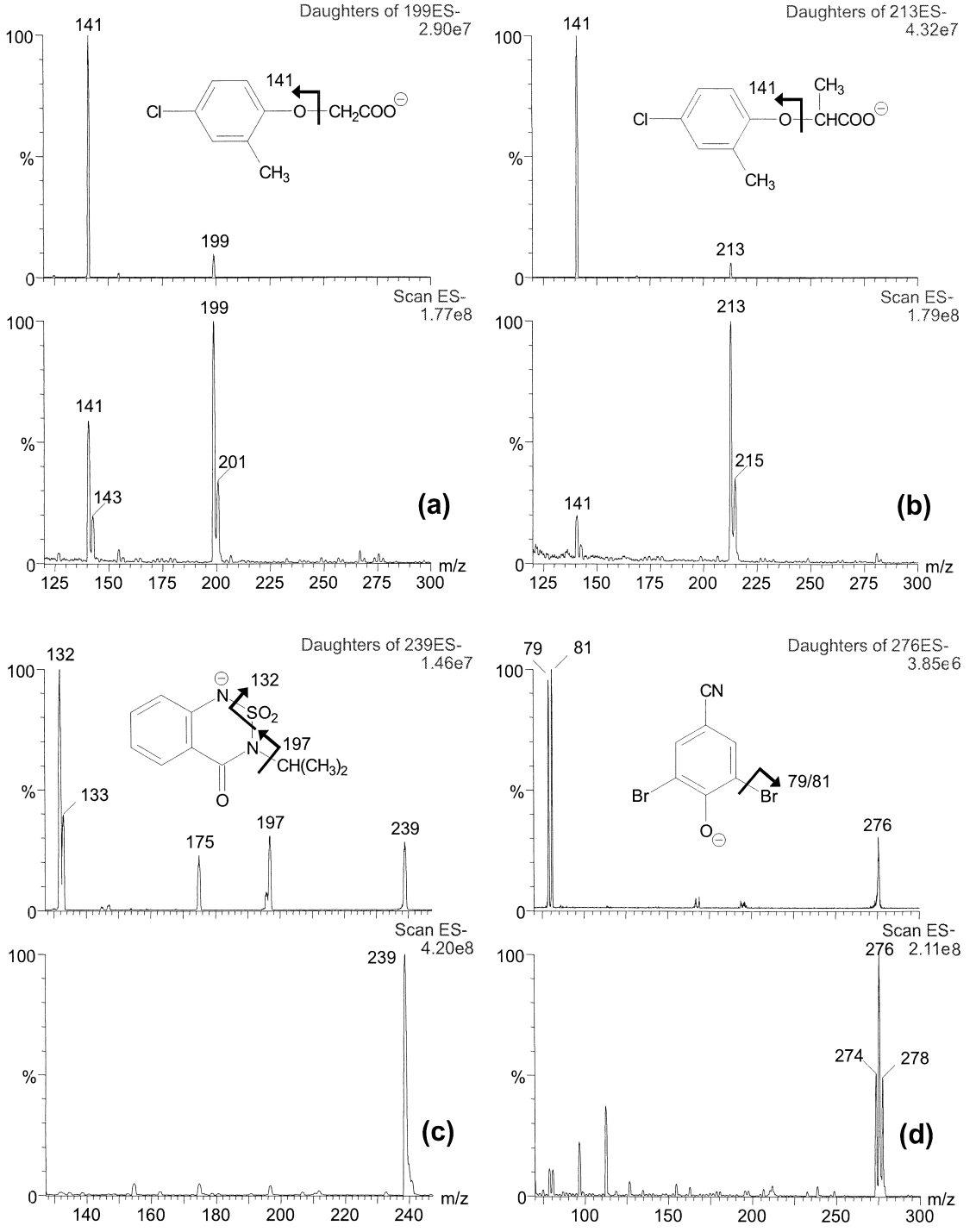

Fig. 2. The negative ion electrospray full scan mass spectra (bottom) and product ion spectra of pseudo-molecular ion (top) of (a) MCPA, (b) MCPP, (c) bentazone and (d) bromoxynil.

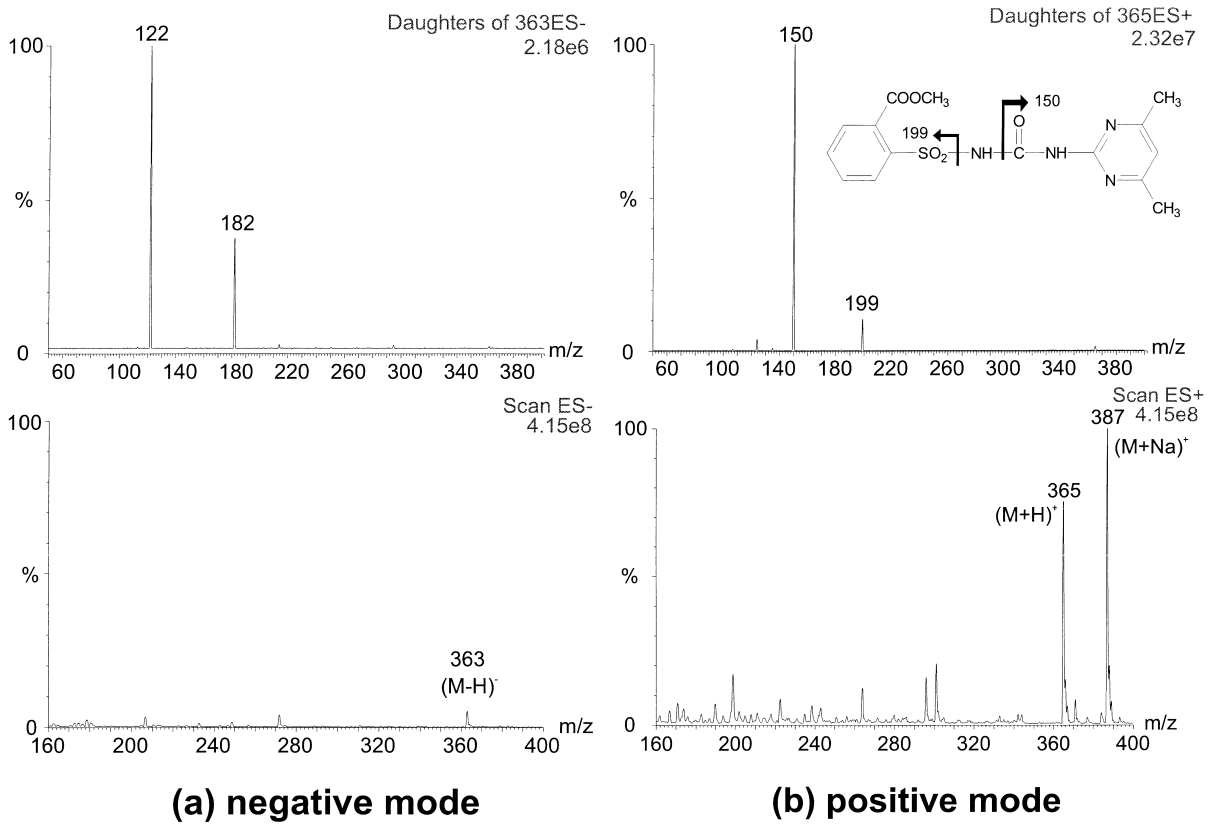

Fig. 3. The electrospray full scan mass spectra (bottom) and product ion spectra (top) of sulfometuron-methyl ion in (a) negative mode and (b) positive mode.

volumes without breakthrough of the first analyte. The eluotropic strength of the second mobile phase (M-2) was always higher than that of the first one (M-1) in order to enhance sensitivity by means of peak compression and to reduce time of analysis.

As regards sample injection, scouting experiments indicated that under the selected LC–MS–MS conditions (see Experimental) injection volumes of 0.25 and 2.0 ml of the water sample provided sufficient sensitivity for ESI-MS–MS and APCI-MS–MS, respectively.

In order to easily compare and evaluate information, the various LC–MS methods are coded by 'number–lowercase letter–number'. The first number stands for the LC mode, viz. single-column operation (=1), PC-LC (=2) or LC–LC (=3). The lowercase letters, e (electrospray) and a (APCI), correspond to the ionization mode, and the last number of the code indicates a specific column combination in a specific LC–MS mode involving column switching.

3.4. Results

Each LC–MS method given in Table 2 was tested by the analysis of a small series of samples consisting of purified Milli-Q water (pw) and tap water (tw), both containing 12 mg/l DOC and spiked with the analytes at a level of 0.4 μg/l. The performance of the method was expressed by the recovery values obtained with external calibration involving the analysis of standards in acidified purified water placed before and after each series of samples (see Experimental).

The results of all experiments are listed in Table 4. On the basis of visual interpretation one can see a few significant effects of LC methods on the MS

Table 4
Recovery (%), mean recovery (%) and standard deviation (%) from spiked water samples[a]

LC configuration	Method code[b]	No. experiments (*n*)	Bromoxynil	Bentazone	MCPP	MCPA	Sulfometuron-methyl
LC	1e/pw	5	63±6	89±3	108±12	103±12	77±6
	1e/tw	4	51±3	60±2	107±4	88±3	43±1
	1a/pw	1	119	111	128	154	93
	1a/tw	1	136	125	140	103	5
PC-LC	2e1/tw	4	67±10	73±10	101±11	94±11	62±3
	2e2/tw	3	87±26	96±10	156±4	163±3	97±11
	2e3/tw	3	81±9	106±7	107±14	107±15	62±7
	2a1/pw	1	126	129	151	120	121
	2a1/tw	1	120	141	117	127	83
LC–LC	3e1/tw	5	57±10	64±8	104±3	100±5	95±1
	3e2/tw	3	40±2	71±2	109±1	99±6	109±11
	3e3/tw	4	110±4	106±4	118±2	116±1	106±6
	3e4/tw[c]	3	38±35	55±40	49±16	48±13	33±21
	3e5/tw[c]	4	54±9	76±4	73±2	51±4	39±6
	3a1/pw	3	132±10	122±5	140±5	144±14	100±11
	3a1/tw	3	131±11	123±6	134±7	131±2	81±1

[a] Spiking level, 0.4 μg/l each analyte.
[b] Methods, see Table 2; pw, purified Milli-Q water; tw, tap water.
[c] Experiments excluded from statistical evalutaion.

calibration performed at the 0.4 μg/l level. An important observation is that the matrix effect of salinity on calibration is distinctly more significant than that of the DOC material.

The LC approach (method codes 1e and 1a) clearly shows a significant reduction in the response of the first eluting analyte (sulfometuron-methyl) in tap water samples; the reduction is most dramatic in the APCI mode.

In the PC-LC approach (method codes 2e and 2a) such a signal reduction is hardly observed indicating that salinity largely contributes to this effect. The average recoveries and corresponding low RSD values of method 2e3 illustrate that the clean-up volume hardly effects the method.

LC–LC with ESI-MS (methods with code 3e) shows good performance in terms of repeatability. However, for the experiments using the standard C_{18} column combinations (codes 3e1 and 3e2, using column B as C-1 and C-2) the recovery for both bromoxynil and bentazone is rather low. More favorable results were obtained by using the column combination B/C (method code 3e3). Apparently, the Supelco ABZ+ material contributes to a more favorable separation between the analytes and interferences and, thus, eliminates the effects of the matrix on quantification for bentazon and bromoxynil.

Unfortunately, poor recovery and repeatability were obtained for the column combinations employing a RAM column as C-1 (method codes 3e4 and 3e5), an observation for which we have no explanation yet.

When comparing the influence of matrix on ESI and APCI, one can notice that for both the PC-LC and LC–LC mode, in most APCI cases, the matrix produces a significant enhancement of the response and, hence, of the recoveries.

In conclusion, the use of LC–LC with the C_{18}/Supelco ABZ+ column hyphenated to ESI-MS–MS (method code 3e3) appears to be the most favorable approach for the on-line analysis of this set of various acidic pesticides in DOC containing tap water. The good performance of this approach is displayed in Fig. 4 showing the high-speed LVI–LC–LC–ESI-MS–MS analysis of a 250 μl sample spiked with the analytes at a level of 0.4 μg/l.

Other approaches, such as PC-LC in both the ESI

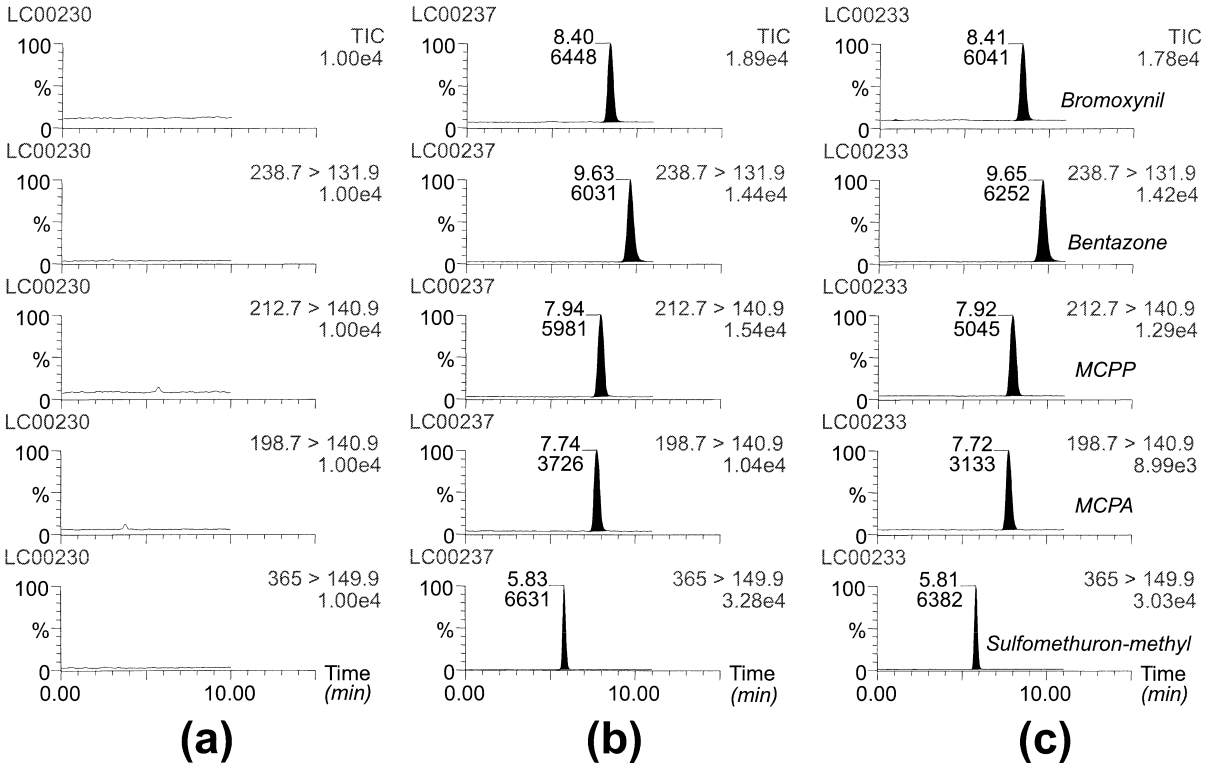

Fig. 4. LVI–LC–LC–ESI-MS–MS of 250 μl of (a) a tap water sample containing 12 mg/l DOC, (b) spiked with the acidic pesticides at a level of 0.4 μg/l and (c) standard of acidic pesticides at a level of 0.4 μg/l. LC–MS conditions, see method 3e3 (Table 2).

and APCI modes, and LC–LC in the APCI mode are also feasible (Fig. 5, LVI–PC-LC–APCI-MS–MS and Fig. 6, LC–LC–APCI-MS–MS). However, they will require a more suitable calibration procedure, e.g., the use of matrix-matching standards.

During this study no significant decrease of the signals or shifts in retention times of analytes were observed for the various column-switching LC–MS–MS procedures, emphasizing the robustness of the methodologies.

3.5. Statistical evaluation

In order to verify the conclusions based on the relatively large number of various LC–MS configurations tested with a rather limited number of experiments, the data listed in Table 4 were statistically evaluated. Because of the unexplained low recoveries, the data involving RAM columns were not included. In the statistical evaluation, five major parameters, listed in Table 5, were selected and coded with two values: '0' (default configuration) and '1' (alternative configuration).

The statistical evaluation for the determination of the effects of the five parameters and the first order interactions, consisted of a backwards ordinary least-squares (OLS) regression on the results of the experiment using the software package Matlab [22] in combination with the Econometrics function library [23].

The backwards regression starts with all main effects and interactions. If the least significant effect/interaction has a *p*-value with a probability larger than 5%, this one is removed. The procedure is repeated until all remaining effects/interactions have *t* values below 5%. The contributions of these parameters/interactions are considered significant.

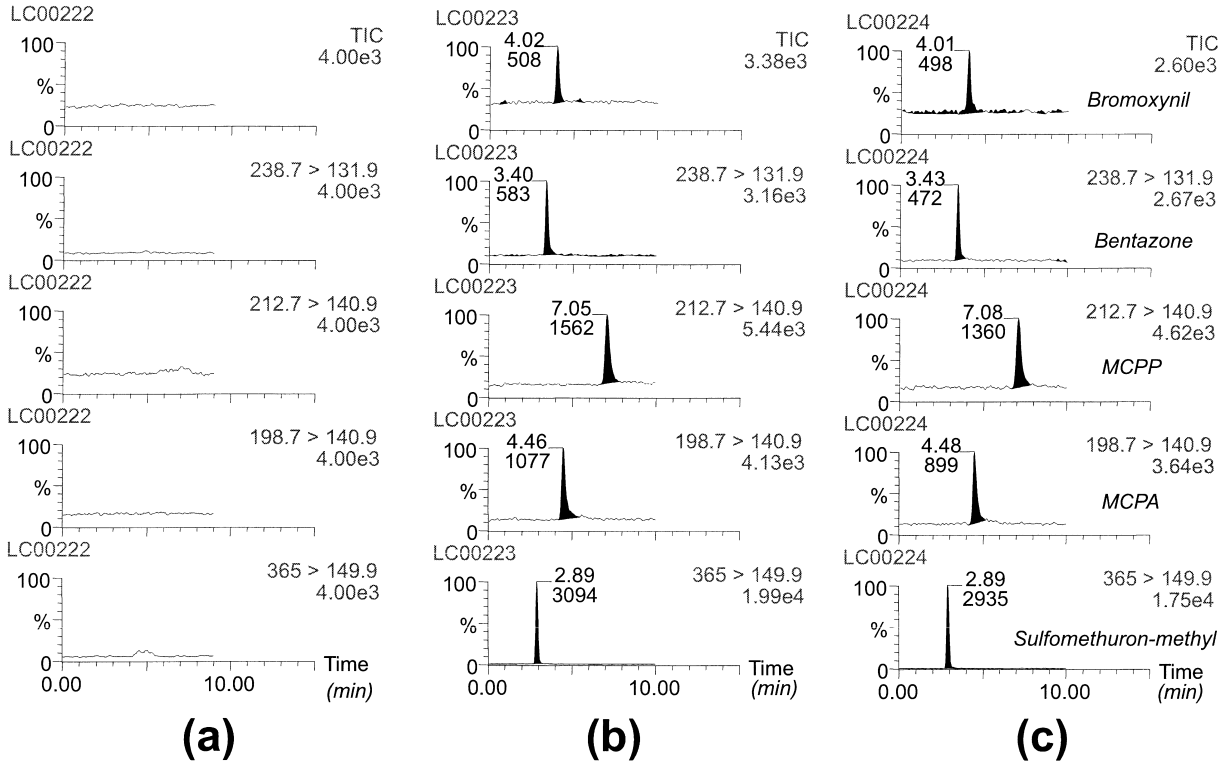

Fig. 5. LVI–PC-LC–APCI-MS–MS of 2000 μl of (a) a tap water sample containing 12 mg/l DOC, (b) spiked with the acidic pesticides at a level of 0.4 μg/l and (c) standard of acidic pesticides at a level of 0.4 μg/l. LC–MS conditions, see method 2a1 (Table 2).

The results of the backward regression for each analyte is shown in Table 6.

The 'Default value' in Table 6 is the calculated recovery percentage of each component when the default configuration is used for each of the five parameters. The contribution shows the effect on the calculated recovery percentage of each analyte when the alternative configuration is used instead of the default configuration.

The results of the statistical evaluation are in good agreement with the conclusions made above. For example, significant effects are estimated for combinations involving type of water and LC modes and no effect is obtained for the combination water× switching mode. In other words, either the absence of salinity or its removal by on-line clean-up with column switching significantly improves the MS detection of the most polar compounds.

The statistical evaluation estimates that for the LC–MS systems tested, the use of APCI or ESI is analyte dependent as regards optimal MS detection.

4. Conclusions

Based on the LC–MS–MS experiments, several conclusions can be made as regards the effects of matrix on calibration in the direct trace analysis of five different acidic pesticides at a level of 0.4 μg/l in DOC containing tap water:

(1) Salinity of the sample severely hampers the determination of early eluting analytes when direct on-line trace analysis without column switching (LC mode) is used as clean-up.

(2) In column-switching modes, PC-LC and LC–LC, direct analysis of each analyte down to a level of 0.1 μg/l was possible with both APCI and ESI-MS–

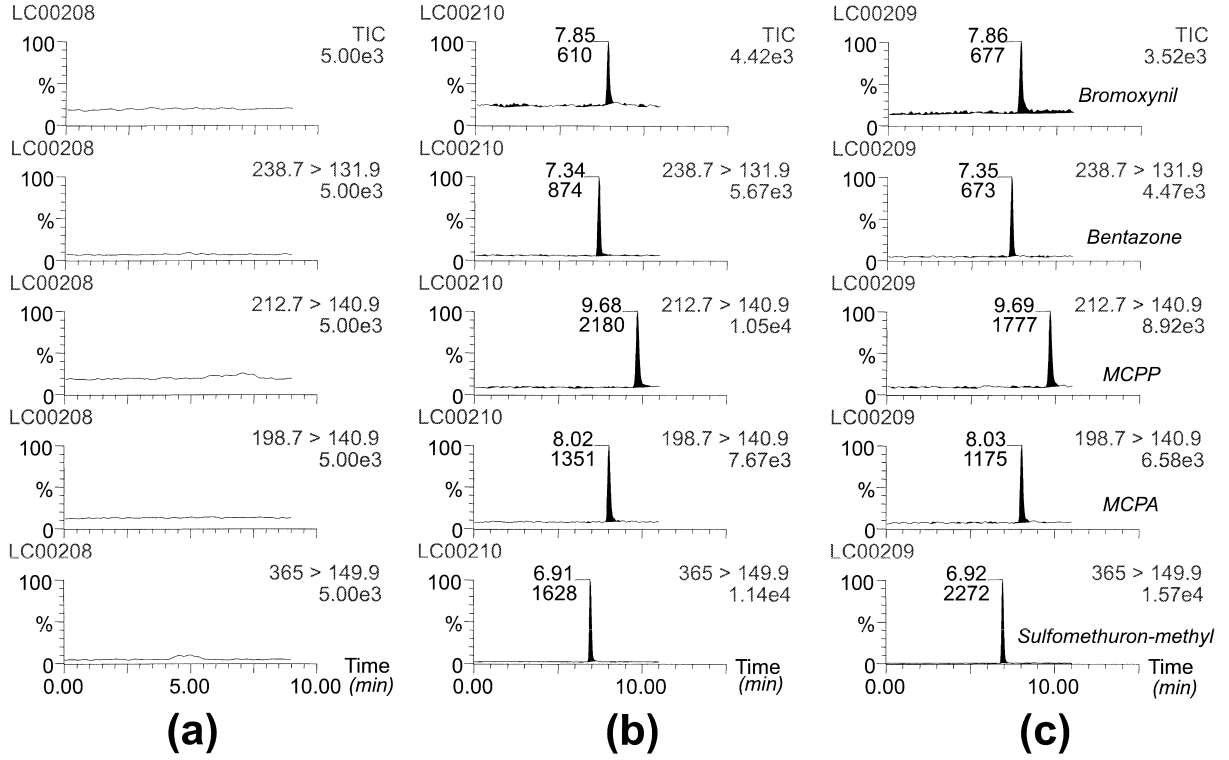

Fig. 6. LVI–LC–LC–APCI-MS–MS of 2000 μl of (a) a tap water sample containing 12 mg/l DOC, (b) spiked with the acidic pesticides at a level of 0.4 μg/l and (c) standard of acidic pesticides at a level of 0.4 μg/l. LC–MS conditions, see method 3a1 (Table 2).

MS when sample injection volumes of 2.0 and 0.25 ml, were used.

(3) The possibility of simultaneously detecting and confirming a group of acidic pesticides in water down to a level of 0.1 μg/l in less 10 min illustrates that MS–MS is attractive for cost-effective analysis.

(4) The approach of LC–LC–ESI-MS–MS using Supelco ABZ+ as a second column appeared to be the most favorable to exclude matrix effects and allowed reliable quantification of the analytes at the level of 0.4 μg/l with external calibration.

(5) Other configurations, such as PC-LC in both ESI and APCI modes and LC–LC with APCI are feasible, but will require some adjustment of the calibration procedure.

(6) The observed major effects on calibration, viz.

Table 5
Selected LC–MS parameters for statistical evaluation

Parameter	Value	
	0 (default)	1
Type of water[a]	Milli-Q water (pw)	Tap water (tw)
MS mode	APCI	ESI
LC mode	Without column switching	With column switching
Column-switching mode	PC-LC	LC–LC
Type of second column	C_{18}	ABZ+

[a] Containing 12 mg/l DOC.

Table 6
The major estimated contributions of LC–MS parameters and first-order interactions to recovery

Parameter	Contribution (%) and p-value[a] for				
	Bromoxynil	Bentazone	MCPP	MCPA	Sulfometuron-methyl
Default value	129; 0.000	111; 0.000	136; 0.000	135; 0.000	103; 0.000
Type of water	–; –	–; –	–; –	−23; 0.022	−98; 0.000
MS mode	−61; 0.000	−23; 0.018	−29; 0.000	−26; 0.000	26; 0.000
LC mode	–; –	16; 0.016	–; –	–; –	
Switching mode	–; –	–; –	–; –	–; –	
Type of C-2	–; –	28; 0.003	–; –	–; –	−18; 0.026
Water×MS mode	–; –	−25; 0.005	–; –	23; 0.010	64; 0.000
Water×LC mode	–; –	–; –	–; –	–; –	77; 0.000
Water×switching mode	–; –	–; –	–; –	–; –	–; –
MS×LC mode	–; –	–; –	21; 0.009	–; –	−39; 0.006
MS×switching mode	–; –	–; –	19; 0.012	–; –	22; 0.001

[a] Probability based on t-value.

salinity and LC mode for the early eluting analytes, were verified with a statistical evaluation of the data.

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