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Determination of acid herbicides in water by LC/MS/MS

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ABSTRACT

This work describes the evaluation of a method using solid-phase extraction (SPE) followed by high performance liquid chromatography coupled to electrospray ionization (ESI) tandem mass spectrometry, LC-MS/MS to screen traces of acid herbicides from water samples. Calibration conditions of LC-MS/MS in MRM mode, showed excellent linearity for the six herbicides studied (2,4-D, 2,4,5-T, 2,4-DP, MPCA, MCPP and bentazone) in the range from 1 to 50 μg/l. Instrumental precision, expressed as relative standard deviation (R.S.D.), was below 3.4%, while sensitivity ranged from 2 to 4 pg on-column for all herbicides. Good average recoveries of the analytes were obtained from three spiked water matrices at two concentration levels 0.1 and 0.01 μg/l, namely ultra pure water (75–88%), mineral water (61–103%) and surface water (70–120%). The method limit of detection (0.003 μg/l) and the above performance characteristics guaranty the correct determination of acid herbicides at low concentrations, much lower than the maximum concentration (0.1 μg/l) admissible for pesticides in drinking water samples, established by the European Union directive. Furthermore, application of this method to surface and coastal water samples from Greece has shown that, in most cases the water samples were free from acid herbicides. MPCA and 2,4-D were detected once, while low levels of bentazone (less than 0.1 μg/l) occurred only in two samples.

Keywords: Acid herbicides; Priority substances; Water quality; LC/MS/MS

1. Introduction

Natural waters are frequently exposed to exceeding concentrations of toxic substances, which makes the quality status of aquatic ecosystems and water resources a permanent issue [1–4]. Among them pesticides constitute a major threat to water quality as their extensive use in agriculture and industrial emission during their production has led to their substantial occurrence in waters worldwide. The volatile non-polar pesticides which have been used in the early days caused bioaccumulation and global transport. Nowadays are replaced by more polar, thermo-labile less volatile easily degradable compounds. Acid herbicides fall in this class and found wide use for their relative cheapness and effectiveness as broad-leaf weed killers in grain crops. They readily dissolve into the water due to their polarity. As water drains from the field, they can leach from soil to ground water or to nearby surface waters as a result of run-off, especially after irrigation or rainfall [5] with the potential to cause contamination to natural waters. Their rate of degradation biological or photolytic results in half lives in soil and water ranging from weeks to months depending on the environmental conditions such as pH and light [6–8]. Although they are relatively readily degradable in soil their degradation products include chlorophenols which are also toxic and frequently occur in the aquatic environment [9].

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The most frequently found acid herbicides in European surface waters (rivers and lakes) are MCPP, MCPA, 2,4 D and bentazon [9–11]. Their concentrations ranged from ng/l to μg/l depending on the area and the season. For example 2,4 D in the Tiber valley (Italy) ranged from <0.1 ng/l in January to 451 ng/l in July however bentazon in the Ebro river delta (Spain) during the main rice growing season reported as high as 127 μg/l. Similar trends were reported for Greek rivers and lakes regarding the compounds observed and their concentration levels [12]. The available data go back from 1991 to 2004. However since the application patterns of pesticide use are constantly changing, updated information is needed through continuous monitoring.

The currently employed methods for acid herbicides analysis in water use a concentration step either by liquid–liquid extraction or solid-phase extraction (SPE) followed by gas chromatography mass spectrometry (GC/MS) or liquid chromatography mass spectrometry(LC/MS) [13–15]. Acid herbicides are polar thermally unstable compounds, their analysis by GC requires prior derivatisation. The derivatisation is effected through sililation or alkylation but difficulties were encountered when GC had to be combined with SPE in order to achieve low detection limits in the (ng/l) range [16]. Instability of the derivatives and low yields account for relatively high RSDs (up to 40%).

On the contrary liquid chromatography overcomes this drawback as the polar acid herbicides are well chromatographically separated without derivatisation. The lack of selectivity and sensitivity of the DAD detectors previously used was the main obstacle in the use of LC systems. The development of direct coupling of LC to mass spectrometry (LC/MS) provided the selectivity and sensitivity of the GC/MS systems, making it the preferred method for the analysis of acid herbicides. Atmospheric pressure ionization techniques (API) either by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) proved very popular interfaces. Both techniques are soft ionization methods giving rise to the protonated $[M+H]^+$ or the deprotonated $[M\text{-}H]^-$ molecular ions when used in the positive or negative modes respectively. Acid herbicides deprotonate readily and are most suited for negative ion mode. The ESI mode offers improved sensitivity over the APCI mode for acid herbicides and is therefore the preferred one [17].

The latest development of LC-MS/MS systems makes use of three quadrupoles where a selective fragmentation of the initially formed molecular ion takes place in the collision cell between the first and second quadrupole. In this way the reactions of the parent ion to specific product ions (Multiple Reaction Monitoring) are monitored providing a very high degree of confidence in the identification of the analyte [9,10,18,19]. The sensitivity is also highly improved. Very low detection limits at the pg level can be achieved allowing in some cases even direct injections [17]. This is particularly important since the maximum acceptable concentration for pesticides in drinking waters set by the European Union directive 98/83 CE is as low as 0.1 μg/l [20]. Nowadays, for the positive identification of pesticides in environmental samples strict criteria have to be satisfied and the use of two transitions per analyte is strictly recommended requiring the use of LC/MS/MS [13]. Few methods employing SPE followed by LC/MS/MS have been reported up-to-date for the analysis of acid herbicides. They have made use of either a post column neutralizing addition solution to improve sensitivity [10] or an on line SPE-LC/MS/MS system with low sample volume (10 ml) to reduce sample volume depended matrix interferences [11].

In this work, we evaluate the performance of a simple method, employing SPE and LC-MS/MS, for the analysis of acid herbicides in water samples, in terms of sensitivity, recovery and reproducibility. Six acid herbicides, namely 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4-dichlorophenoxypropionic acid (2,4-DP), 2-(2-methyl-4-chlorophenoxy) propionic acid (MCPP), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and 3-isopropyl-1H-2,1,3-benzothiathiadiazin-4(3H)-one dioxide (bentazone), were selected. Furthermore, another objective of this study was to update information on the occurrence of acid herbicides in Greek surface and coastal waters.

2. Experimental

2.1. Reagents, chemicals and working solutions

Analytical standard solutions of 2,4,5-T, MCPA, MCPP, 2,4-DP and bentazone at 100 mg/l in acetone were supplied by Chemservice. 2,4-D standard solution at 1,000 mg/l in methanol was supplied by Restek. Fig. 1 shows the chemical structures of the herbicides studied. A mixed standard solution of 100 mg/l in methanol was purchased from Ultra Scientific. Other chemicals used in this study were: methanol LC-MaScan grade (Lab-Scan), water LC-MS Chromasolv® grade (Riedel-de Haën), acetone Chromasolv® grade (Sigma-Aldrich) and ammonium formate puriss p.a. eluent additive for LC-MS grade (Fluka).

Individual herbicides as well as mixed stock solutions containing 1 mg/l of the targeted compounds were prepared in methanol and stored in the dark at −18 °C. Working mixed standard in methanol,

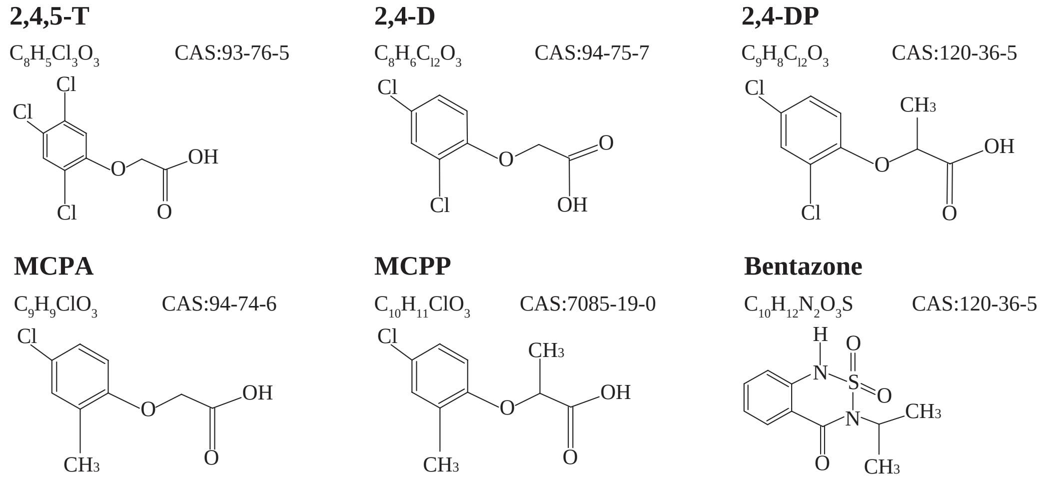

Fig. 1. Chemical structures of the herbicides studied.

containing 100 μg/l for each herbicide was used for spiking samples. Calibration standard solutions of 1–50 μg/l were prepared in methanol:water (1:9, v/v).

2.2. Sample preparation

Three water matrices were used in this study, ultra pure water (conductivity 18.2 μS/cm at 20 °C) produced by a MilliQ system (Waters), mineral bottled water under the commercial name ''Zagori'' (conductivity 385 μS/cm at 20 °C) and surface water from Ilissos river (Attiki) near its fountains.

Water samples were extracted by solid-phase extraction (SPE) using CHROMABOND® EASY extraction cartridges (polar modified polystyrene-divinylbenzene copolymer) from Macherery-Nagel. The extraction cartridges were fitted into a 20-port vacuum manifold IST VacMaster connected to a Millipore vacuum pump. Water samples were forced through the cartridges under reduced pressure. Cartridges were conditioned with acetone (2 × 2 ml) and water (2 × 2 ml) at 4 ml/min. After the conditioning step, aliquots of 500 ml of water samples, acidified at pH = 2.0, were loaded through the cartridges at a flow rate of 10 ml/min. The cartridges were then dried under nitrogen stream and eluted with 2 × 2 ml methanol:acetone (1:1, v/v). Consequently, the eluents were evaporated to dryness using Turbo-Vap®LV Concentration Workstation (Caliper Lifescience) dissolved again to a final volume of 1 ml in methanol:water (1:9, v/v) and vortexed for 1 min.

2.3. Instrumentation

The LC analyses were carried out on an Agilent 1200 LC system comprising a quaternary pump, an autosampler and a column oven. A Synergi column, 4 μm particle size, Fusion-RP 80A, 50 × 2 mm (Phenomenex) was used. The mobile phase consisted of 5 mM ammonium formate aqueous solution in water (solvent A) and 5 mM ammonium formate aqueous solution in methanol (solvent B). The initial mobile phase was 95%A–5%B. The gradient applied was: 0–1.5 min: 5–40%B, 1.5–8 min: 40–60%B, 8–10 min: 60–90% B, 10–14 min: 90% B and 14–15 min: 95–5%B. The flow rate was 0.35 ml min^{-1}, the injection volume was 20 μl and the column oven temperature was set at 25 °C.

Mass spectrometry was performed on an API 3200™ LC/MS/MS system (Applied Biosystems/MDS SCIEX) fitted with a TurboIonSpray® source and controlled by Analyst software (version 1.4.2). Electron Spray Ionization (ESI) was carried out in negative mode. The interface conditions were as follows: nebulizer gas 40 psi, drying gas 60 psi, curtain gas 30 psi, ionspray source voltage −4,200 V and dissolvation temperature 550 °C. The data acquisition was performed under the Multiple Reaction Monitoring (MRM) mode. Selection and tuning of MRM transitions was based on direct infusion on the MS detector of a 1 mg/l standard solution of each analyte.

3. Results and discussion

3.1. MS/MS optimization

Acid herbicides due to their acidic properties are most suited for negative ion mode and this was the selected mode of ionization. The full-scan mass spectra of the analytes were first obtained by direct infusion on the MS and the strong presence of their negative molecular ion $(M\text{-}H)^-$ was confirmed and used as precursor ion. The pre-collision cell voltages namely Declustering

Table 1
Selected MS-MS conditions for multiple reaction monitoring (MRM)

Compound	MW	Q1 Mass (amu)	Q3 Mass (amu)	DP(V)*	EP (V)*	CE (V)*	CXP (V)*
2,4,5-T (quantifier)	255.49	252.90	194.90	−20.00	−4.50	−20.00	−12.00
2,4,5-T (qualifier)		254.90	196.90	−20.00	−4.50	−20.00	−12.00
2,4,-D (quantifier)	220.00	218.90	161.00	−25.00	−10.00	−20.00	−14.00
2,4,-D (qualifier)		220.90	163.00	−25.00	−10.00	−20.00	−14.00
2,4-DP (quantifier)	234.00	233.00	161.00	−20.00	−4.50	−22.00	−14.00
2,4-DP (qualifier)		235.00	163.00	−20.00	−4.50	−22.00	−14.00
MCPA (quantifier)	200.00	199.00	141.00	−25.00	−10.00	−20.00	−12.00
MCPA (qualifier)		201.00	143.00	−25.00	−10.00	−20.00	−12.00
MCPP (quantifier)	214.00	213.00	141.00	−25.00	−4.50	−22.00	−12.00
MCPP (qualifier)		213.00	71.00	−25.00	−4.50	−14.00	−9.00
Bentazone (quantifier)	240.00	239.00	132.00	−60.00	−10.00	−28.00	−4.00
Bentazone (qualifier)		239.00	197.00	−45.00	−4.50	−26.00	−16.00

*DP = Declustering potential; EP: entrance potential; CE: Collision energy; CXP: Cell exit potential.

potential (DP) and Entrance potential (EP) were optimized at this stage, by ramping from low to high voltages and determining the optimum value which gave the highest intensity of the precursor ion. Next, the product ion mass spectra were obtained revealing the most intense fragment ions for each compound and allowed the selection of the most intense one to be used for the MRM. In the MRM mode the reaction of the parent ion to the product ion is monitored so that the highest sensitivity of the product ion is obtained on the detector. This is achieved by optimizing the parameters Collision Energy (CE) and Cell Exit Potential (CXP) for each product ion. Two MRM transitions were used for each analyte. The most intense transition was used as quantifier and the next best as qualifier. The selected optimized conditions used for each herbicide are given in Table 1.

The confirmation of the analytes in an unknown sample was based on the presence of the two MRM transitions at the expected retention times and expected ratios as in the standards. According to European guidelines [21] for a positive identification the following criteria had to be met: the LC retention time of the analyte in the sample must be within 2.5% of the retention time in the standard and the relative abundance of the MRM transitions signals must be within 20% of the ratio obtained for the standards.

3.2. Linearity and precision

Analytes were quantified using external standard calibration. Calibration standard mixtures of 1, 2, 5, 10, 20, 50 μg/l of each analyte were analyzed and the responses showed excellent linearity for all compounds, as shown by their respective correlation coefficients (Table 2).

In order to evaluate the instrumental precision, six replicates of a standard mixture at 5 μg/l level of each herbicide were carried out. The data obtained from the repeatability studies allowed a relative standard deviation (R.S.D.) below 3.4% for all analytes.

Moreover, repeatability of the retention times was also evaluated to ensure the reliability of this identification criterion. Relative standard deviation ranged between 0.47 and 0.58% (Table 2) that is variations

Table 2
Retention time, linearity, instrumental precision

Compound	Retention time (min)	% RSD of retention time ($n = 6$)	Linearity, r (1–50 μg/l)	Precision, % RSD (5 μg/l, $n = 6$)
Bentazone	3.98	0.58	1.0000	3.2
2,4,-D	5.11	0.49	0.9994	2.7
MCPA	5.17	0.49	0.9999	2.9
MCPP	5.87	0.47	0.9998	2.2
2,4-DP	5.88	0.56	0.9997	0.9
2,4,5-T	6.26	0.55	0.9993	3.4

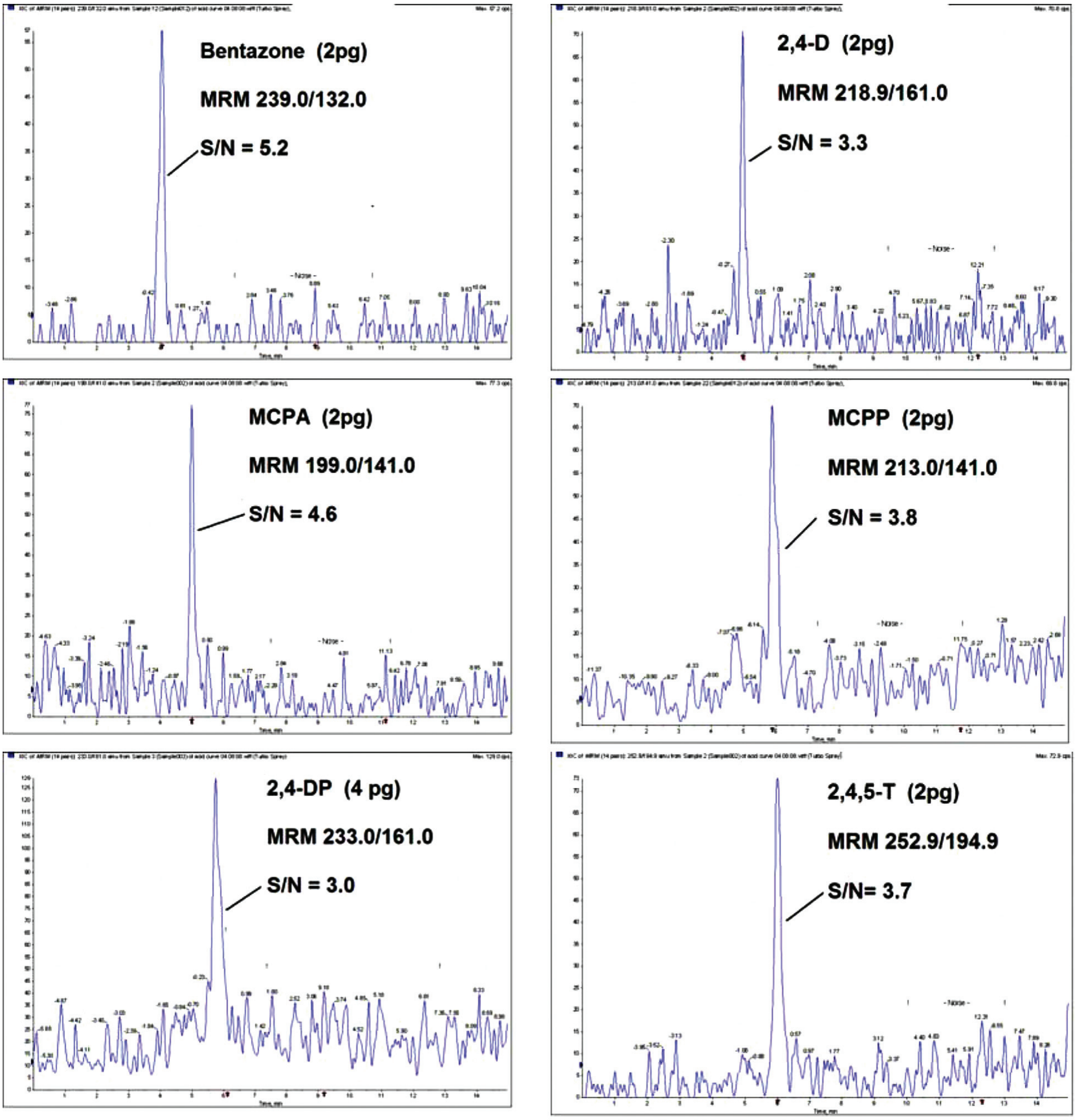

Fig. 2. Extracted MRM traces of the herbicides studied.

of ± 0.02 min to ± 0.03 min as absolute values, showing good stability in the elution conditions.

3.3. Sensitivity, limit of detection and quantification

The instrumental limit of detection for each herbicide was defined as the concentration for which a signal-to-noise (S/N) ratio of 3 (the ratio of the peak intensity to the intensity of the average noise) is observed. In practice, this was studied through the injection of a series of dilutions of standard mixtures of herbicides and establishing the lower concentration where a (S/N) ratio of ≥3 is achieved. The instrumental limits of detection (20 μl injection) achieved were less than 0.10 μg/l for all herbicides studied, except for 2,4-DP with an instrumental limit of detection of 0.2 μg/l. This corresponds to a sensitivity of less than 2 pg (4 pg for 2,4DP) for all analytes on-column as shown in Fig. 2.

The method detection limit (LOD) was obtained by analysis of 500 ml of a surface water sample spiked with

Table 3
Average recoveries of acid herbicides from three matrices at 0.1 and 0.01 μg/l level

Compound	Recovery (% ± R.S.D.) ($n = 3$)					
	Ultra-pure water (0.1 μg/l)	Mineral water (0.1 μg/l)	Surface water (0.1 μg/l)	Ultra-pure water (0.01 μg/l)	Mineral water (0.01 μg/l)	Surface water (0.01 μg/l)
Bentazone	83 ± 11	74 ± 10	117 ± 6	76 ± 16	82 ± 3	101 ± 4
2,4-D	79 ± 4	68 ± 9	72 ± 3	75 ± 14	61 ± 2	70 ± 6
MCPA	82 ± 4	75 ± 12	93 ± 3	88 ± 21	76 ± 4	87 ± 3
MCPP	85 ± 7	85 ± 6	111 ± 2	87 ± 19	97 ± 9	120 ± 5
2,4-DP	84 ± 1	84 ± 6	112 ± 4	87 ± 18	103 ± 6	105 ± 6
2,4,5-T	78 ± 4	72 ± 13	86 ± 5	81 ± 16	77 ± 6	87 ± 10

the analytes at several concentration levels up to 0.01 μg/l level and establishing the lower concentration where a (S/N) ratio of ≥3 is achieved for all analytes. The LOD achieved was 0.003 μg/l for all herbicides. This value is at least 10 times lower than LOD's reported for acid herbicides analyzed by GC [5]. The method quantification limit (LOQ) for each herbicide was calculated using signal-to-noise (S/N) ratio of 10. The LOQs ranged from 0.005 μg/l for MCPA and MCPP to 0.01 μg/l for the rest herbicides. These levels are well below the respective compliance levels (0.1 μg/l) proposed by the European Community for drinking water.

It has been reported that sensitivity can be negatively influenced by liquid chromatographic conditions worsening the method detection limits [17]. Elution under acidic conditions (0.6% formic acid) although improves chromatographic separations of acid herbicides does not favour the formation of their molecular ions in the gaseous phase and post-column neutralization of the LC eluent is necessary to achieve sensitivity of 3 ng/l for acidic analytes [10]. We overcome the need for post-column neutralization by the use of 5 mM ammonium formate instead of formic acid in the mobile phase which resulted in good chromatographic separations of the target compounds and did not impair the sensitivity of our method.

3.4. Recovery studies

The data in Table 3 are the average recoveries of 3 replicates of spiked samples. Blank (unspiked) samples of the three matrices were analyzed, showing no relevant interference substances from the analytical procedure used, including matrices, cartridges and reagents.

Recoveries from surface water ranged from 70% to 120% and compare well for the two concentrations. The highest mean recovery was found for MCPP (120%) while 2,4-D had the lowest mean recovery of 70%. RSDs ranged between 2 and 10% for the two concentrations. These values meet the criteria for method performance (mean recoveries in the range 70–120% with a RSD ≤ 20% set for pesticide residue analysis [22]. Comparable recoveries were obtained in another study employing SPE and LC/MS/MS from a lake water sample for 3 acid herbicides (10). The reported values were MCPA(105%), MCPP(107%) 2,4-D (112%).

Mineral water recoveries ranged from 61% (2,4 D) to 103% (2,4-DP) and Ultra pure water from 75% (2,4 D) to 88% (MCPA) and compare well for the two concentrations. The relative standard deviations (RSDs) ranged between 1 and 13% for all compounds in the above two matrices with the exception of recoveries from the 0.01 μg/l of Ultra pure water with RSD's ranging from 14 to 21%.

In general, average recoveries of acid herbicides were good and reproducible from all water matrices, evidencing that the present methodology is suitable for acid pesticides' screening.

Matrix effects leading to either suppression or enhancement of the analyte signal in the GC detector have been reported in the analysis of acid pesticides [11,13]. The phenomenon is complex and very much matrix depended. A comparison of the relative recoveries obtained between the three matrices indicates that in some analytes (MCPP, bentazon and 2,4-DP) the surface water offers significantly higher recoveries to those of ultra-pure and mineral water. This may well be an effect of matrix enhancement on the above analytes. Several approaches have been used to overcome it such as matrix matched calibration solutions or isotope labelled internal standards. As this is very much a matrix depended effect, in the future, we will evaluate the extent of it on several matrices of our interest (rivers and lakes from allover Greece) included in our monitoring survey program (Table 4).

3.5. Acid herbicides levels from Greek surface and coastal water

In order to determine the levels of acid pesticides in Greek surface and coastal water samples, twenty

Table 4
Concentrations of acid herbicides (μg/l) (LODs: 0.003 μg/l, LOQs for MCPA and MCPP: 0.005 μg/l, LOQs for the rest herbicides: 0.01 μg/l)

Sampling point	Water type	Bentazone	2,4-D	MCPA	MCPP	2,4-DP	2,4,5-T
Aheloos river- Matsuki bridge	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Aheloos river- Katohi bridge	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Alfios river	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pinios river (Ilia)	river	<LOD	<LOD	**0.006**	<LOD	<LOD	<LOD
Glafkos river	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Lake Trihonida	lake	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Viotikos Kifissos river	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sperhios river	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pinios river (Thessalia) – Megala Kalivia	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pinios river (Thessalia) – Pyrgetos bridge	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Lake Kastoria	lake	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Evros river – Dikaia	river	**0.036**	<LOD	<LOD	<LOD	<LOD	<LOD
Evros river – Kipoi	river	**0.062**	**<LOQ**	<LOD	<LOD	<LOD	<LOD
Ardas river	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Nestos river	river	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Lake Vistonida	lake	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Eastern Pagasitikos gulf	Coastal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Central Pagasitikos gulf	Coastal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pagasitikos gulf – Port	Coastal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pagasitikos gulf – cape Agistri	Coastal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

sampling points were established allover Greece. The monitoring survey covered nine rivers (Aheloos, Alfios, Pinios (Ilias), Glafkos, Pinios (Thessalias), Viotikos Kifissos, Sperhios, Ardas and Evros), three lakes (Trihonida, Kastoria and Vistonida) and one gulf (Pagassitikos). The sampling took place from November to December 2008. The water samples were collected in glass bottles, stored in a fridge at 4 °C and extracted within two weeks after sampling.

The concentration levels of the six acid herbicides in surface and coastal water samples are presented in Table 4. The herbicides 2,4,5-T, 2,4,-DP and MCPP have not been detected in the water samples. 2, 4-D has been detected in the sample from Evros river- Kipoi, but in concentration below the limit of quantification (LOQ = 0.01 μg/l), while MPCA was found in concentration near LOQ (0.005 μg/l) in Pinios river (Ilia). 2,4D and MCPA were also found in some Greek rivers of the north at concentrations of up to some μg/l in the past (data from 1992 to 1998) [12]. On the other hand, low levels of bentazone, 0.036 and 0.062 μg/l occurred in the two sampling points of river Evros. However the levels may be higher if the sampling season was close to summer coinciding with the application of the herbicide on the crops. The presence of bentazon in Evros was not reported in a recent study carried out during 1999–2007 involving 147 copmounds mostly pesticides monitored in surface river waters near Greek/Bulgarian/Turkish borders [23]. However, this may be due to limitations in the analytical method employed in the study (GC-MS) which is not suited for trace analysis of acidic pesticides as discussed earlier.

4. Conclusions

The method presented, employing a simple SPE concentration step and an optimized LC-MS/MS analysis, proved to be suitable for screening of acid herbicides from water matrices. Excellent linearity, high sensitivity, good reproducibility and low limits of detection (0.003 μg/l) were among the method performance characteristics. Application of this method to Greek surface and coastal water samples revealed that most water samples were unaffected from acid herbicides inputs. Only, Evros river presented low concentrations of bentazone (less than 0.1 μg/l).

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