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Application of ultra performance liquid chromatography–tandem mass spectrometry to the analysis of priority pesticides in groundwater☆

Milagros Mezcua[a], Ana Agüera[a,*], Josep Lluís Lliberia[b], Miguel Angel Cortés[b], Bàrbara Bagó[b], Amadeo R. Fernández-Alba[a]

[a] *Pesticide Residue Research Group, Faculty of Sciences, University of Almería, 04120 Almería, Spain*
[b] *MS Applications Laboratory, Parc Tecnologic del Vallés 08290 Waters Corporation, Spain*

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Abstract

Ultra performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry (UPLC™-MS/MS) has been applied for the accurate and rapid analysis of nine trace level priority pesticides in water. The UPLC™ technology, based on the use of columns packed with 1.7 μm porous particles combined with higher pressures than those conventionally applied in HPLC, enabled to improve in peak resolution, sensitivity and speed of analysis. UPLC™ chromatograms showed very sharp peaks with less than 2 s wide at the base, except for alachlor. This enhanced efficiency resulted in an increased separation speed of the whole UPLC™-MS/MS procedure that required less than 5 min. Limits of detection, determined for 300 ml water samples after SPE preconcentration were in the range between 0.1 and 20 ng/L. The presence of matrix effects or ion suppression was checked by the obtaining of calibration curves in both pure solvent and matrix matched standards. Other performance characteristics of the method, such as linearity and precision were also satisfactory. Finally, the method was successfully applied to the analysis of two water samples from an inter-laboratory exercise.

Keywords: Priority pesticides; Ultra performance liquid chromatography; UPLC™-MS/MS; Water analysis

1. Introduction

In the last decades, liquid chromatography–mass spectrometry (LC–MS) and LC–tandem MS have experienced an impressive progress, both in terms of technology development and application [1–3]. The introduction of atmospheric pressure ionization (API) interfaces in the 1990s was the start point that made unforeseen analytical capabilities available for polar compounds and metabolites determination. Despite the impressive potential of modern LC–MS based systems, chromatographic efficiency (peak capacity and resolution) of LC methods is still limited when using conventional columns packed with 5.0–3.5 μm stationary phase. The use of gradient methods increase the separating power of the LC methods, allowing the separation of analytes with a wide range of polarities, but also increase the analysis time and reduce sample throughput. Recently, alternative strategies have been developed to obtain increased efficiency together with short times of analysis by the use of 1.7 μm porous stationary phases, mobile phases at high linear velocities and instrumentation that operates at high pressures (ca. 15.000 psi). This new technology has been called ultra performance liquid chromatography (UPLC™) to distinguish this procedure from conventional HPLC [4]. UPLC™ provides higher peak capacity, greater resolution, increased sensitivity and high speed of analysis [5]. The overall enhancement in the chromatographic resolution also leads to a reduction in the number of co-eluting species so providing a wider knowledge of the analyzed samples, a higher spectral purity and a reduction in ion suppression effects.

Because of the recent development of this technology, very few applications have been reported until now, but it is predictable an increase in the near future because of the significant advantages that UPLC™ offers over conventional HPLC [4,6]. Most of the reported applications are in the field of drug

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* Corresponding author. Tel.: +34 950 01 55 31; fax: +34 950 01 54 83.
E-mail address: aaguera@ual.es (A. Agüera).

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metabolism [7–10] and metabonomics because of the complexity of the samples such as biofluids or tissue extracts and the need of an exhaustive detection and characterization of all the metabolites from a drug safety and patent protection perspective.

In this paper the UPLC™ technology coupled with triple quadrupole tandem mass spectrometry has been applied to the analysis of priority pesticides in water. In the last few years, several applications to monitor these compounds from natural and drinking waters have been proposed in the literature using LC–MS analytical systems [11–16]. The API and the use of tandem mass spectrometric detection have allowed the development of each time more sensitive and selective methods. Detection limits in the range of nanogram per liter have become usual with extracted water volumes varying between 500 and 1000 mL. Despite the improvements in sensitivity it is steel in the aim of the researchers the need of a reduction in the analysis time that allows increase the sample throughput. The use of on-line SPE-HPLC coupling techniques have allowed obtaining faster methods by reducing the sample preparation time [17,18], however the chromatographic analysis steel takes a vast part of the time in the whole method, from 20 to 40 min in many cases.

An attempt to reduce the analysis time has been developed by Asperger et al. [19] by the application of high-speed on-line SPE-LC–MS/MS using turbulent-flow chromatography columns for enrichment and a short monolithic column for fast chromatographic separation. In the present work an UPLC™-MS/MS method has been developed as an alternative to provided very fast chromatographic determinations. The results presented demonstrate the potentiality of the technique in this new field of application.

2. Experimental

2.1. Reagents, chemicals and working solutions

Atrazine, trifluoralin, simazine, chlorpyrifos, diuron, terbutilazine, pentachlorophenol, isoproturon and alachlor analytical standard (purity >98%) were supplied from Dr. Ehrenstorfer (Augsburg, Germany). HPLC grade methanol and acetonitrile were supplied from Merck (Darmstadt, Germany). Formic acid (98%) was obtained from Fluka (Buchs, Switzerland).

Individual pesticide stock solutions containing 200–250 mg/L of the target compounds were prepared in methanol and stored in the dark at −18 °C. Working standard mixtures in methanol, containing 10 mg/L for each pesticide were used for spiking samples. Calibration standards were prepared in both methanol solutions and blank water extracts. In the last case, 200 μL aliquots of the extract were evaporated to dryness under a gentle stream of nitrogen and dissolved again, with sonication, in 200 μL of methanol containing the pesticides studied.

2.2. Sample preparation

Water samples used in the validation studies were collected from a well which provide drinking water for a population of about 2000 inhabitants. Water samples were extracted by solid-phase extraction using Oasis™ HLB (divinylbenzene/*N*-vinylpyrrolidone copolymer). Extractions were carried out with an automatic extraction system (ASPEC) following the next scheme. Cartridges were conditioned with MeOH (4 mL) and water (4 mL) at 4 mL/min. After the conditioning step, aliquots of 300 mL of water samples, acidified at pH ∼ 4.0, were loaded through the cartridges at a flow of 10 mL/min. Before elution, cartridges were dried under nitrogen stream and eluted with 4 mL × 4 mL of MeOH (3 mL/min). The eluates obtained were concentrated to a final volume of 1 mL under gentle nitrogen stream. The final extract was filtered through 0.2 μm before UPLC™ analysis.

2.3. Instrumentation

LC separations were performed in a Waters ACQUITY UPLC™ system (Waters Corp., Milford, USA) using a 100 mm × 2.1 mm ACQUITY BEH C18 1.7 μm column. A sample volume of 10 μL was injected with a Acquity UPLC™ autosampler. The mobile phase was composed of Solvent A (0.1% formic acid in water) and solvent B (acetonitrile) at a constant flow of 0.5 mL/min. The gradient was programmed to increase the amount of B from an initial 20–100% in 5 min, returning to the initial conditions (20% B) in 0.1 min (from 5 to 5.1 min). These conditions were maintained until 6 min.

Mass spectrometry was performed on a Micromass Quattro Premier LC/MS/MS (Waters, Manchester, UK) fitted with an ESI interface and controlled by MassLynx software (version 4.0 SP4). Typical interface conditions were optimised for maximum intensity of the precursor ions as follows: nebulizer and desolvation (drying gas) N_2 flows were set at 650 and 150 L/h, respectively, source block and desolvation temperatures were 100 and 350 °C, respectively. The ESI polarity ionisation mode was set individually for each target compound, the switch time between positive and negative mode was 20 ms.

MS–MS: Argon was used as collision gas at a pressure of 3.7×10^{-3} mBar QuanOptimize software was used for data acquisition under multiple reaction monitoring (MRM) mode. Selection and tuning of MRM transitions were performed individually for each analyte. For optimising the mass spectrometer, direct infusion of a 100 μg/L standard solution of each analyte was used. Optimal conditions for each pesticide are summarized in Table 1.

2.4. Validation studies

The performance characteristics of the method were established by a validation procedure by using methanol standard solutions and spiked samples. Linearity, matrix effects, trueness, precision and detection limits were evaluated. Linearity and matrix effects were assessed by solvent and matrix-matched calibration curves. The linearity in the response was investigated by using calibration solutions at six concentration levels, ranging from 15 to 300 μg/L. The integrated area data of the selected quantification masses were used to construct the curves.

Trueness of the method was investigated through mean recoveries obtained for the three replicates of spiked samples at two different concentration levels (see Table 2). Water samples

Table 1
MS–MS conditions for multiple reaction monitoring

Pesticide	Ionisation mode	Cone voltage (V)	Precursor ion (*m*/*z*)	Collision energy (eV)	Product ion (*m*/*z*)	Dwell time (ms)
Simazine	ESI+	35	202	20	132	50
Atrazine	ESI+	32	216	20	174	50
Isoproturon	ESI+	25	207	20	72	50
Diuron	ESI+	30	233	16	72	50
Terbutylazine	ESI+	30	230	20	174	50
Alachlor	ESI+	15	270	10	238	50
Pentachlorophenol	ESI−	45	265	15	265	50
Chlorpyrifos	ESI+	30	352	20	200	50
Trifluralin	ESI+	25	336	15	236	50

spiked with all the pesticides were extracted by applying the SPE method described above. Analytical signal was compared with the signal of a blank water extract spiked after solid phase extraction with the target compounds. Spiked water extracts were also used for evaluating precision and limits of detection and quantification of the method. Intra-laboratory precision under repeatability conditions was expressed in terms of relative standard deviation for 10 replicates of a spiked sample at 0.1 μg/L level. The limits of detection (LOD) were determined as the analyte concentration that gave a signal-to-noise ratio (S/N) of 3.

3. Results and discussion

3.1. Ultra performance liquid chromatography

The primary objective of this study was to evaluate the potential of this new UPLC™ technology in the analysis of priority pesticides in groundwater. The main requirements for this application were a high sensitivity, in order to meet the strict quality standards for water destined for human consumption (Council Directive 98/83/EC) and short analysis time, because of the need of a high sample throughput. Both requests could be fulfilled by UPLC™ that provided a high peak capacity derived from the use of 1.7 μm columns. In this work a 100 mm long column with a flow rate of 0.5 mL/min and a gradient time of 5 min was used to obtain the chromatograms shown in Fig. 1. These chromatograms correspond to a water sample spiked with all the target pesticides at concentrations from 0.8 to 20 ng/L. The selected ion plots for the MRM transitions are represented. Retention times are showed in Table 2. As it can be seen, very sharp peaks were obtained with peak widths at half height of around 1.5 s. The flow rate and gradient time applied during the analysis allowed the elution of the nine compounds in less than 5 min with an acceptable separation. These two parameters, together with the column length, greatly affect the peak capacity and thus the chromatographic resolution [5].

In the chromatogram only isoproturon and diuron were not completely separated. However, this coelution could be resolved since, though both compounds yield the same product ion at *m*/*z* 72, the precursor ions are different and MS/MS separate detection of both compounds is feasible, allowing their correct identification and quantification.

3.1.1. Sensitivity and matrix effects

The more efficient chromatographic separation afforded by the UPLC™ methodology also conducted to a high sensitivity, consequence of the reduced peak width (∼1.5 s) obtained for the chromatographic peaks. Limits of detection reached with the whole method were in the range of nanogram per liter or lower, as it can be seen in Table 2. Moreover, the enhanced sensitivity usually reported by UPLC™ methods in previous works has been attributed to a general reduction in the number of coeluting peaks and hence to a reduction in ion suppression/enhancement [9] derived from the presence of matrix components. To evaluate

Table 2
Validation data of the UPLC-MS/MS method

Pesticide	Retention time (min)	Linearity (*r*)	LOD (ng/L)	Recovery (%) (*n* = 3)		Repeatability[a] (%)
				0.1 μg/L	0.005 μg/L	
Simazine	1.91	0.994	0.8	93	98	2.0
Atrazine	2.37	0.998	0.6	97	105	2.5
Isoproturon	2.43	0.999	0.1	98	99	3.8
Diuron	2.45	0.999	0.1	99	104	2.7
Terbutylazine	2.92	0.998	0.2	100	110	3.5
Alachlor	3.44	0.999	1.0	95	97	4.1
Pentachlorophenol	3.84	0.992	13	75	n.d	6.3
Chlorpyrifos	4.49	0.999	0.1	100	115	2.0
Trifluralin	4.54	0.998	20	56	n.d.	2.1

[a] Ten replicates injection of a spiked sample at 0.1 μg/L.

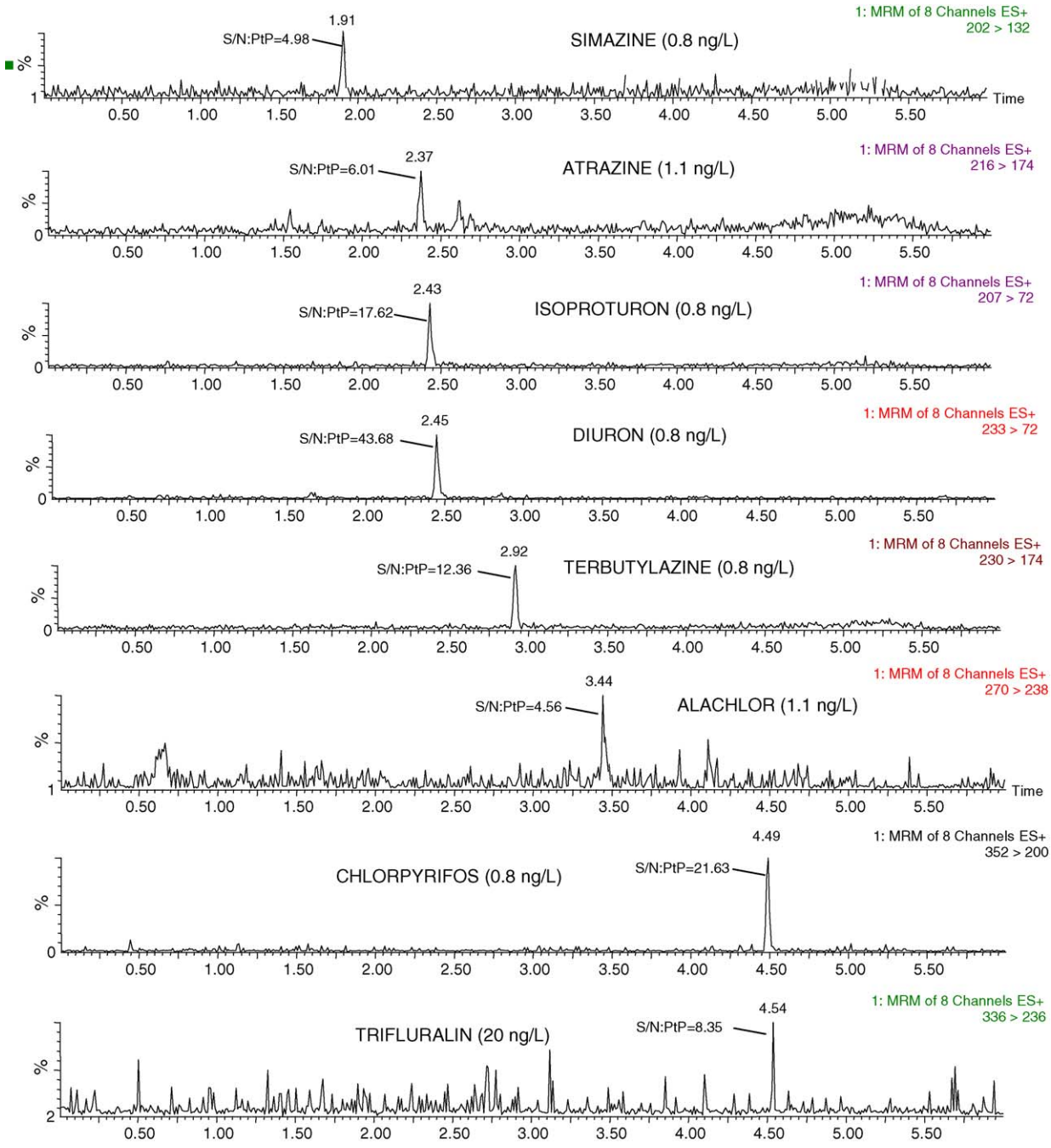

Fig. 1. Extracted MRM traces of the target pesticides obtained from UPLC-MS/MS analysis of a spiked SPE water extract.

the robustness of this instrumental approach and the influence of the water matrix in the MS signal intensity, calibration curves were drawn with both pure methanol and real water extracts spiked with identical amounts of pesticide standards. Variations observed in the slopes of the calibration functions obtained with matrix matched standards were in the range between 80 and 120% compared to pure solvent. Most of the compounds showed signal suppression with the exception of simazine and pentachlorophenol that presented signal enhancement of 10 and 20%, respectively. The suppression of the signal was below 10% for all compounds, except for atrazine and diuron, which showed 17 and 20% signal reduction.

3.1.2. Other performance characteristics of the method

A simple method based on solid phase extraction was applied for the extraction of the group of selected pesticides. The efficiency of the extraction method was evaluated by using matrix spike samples at 0.1 μg/L, the maximum concentration level allowed for individual pesticides by the EU legislation in drinking water, and 5 ng/L. The average recoveries and standard deviations obtained are included in Table 2. Good recoveries (>90%) were obtained for most of the target pesticides at the two fortification levels assayed, with RSD lower than 15%, except for trifluralin and pentachlorophenol which were extracted from the matrix at 56 and 75%, respectively. These low recoveries can be

Table 3
Results obtained in the analysis of water samples containing the target pesticides

Sample 1				Sample 2			
TestQual value (μg/L)		Experimental value (μg/L)		TestQual value (μg/L)		Experimental value (μg/L)	
Atrazine	0.076	Atrazine	0.066	Simazine	0.15	Simazine	0.16
Terbutilazine	0.026	Terbutilazine	0.022	Pentachlorophenol	0.29	Pentachlorophenol	0.40
Diuron	0.035	Diuron	0.030	Isoproturon	0.24	Isoproturon	0.26
				Alachlor	0.28	Alachlor	0.26

attributed to the low polarity of these compounds. Despite these lower recoveries, the other performance data were very good, and so a reliable determination of these compounds is feasible. Intra-laboratory precision expressed in terms of repeatability was considered satisfactory, with standard deviations varying from 2 to 6%. Repeatability of the retention times was also evaluated to ensure the reliability of this identification criterion. No variation in the retention time was observed after 10 consecutive injections of a fortified matrix extract for simazine, atrazine, diuron, terbutylazine and chlorpyrifos. For the rest of compounds relative standard deviations between 0.20 and 0.28%, that is variations of ±0.02 min as absolute value, were obtained, showing a good stability in the elution conditions.

The linearity was also good for all compounds with correlation coefficients always higher than 0.99 over the studied concentration range (0.05–1 μg/L). Limits of detection, obtained with a preconcentration factor of 300, ranged from 0.1 ng/L for isoproturon, diuron or chlorpyrifos to 20 ng/L for trifluralin, which resulted to be the less sensitive compound under the experimental conditions applied. In all the cases, LODs obtained guaranties the correct determination of the pesticides at the maximum concentration admissible for pesticides in water samples established by the European Union.

3.2. Tandem mass spectrometric analysis

The advantages supplied by the UPLC™ system were strengthening with its coupling to a tandem quadrupole mass spectrometer, which provided enhanced selectivity and sensitivity. Fast mass spectral data acquisition was however required to allow a rapid switch between MRM acquisition channels and between positive and negative ionization modes.

Optimal MS/MS conditions were optimized as it is showed in Table 1. Most of the compounds were analysed under positive ionisation mode (ESI+) with the exception of pentachlorophenol that exhibited an increased response in the negative mode (ESI−). Two acquisition periods were set during the analysis, one (0–6 min retention window) including all the pesticides analysed under ESI+ and the other one (2.8–3.7 min) for pentachlorophenol (ESI−). The change of polarity during the acquisition method was performed without any damage in the intensity of the peaks observed. Characteristic source parameters were optimised to get an only and very intense precursor ion. The most important analyte-dependent parameter in this case is the cone voltage. Under the selected conditions the quasi-molecular ion, $[M+H]^+$ or $[M-H]^-$ was obtained for all the compounds. Suitable transitions from these precursor ions to product ions were automatically optimised by the instrument software. Table 1 shows the ions used for MRM and the optimised collision energy values. The complete disappearance of the parent ion was avoided to get more confidence in the identification. A dwell time of 50 ms was selected in the method; however, it would be still possible to reduce this value if a higher number of transitions are included in the analysis.

3.3. Application to water analysis

To evaluate the effectiveness of the proposed method, it was applied to the analysis of two samples from an inter-laboratory comparison test for pesticide residue analysis organized by TestQual. The results obtained are shown in Table 3. All the target compounds were identified by comparing the retention times and product ion masses in both, samples and matrix matches standards. The concentration of the different compounds present varied between 0.026 and 0.29. Good agreement was obtained between the UPLC™ results and the target values with deviations lower than 15% in all the cases, except for pentachlorophenol which presented a higher deviation.

4. Conclusions

The application of the recently developed ultra performance liquid chromatography technology combined with tandem mass spectrometry for the determination of priority pesticides in groundwater has been established. This technique has provided enhanced characteristics regarding resolution, sensitivity and speed of analysis. Separation of the nine target compounds was obtained in less than 4 min with a total run time of 6 min, so providing the very high throughput required in screening analysis. The very narrow chromatographic peaks generated by UPLC™, with peak width lower than 2 s, result in an increase in the chromatographic efficiency and sensitivity, with LODs in the range between 0.11 and 7.8 ng/L. Other performance characteristics of the developed analytical method include good linearity, precision, selectivity and absence of ion suppression effects. Therefore, this analytical UPLC-MS/MS approach can be considered as a promising technique that can easily compete with conventional LC–MS techniques in this field of application.

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