Method Article

Rapid SPE – LC MS/MS analysis for atrazine, its by-products, simazine and S metolachlor in groundwater samples

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A B S T R A C T

A rapid analysis of pesticides using on-line Solid phase extraction LC MS/MS (Agilent Technology) was performed using only 2-mL water samples. SPE cartridge PLRP-s was used for the pre-concentration sample with methanol elution in back flush. Sensitive transitions and mass spectrometry conditions were optimized by direct infusion of individual standard solutions in a positive electrospray mode. Water samples were spiked with internal standards to compensate the matrix effect. The limit of quantification was calculated to be 20 ng L^{-1} using the standard deviation of blank analysis injected ten times and uncertainties were estimated at less than 20% on concentrations. This method was validated to study leaching water samples for which only small quantities of water were available.

- Only 2 mL water sample was used.
- Samples were filtered at 0.2 μm and spiked with individual standard.
- Compounds were separated in an 18.5-min elution time using the dynamic MRM program.

Specification Table

Subject Area	*Chemistry*
More specific subject area:	*Analytical chemistry*
Method name:	*A rapid analytical method to analyse pesticides* using only 2-mL water samples
Name and reference of original method	
Resource availability	

Method details

The method developed was applied to investigate atrazine and its main by-products Desethylatrazine (DEA), Deisopropylatrazine (DIA), Hydroxyatrazine (HA), simazine and S-metolachlor in 2-mL water samples. These samples were collected in a quarry located in Saint-Martin-le-Noeud, 70 km north of Paris. This site is an exceptional natural lysimeter, 1.2 km long, with no disturbed soils and an unsaturated layer above the quarry [1,2]. The study sites correspond to 15 different lakes isolated from each other. Both percolation water and groundwater were sampled. The percolation water was sampled in beakers attached at the roof of each site and groundwater directly into the lake. Depending on percolate flow, it is possible to collect from 5 mL to more than 200 mL in the beaker for major element analysis, as well as tritium and other trace elements. A limited water volume was available for pesticide analysis. Pesticide uses have been investigated directly near farms for the last 40 years and we focused on pesticides that were the most widely used: atrazine, simazine, S-metolachlor.

The on-line method was developed to analyze these pesticides using LC-MS/MS (LC-1200, QQQ-G6410B, Agilent Technologies). LC-MS/MS is commonly used to analyze these pesticides in different matrices [3–6]. On-line solid-phase extraction methods were also developed, using different SPE cartridges such as OASIS HLB, Waters [7], Strata-X extraction cartridge, Phenomenex [8] or PLRPs, Agilent [9]. In these three studies, water sample volumes were 5, 20 and 5 mL and limits of quantification (LOQ) were <1, 6–15, and 57–508 ng L^{-1} respectively. Highest LOQ [9] were obtain with the residual standard deviation method. An on-line SPE method had already been developed for antibiotic analysis with only 2-mL samples [10]. Such a method is essential because it was not possible to devote a larger volume to the analysis of pesticides in our study, especially for percolate water. Here we adapted this method for pesticides, using only 2 mL water sample filtered and directly analyzed by SPE – LC MS/MS.

Reagents and standards

LC/MS-grade methanol (MeOH) and LC/MS-grade acetonitrile (MeCN) were purchased from Merck (Guyancourt, France) and analytical grade formic acid (99%) from Carlo Erba (Val de Reuil, France). Ultrapure water 18 Mohm (UP water) was dispensed from a Pure Lab Chorus water purification system (Veolia Water STI, France).

Unlabeled standards at 100 μg mL^{-1} (purity between 96.8 and 99%) were purchased from LGC (Molsheim, France): atrazine, DEA, DIA in MeCN and simazine and HA in MeOH, and from CIL Cluzeau (Saint Foy la Grande, France): S-Metolachlor in acetonitrile. Ring-labeled internal standards at 100 μg mL^{-1} (purity between 96.8 and 98% and isotopic value between 99 and 99.9%) were purchased from LGC (Molsheim, France): Atrazine Ring-^{13}C3 in acetone, DEA Ring-^{13}C3 in MeCN and HA ring-^{13}C3 in 80% water/20% diethylamine. All individual stock standard solutions were stored at −18 °C.

Mixed unlabeled solution was prepared in UP water containing 1 mg L^{-1} of each compound. The mixed labeled solution was also prepared at 1 mg L^{-1} in UP water. Both solutions were stored at 4 °C for 1 week maximum. All working solutions were prepared immediately before each analytical series by appropriate dilution of the mixed solution with UP water.

Sample preparation

Two milliliters of water samples were filtered at 0.2 μm using polytetrafluoroethylene (PTFE) filters (4 mm, 0.2 μm for the syringe, Interchim (Montluçon, France) using a Luer syringe and spiked with
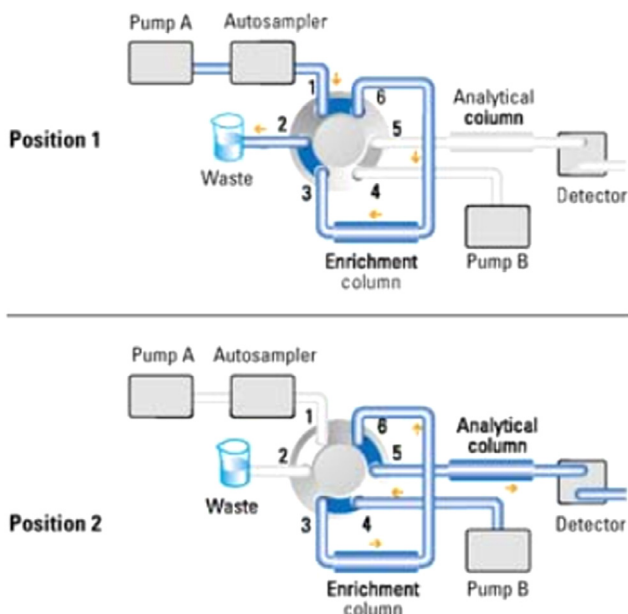
Fig. 1. Scheme of sample enrichment method [11].

Table 1
Quaternary pump time table.

Time	Flow	Pressure	Solvant ratio B (UP-water)%
0	1	400	100
0.1	0.5	400	100
2	0.5	400	100
2.1	1	400	0
8	1	400	0
8.1	0.5	400	100
18.5	0.5	400	100

0.1 mL of the internal standard (ISs) mixture at 10 µg L^{-1}. Since water had no suspended matter, there was no need to previously filter it at 0.45 µm.

On-line enrichment

The on-line SPE system consisted of an automated liquid sampler (ALS) fitted with a 900-µL injection loop with multidraw capability. A programable six-port/two-position valve was used to switch between the load or elution modes (Fig. 1). PLRP-s cartridges (15–25 µm, 2 × 10 mm, Serlabo, Entraigues, France) were first conditioned for 10 min of methanol (A) and 10 min of UP water (B) at a flow rate of 0.3 mL min^{-1} for a series of three blanks, seven standards at different concentration levels and 16 samples.

A quaternary pump delivered the loading buffer at a flow rate of 1 mL min^{-1} (100% UP-water) and 1800-µL (i.e., twice 900 µL) samples were loaded onto the cartridge (Table 1). At 0.1 min after injection, the valve switch position and the analytes retained on the PLRPs cartridge were progressively transferred to the LC analytical column in back-flush mode, using the LC solvent gradient described below (Table 2).

Table 2

LC Gradient to separate analytes.

Step	Time	A (UP water+ 0.1% formic acid) %	B (Acetonitrile+ 0.1% formic acid) %	Flow mL min^{-1}
1	0.00	5%	95%	0.300
2	0.50	5%	95%	0.300
3	10.00	100%	0%	0.300
4	14.00	100%	0%	0.300
5	15.00	5%	95%	0.300
6	18.50	5%	95%	0.300

Table 3

MS/MS optimized conditions for selected pesticides and internal standards.

Compound Name	Prec Ion	Prod Ion	Frag (V)	CE (V)	Ret time
HydroxyAtrazine 13C	201.2	159.1 (Q)	110	14	5.2
HydroxyAtrazine 13C	201.2	116.2	110	18	5.2
Hydroxyatrazine	198.1	142.1	115	12	5.2
Hydroxyatrazine	198.1	86.1 (Q)	115	24	5.2
DIA	174.1	96	110	16	5.9
DIA	174.1	68.2 (Q)	110	28	5.9
DEA 13C	191.6	149.1 (Q)	110	14	6.8
DEA 13C	191.6	106	110	26	6.8
DEA	188	146 (Q)	105	16	6.8
DEA	188	104	105	24	6.8
Simazine	202	132.1	100	20	8.05
Simazine	202	104.1 (Q)	100	20	8.05
Atrazine 13C	219.7	106	110	30	8.9
Atrazine 13C	219.7	70 (Q)	110	42	8.9
Atrazine	216	174.1 (Q)	130	16	8.9
Atrazine	216	104.1	130	30	8.9
S-metolachlor	284	252 (Q)	95	8	10.65
S-metolachlor	284	176.2	95	24	10.65

Chromatographic separation was achieved using an Agilent Zorbax Eclipse XDB-C 18 column (4.6 mm I.D. × 50 mm, 1.8-m particle size; Agilent, Les Ulis, France) with a 0.2-μm prefilter upstream to protect the analytical column. Mobile-phase solvents were UP water + 0.1% formic acid (B) and acetonitrile + 0.1% formic acid (A) in an initial ratio (B:A) of 95/5. Separation was achieved at 35 °C using a flow rate of 0.3 mL min^{-1} with the gradient described in Table 2. Then the system was equilibrated for 1 min prior to the next injection (total run time: 18.5 min).

During MS/MS analysis, the sample injection loop was flushed to prevent cross-contamination: 100% B for 2 min, 100% A for 6 min (Table 1).

Procedural blanks consisting of UP water spiked with ISs were analyzed as a control of contamination during sample handling in the laboratory and to assess a potential memory effect in the on-line SPE apparatus.

Mass spectrometry

The Agilent 6410B triple quadrupole mass spectrometer was equipped with an electrospray ionization (ESI) source and was operated in positive mode. Nitrogen (99.9%, Air Liquide, Paris, France) was used as collision gas while nitrogen used as the nebulizing gas (11.0 L h^{-1}, nebulizer pressure 35 psi) was produced via a nitrogen generator (Claind, France). The source temperature was set at 350 °C and MS/MS signal acquisition was performed in dynamic multiple reaction monitoring (dMRM) mode. For MS/MS optimization, individual standard solutions were directly infused in the triple quadrupole. Method selectivity was provided by the monitoring of two transitions per compound at the retention time of the analyte, corresponding to the transition between the precursor ion and the two most abundant product ions (Table 3). The most abundant one was used for quantification while the other one was used for confirmation. The ratio of quantification transition area to the confirmation

Table 4

Mean, standard deviation (SD) and calculated LOD and LOQ.

Compound	Atrazine	DEA	DIA	HA	Simazine	S-metolachlor
Mean (ng L^{-1})	4.52	3.56	1.73	10.86	0.99	14.75
SD (ng L^{-1})	0.59	2.75	1.01	5.26	0.58	1.01
LOD (ng L^{-1})	3	10	4	21	2	4
LOQ (ng L^{-1})	10	31	12	63	7	12

transition area was compared to that obtained with an authentic standard (margin applied: 20%). Data were collected using Mass Hunter software from Agilent Technologies, which was also used for analyte quantification.

To correct potential analyte losses, matrix effect or volume variation, ISs were used to quantify one or several analytes: Atrazine ring[13]C3 was used for atrazine, simazine and S-Metolachlor, HA[13]C3 was used for HA, and DEA [13]C3 was used for DEA and DIA. Quantification was carried out by calculating the response factor of each analyte relative to its corresponding IS and the concentrations were determined using a least-square linear regression analysis of the peak area ratio versus the concentration ratio.

Method validation

As high temporal and spatial variation of concentrations were measured in samples, the linearity was studied by injecting seven different concentration levels in the range 0–0.01–0.05–0.1–0.5–1–5 µg L^{-1}. In all cases, the calibration curves showed correlation coefficients (*r*) greater than 0.999 with a linear regression, based on relative responses between the analyte peak area and its selected IS peak area.

Limits of detection and quantification were calculated using the blank method. It consists in injecting blank (here UP water spiked with IS) ten times and integrating a peak at the retention time of each analyte [12]. When no signal was detected, the noise was integrated and limits of detection and quantification were calculated according to Eqs. (1) and (2), respectively.

$$LOD = X_{Bl} + 3\,SD_0 \tag{1}$$

$$LOQ = X_{Bl} + 10\,SD_0 \tag{2}$$

with LOD = limit of detection,

LOQ = limit of quantification,

X_{Bl} = the mean concentration calculated for the ten blanks

SD_0 = standard deviation from the average concentration

However, if mean values were greater than 10 ng L^{-1}, we considered that the sample was not a blank value and the limits of detection and quantification were determined by Eqs. (3) and (4), respectively:

$$LOD = 4\,SD_0 \tag{3}$$

$$LOQ = 3 * LOD \tag{4}$$

The mean values and standard deviations are reported in Table 4. For S-metolachlor and HA, mean blanks were 15 ng L^{-1} and 11 ng L^{-1}, respectively, and the LOD and LOQ were calculated with Eqs. (3) and (4). For S-metolachlor, the concentrations were confirmed by the qualifier transition and a signal-to-noise ratio (SNR) above 10. Since S-metolachlor was also detected in drinking water, we suggest that the UP-water system was not sufficiently efficient for this herbicide and linear regression was forced to zero for sample quantification. For HA, SNR was below 3 and the qualifier was too weak to be detected. Moreover, it was not detected in UP water without ISs, suggesting that ISs were the source of this detection. Isotope Ring-labeled HA[13]C3 was 99% pure and the concentration of ISs in

Table 5

Average, standard deviation of natural and spiked samples and accuracy at 2.5 µg L^{-1}.

Compound	Atrazine	DEA	DIA	HA	Simazine	S-metolachlor
Average natural sample (ng L^{-1})	ND	ND	ND	1344	ND	ND
SD (%)				6		
Average of spiked samples (ng L^{-1})	2121	2160	2202	4038	2097	2249
Standard deviation	13	13	14	7	13	11
Recovery (%)	85	86	88	92	84	90

Table 6

Average, standard deviation of natural and spiked samples and accuracy at 0.250 µg L^{-1}.

Compound	Atrazine	DEA	DIA	HA	Simazine	S-metolachlor
Average natural sample (ng L^{-1})	76	294	ND	ND	ND	ND
Average of spiked samples (ng L^{-1})	257	256	265	271	253	265
Standard deviation	1	2	2	2	5	2
Recovery (%)	103	103	106	108	101	106

vials was 500 ng L^{-1}. This detection at 10 ng L^{-1} in all blanks is certainly due to labeled HA^{13}C3 used as EI, and linear regression was then calculated with the blank offset method.

For all the other analytes, blank quantification corresponded to noise integration, and Eqs. (1) and (2) were used to calculate LOD and LOQ. Limits of quantification were higher than using the signal-to-noise ratio.

To achieve accurate quantification of the analytes, triplicates of both natural and spiked samples were injected to determine accuracy and repeatability. To avoid volume variation of the spiked sample, 10 µL of a mix of standard solution (500 µg L^{-1}) was added to the 2-mL sample prior to analysis, which corresponds to a final concentration of 2.5 µg L^{-1} of each compound (Table 5). This protocol was repeated to obtain a final concentration of 0.25 µg L^{-1} with another sample (Table 6). A sampling site that was not too contaminated was spiked to avoid concentrations higher than 5 µg L^{-1}.

Repeatability was estimated using the standard deviation of triplicates of natural (when available) and spiked samples. Accuracy was the difference between the concentration in spiked samples and the theoretical concentration of 2500 ng L^{-1}. For HA, the average in the natural sample concentration was removed to calculate accuracy.

For all analytes, this method was validated with a mean LOQ of 20 ng L^{-1}. This value is high considering the analytical performance of the LC-MS/MS, due to the calculation method. Lower LOQ can be calculated using SNR, especially in pure or tap water but it does not reflect the accuracy requirements for analytical methods. Concentrations lower than 20 ng L^{-1} were then considered to be not quantified and the first level of analytical curve was kept at 10 ng L^{-1}. Even if only 2 mL of water is sampled, this method show that we still have a good recovery and accuracy compared to previous studies. Uncertainties are estimated to be less than 20% in both low and high spiked concentrations, including uncertainties on the spiked volume.

Declaration of Competing Interest

Authors declare no conflict of interest.

Acknowledgments

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