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Trace analysis of pesticides in paddy field water by direct injection using liquid chromatography–quadrupole-linear ion trap-mass spectrometry

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ABSTRACT

A multiresidue method was developed for the quantification and confirmation of 70 pesticides in paddy field water. After its filtration, water was injected directly in a liquid chromatograph coupled to a hybrid triple quadrupole-linear ion trap-mass spectrometer (QqLIT). The list of target analytes included organophosphates, phenylureas, sulfonylureas, carbamates, conazoles, imidazolinones and others compounds widely used in different countries where rice is cropped. Detection and quantification limits achieved were in the range from 0.4 to 80 ng L^{-1} and from 2 to 150 ng L^{-1}, respectively. Correlation coefficients for the calibration curves in the range 0.1–50 μg L^{-1} were higher than 0.99 except for diazinon (0.1–25 μg L^{-1}). Only 9 pesticides presented more than 20% of signal suppression/enhancement, no matrix effect was observed in the studied conditions for the rest of the target pesticides. The method developed was used to investigate the occurrence of pesticides in 59 water samples collected in paddy fields located in Spain and Uruguay. The study shows the presence of bensulfuron methyl, tricyclazole, carbendazim, imidacloprid, tebuconazole and quinclorac in a concentration range from 0.08 to7.20 μg L^{-1}.

1. Introduction

The widespread use of pesticides, not only in the agriculture but also in domestic and industrial activities resulted in the presence of residues of these products and their metabolites in the environment. Many of these pesticides show a strong persistence in the soil–water environment and also in fatty tissue as they tend to bioaccumulate [1,2]. Moreover, due to their physicochemical properties, pesticides can leach from agricultural fields to ground and surface waters being a potential risk for ecosystems as well as for drinking water quality [1].

During the last decade, the public/government concern on environmental pollution caused by pesticides use has been growing. Many regulatory organisms, like the European Commission (EC), have adopted strict regulations trying to hamper or minimize the negative effects in the environment. In the water policy field, the European Union (EU) established different directives such as the Water Framework Directive 2000/60/EC whose main objective is to protect and prevent water quality [3]. In 2008, the Directive No. 2008/105/EC was set amending the Directive 2000/60/EC and establishing a list of 33 priority substances in water to be controlled, where the third part of the list are pesticides [4]. Two other possible candidates, bentazone and glyphosate, are also under review to be included in the list as it is presented in the Annex III of this Directive [4]. The maximal concentrations authorized for these contaminants vary from 4 ng L^{-1} to 20 μg L^{-1} depending on the chemical nature of the compound [4]. Moreover, environmental quality standards have been proposed for a number of pesticides and other contaminants in inland and other surface waters [5].

In order to detect the contamination of water resources by pesticide residues that can threaten environment preservation it is mandatory to develop simple, fast and reliable analytical tools which can be used to determine a wide range of pesticides at such low concentrations. Although there are several methodologies developed for the analysis of pesticides residues in agriculture waters these techniques require a first step of extraction followed by a clean-up and pre-concentration step in order to detect a threshold down to 0.1 μg L^{-1}.

The main analytical techniques reported for the analysis of pesticide residues in water samples are solid phase extraction (SPE) and liquid–liquid extraction (LLE). SPE has been developed as an alternative for LLE, owing to its simplicity and economy in terms of analysis time and solvents consumption [6]. Nevertheless, still it is a quite laborious and expensive technique, as many work-up steps are involved and cartridges cost is quite high. Another important disadvantage of SPE is the high amount of co-extractives presented in the final extract, as generally 50- to 750-fold pre-concentration is needed to reach appropriate limits of detection (LODs) [1,2,7–10].

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In the last years, the new LC–MS/MS technology has improved the instrumental detection limits of LC–MS systems from nanograms to sub-picograms levels, turning LC/MS–MS an invaluable tool for the detection of polar contaminants in aqueous environmental matrices [11].

Taking advantage of the high performance of new equipments, this work reports the development of a multiresidue method for the analysis of 70 pesticides from different chemical classes in paddy fields water by direct injection analysis avoiding some of the typical sample treatment steps employed in water analysis. The equipment used in this study was a last generation hybrid triple quadrupole-linear ion trap (QqLIT) spectrometer.

The selected compounds for this study are the most commonly pesticides applied over rice fields worldwide and some of their degradation products. Other substances included on the priority list of the EU such as diuron and isoproturon were also included in this methodology [4].

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile HPLC grade (MeCN) was supplied by Merck (Darmstadt, Germany). Water used for LC–MS analysis was obtained from a Direct-Q5 Ultrapure Water System from Millipore (Bedford, MA). Formic acid (purity, 98%) was obtained from Fluka (Buchs, Germany). Analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Riedel-de-Haën (Seelze, Germany). The purity of all the standards was greater than 97%. Individual stock standard solutions of the target compounds were prepared in pure MeOH or MeCN, according to the solubility properties of each compound, and stored at −20 °C. Working solutions were prepared by an appropriate dilution of the stock solutions in MeCN and used for both procedures, the spiking and the calibration curves preparation. Nicotine-d_3 (Sigma–Aldrich, Steinheim, Germany) was used as surrogate standard for positive electrospray ionization mode (ESI).

2.1.1. Selected analytes

The analytes included in this method were chosen on the basis of previous experience and published literature concerning pesticide used in paddy fields [12–16]. They comprise a group of 78 compounds belonging to different chemical classes such as phenylureas, strobilurins, organophosphorous, carbamates, ureas, triazoles, phenoxyacids, including some metabolites. Occurrence of many of these compounds has been already reported in environmental waters [13,14,17–19].

2.2. Liquid chromatography–QqLIT-MS analysis

A hybrid Triple Quadrupole-Linear Ion Trap-Mass Spectrometer (5500 QTRAP® LC/MS/MS system, AB Sciex Instruments, Foster city, CA) was used for the analysis of the target compounds. The system was equipped with a turboionspray source operating in both, positive and negative ionization modes. Chromatographic separation was carried out using an HPLC system (Agilent Series 1200) provided with a Zorbax Eclipse XDB-C-8 150 length × 4.5 mm i.d., 5 μm particle size column (Agilent Technologies). The mobile phase consisted of acetonitrile (solvent A) and 0.1% formic acid in water (solvent B). For the positive mode the initial proportion of solvent A was 10%, which was kept constant for 1 min, increased to 100% within 15 min, kept constant for 10 min and reduced to 10% in 0.1 min. The run time analysis was 25 min and the post-run equilibrium time was 10 min. The gradient program used in the negative mode started with 20% A for 50 s, then linearly increased to 100% in 5 min, which was maintained constant for 5 min and reduced to 20% in 0.1 min. The total run time for the analysis in the negative mode was 15 min and the re-equilibration time was 5 min. The injection volume was 5 μL and the flow rate was kept constant at 0.6 mL min^{-1} in both modes.

The turboionspray source settings were: Ionspray voltage, 5000–3500 V; curtain gas, 20 (arbitrary units); GS1 and GS2, 50 psi; probe temperature, 500 °C. Nitrogen served as nebulizer gas and collision gas in both modes. Mass calibration and resolution adjustments on the resolving quadrupoles were performed automatically by using a 10–5 mol L^{-1} solution of polypropylene glycol introduced via a syringe pump and connected to the interface.

Applied Biosystems/MDS Sciex Analyst software was used for data acquisition and processing.

2.2.1. Selected reaction monitoring (SRM) parameters

SRM experiments were carried out to obtain the maximum sensitivity for the detection of the target molecules. The optimization of MS parameters, declustering potential (DP) and entrance potential (EP) for precursor ions, and collision energy (CE) and collision cell exit potential (CXP) for product ions, was performed by flow injection analysis (FIA) of 1 mg L^{-1} of each compound separately. Table 1 shows the optimized parameters and the selected SRM transitions.

For all the compounds, the protonated molecule $[M+H]^+$ was the most abundant, and so it was chosen as the precursor ion. Afterwards, in the product ion mode, two product ions for each pesticide were selected, along with their corresponding CE.

Some compounds yielded low mass product ions. This was the case for tebuconazole, triadimenol (70*m/z*), triflumizole (72.9*m/z*), cyproconazole (70.1*m/z*), chlorotoluron, pirimicarb (72.1*m/z*), difenoxuron, diuron and isoproturon (72*m/z*). Obtaining such low masses represents a disadvantage as it entails a decrease in specificity. Nevertheless these ions were chosen as product ions as no other higher mass were sensitive enough.

In the present work, two different algorithms: Standard and the Scheduled MRM™ mode [20] were compared in terms of limits of detection (LODs), reproducibility (RSD) and number of data points across a chromatographic peak.

The dwell time for the Standard mode was optimized and set as 5 and 100 ms for the positive and negative mode, respectively, whereas working in the Scheduled mode no dwell time is needed. Instead of that, the Scheduled mode monitors SRM transitions only when they need to be monitored and not continuously thorough the chromatographic run. Therefore the retention time of each pesticide must be accurately known and determined before data acquisition [20,21].

2.2.2. Information-dependent-acquisition (IDA) conditions

An IDA experiment in the positive mode was programmed combining SRM as the survey scan and an enhanced production (EPI) scan as the dependent scan, in the same injection. This experiment was developed for the confirmation of quinclorac, where further structural information was necessary for confirmatory purposes due to the absence of its second transition (SRM2) at the concentration range studied.

SRM parameters used in the optimized survey scan are shown in Table 1 for the positive amenable pesticides. IDA parameters included the acquisition of one ion which peak height exceeded 500 counts per second and without exclusion after dynamic background subtraction of survey scan. EPI scan was performed with Q1 set at Low resolution and the linear ion trap scanning from 50 to 270 amu at a scan rate of 10,000 amu s^{-1}. The dynamic fill-time option was selected on the ion trap. The CE, DP and EP used for the EPI scan were 30 eV, 65 V and 10 V, respectively. The complete SRM–IDA–EPI cycle time was 1.19 s.

Table 1
Optimized parameters for the QqLIT/MS analysis of the target pesticides (precursor ion, DP, product ions and their CE), SRM ratio and their corresponding variation coefficients (% RSD).

Pesticide	tR	Precursor ion	SRM1	CE1	SRM2	CE2	DP	SRM2/SRM1[a] (% RSD)
Positive mode								
Azimsulfuron	12.7	425.0	182.1	29	243.7	28	100	0.03 (16)
Azoxystrobin	14.4	404.2	372.0	21	343.9	33	100	0.4 (9)
Bendiocarb	12.6	223.7	166.9	13	109.0	27	100	0.9 (12)
Bensulfuron methyl	13.3	411.2	182.2	29	148.9	30	117	0.6 (6)
Bispyribac sodium	13.5	431.2	274.9	21	413.1	28	205	0.2 (18)
Carbaryl	12.8	202.1	145.0	16	127.1	43	130	0.5 (7)
Carbendazim	6.7	192.0	160.1	27	132.0	41	200	0.2 (4)
Carbofuran	12.6	222.0	165.1	18	123.1	31	91	0.9 (4)
Chlorotoluron	10.5	214.0	72.1	32	140.8	19	87	0.04 (6)
Clomazone	13.7	240.7	125.0	30	85.0	34	80	0.01 (6)
Cyhalofop butyl	16.9	357.8	256.0	17	302.2	10	117	–
Cyproconazole	14.1	292.1	70.1	58	125.0	44	170	0.5 (7)
Diazinon	16.7	304.6	169.0	30	153.1	27	100	0.7 (10)
3,4-Dichloroaniline	13.6	162.1	126.7	29	145.1	34	213	–
Diethofencarb	14.3	268.0	226.1	14	180.1	23	76	0.8 (9)
Difenoconazole	15.8	405.9	250.9	36	337.0	24	100	0.5 (5)
Difenoxuron	12.8	287.0	72.0	23	123.3	25	150	0.6(9)
Diflubenzuron	15.0	310.8	158.1	19	141.1	47	115	0.8 (12)
Dimethoathe	10.2	230.0	199.1	15	171.2	20	60	0.5 (4)
Diuron	13.0	233.0	72.0	22	160.0	35	64	0.1 (4)
Edifenphos	15.6	311.0	283.0	17	173.0	25	160	0.4 (8)
Epoxiconazole	14.4	331.0	121.0	30	141.1	28	185	–
Ethiofencarb	13.2	226.1	107.0	28	164.1	11	60	0.4 (11)
Fenobucarb	14.4	208.1	94.9	25	152.1	12	94	0.6 (7)
Fenuron	9.8	165.1	72.0	26	120.0	24	40	0.05 (6)
Flufenoxuron	17.3	489.2	158.1	32	140.6	62	165	0.2 (4)
Fluroxypir	14.7	255.0	208.8	22	237.0	16	135	0.3 (5)
Flutolanil	15.3	323.9	261.8	23	282.2	20	120	0.8 (11)
Hexaconazole	15.2	314.3	69.9	69	245.0	23	180	–
Imazamethabenz methyl	10.1	289.1	229.0	28	257.1	24	280	0.2 (12)
Imazapic	9.5	276.1	231.0	25	163.0	36	240	0.8 (8)
Imazapyr	8.5	262.9	218.1	28	235.0	22	150	0.4 (4)
Imazaquin	11.4	312.0	267.0	28	198.9	36	245	0.4 (11)
Imazosulfuron	13.3	413.1	156.1	31	231.9	24	120	0.2 (10)
Imidacloprid	9.9	256.0	175.1	27	209.1	35	118	0.7 (9)
Iprodione	15.3	330.0	244.8	22	288.0	22	165	–
Isoprocarb	13.6	194.0	94.9	18	152.1	11	60	0.4 (9)
Isoproturon	12.9	207.1	72.0	27	165.1	19	70	0.3 (12)
Kresoxim methyl	15.9	314.0	206.1	12	266.9	8	76	0.9 (9)
Malathion	15.4	331.3	127.0	19	99.0	37	52	1(10)
Metsulfuron methyl	11.9	382.2	166.7	19	141.0	35	100	0.2 (8)
Molinate	15.1	188.0	125.9	23	97.8	48	130	–
Oxydemethon methyl	7.4	247.0	168.8	16	104.9	16	140	0.3 (2)
Picoxystrobin	15.8	368.2	205.2	13	145.0	30	80	0.8 (9)
Pirimicarb	8.3	238.7	72.1	38	182.2	23	50	0.7 (11)
Pirimiphos methyl	16.8	306.1	108	42	164.1	28	110	–
Prochloraz	13.6	377.1	309.2	23	266.7	18	100	0.1 (5)
Promecarb	14.7	208.1	151.1	14	109.0	23	80	0.7 (8)
Propaphos	15.5	304.8	221.0	19	263.0	10	90	0.3 (8)
Propaquizafop	16.9	443.7	100.0	20	371.1	19	115	0.4 (4)
Propiconazole	15.5	342.2	159.0	51	187.0	25	110	0.1 (8)
Propoxur	12.5	210.0	168.1	9	111.2	12	60	0.8 (5)
Pyraclostrobin	16.2	388.2	193.7	16	295.9	20	50	0.3 (6)
Pyrazosulfuron ethyl	14.1	415.2	182.1	19	369.1	19	44	0.2 (11)
Pyridaphenthion	14.8	341.0	189.1	36	205.0	34	100	0.5 (10)
Quinclorac	11.6	242.0	224.0	21	–	–	50	–
Quinoxyfen	17.1	309.1	197.9	40	272.9	40	100	0.6 (3)
Spiroxamine	11.2	298.1	144.0	25	100.1	47	240	0.5 (7)
Tebuconazole	14.8	308.1	70.1	60	125.1	57	80	0.2 (14)
Tebufenozide	15.7	353.4	296.7	10	133.2	23	60	0.1 (9)
Temephos	17.3	467.1	404.7	25	418.8	26	190	0.6 (13)
Tetraconazole	14.7	372.0	159.0	43	70.0	65	110	0.4 (14)
Thiacloprid	11.0	253.0	125.9	34	186.0	19	110	0.2 (9)
Thiamethoxam	8.9	292.1	210.9	19	246.1	14	60	0.1 (3)
Thiodicarb	11.6	355.1	87.9	29	163.2	11	160	0.2 (12)
Thiophanathe ethyl	13.4	371.0	282.0	15	324.8	13	60	0.9 (9)
Triadimefon	14.8	294.0	197.1	20	224.9	20	180	0.1 (11)
Triadimenol	13.9	296.1	70.0	40	227.1	14	50	0.1 (16)
Triazophos	13.3	314.1	162.1	25	286.0	16	120	0.1 (10)
Tricyclazole	10.0	190.1	163.1	31	136.1	40	210	0.9 (6)
Trifloxystrobin	16.9	409.2	185.7	25	206.0	20	150	0.5 (6)
Triflumizole	15.5	346.2	278.2	15	72.9	21	100	0.4 (9)
Triflumuron	15.8	359.0	156.2	27	138.8	42	40	0.5 (10)

Table 1 (*Continued*)

Pesticide	tR	Precursor ion	SRM1	CE1	SRM2	CE2	DP	SRM2/SRM1[a] (% RSD)
Negative mode								
2,4-D	7.5	220.7–218.7	162.8	15	160.8	34	110	0.7 (16)
Bentazone	7.6	238.8	175.2	28	197.1	29	250	0.6 (6)
Fipronil	8.4	437.1	331.0	24	319.1	32	100	0.1 (10)
Propanil	7.9	216.9	160.8	23	125.0	33	110	0.04 (12)
Teflubenzuron	8.7	379.0	339.0	16	359.0	10	110	0.4 (12)

DP, declustering potential (V); CE, collision energy (eV); EP, entrance potential (10 V); CXP, collision cell exit potential (5 V).

[a] The SRM ratio is calculated from mean values obtained from the matrix-matched calibration curves.

Previously, the spectra generated in matrix solutions at 1 and 10 μg L^{-1} concentrations, acquired in EPI mode, were stored in a mass spectral library at the CE selected (30 eV), which enables the confirmation of quinclorac in real positive samples. In this case, confirmation criteria applied to quinclorac in the water samples were: the presence of the characteristic SRM transition at the correct retention time, and a library search fit value higher than 70%.

2.3. Sampling and sample preparation

Paddy field water samples used in this study were taken from two different regions were different pesticides are currently applied, namely – South America, Uruguay and Europe, Spain. A total of 59 samples were analyzed.

Water samples were collected in clean amber glass bottles and stored in the dark at −20 °C. After collection, the samples were adjusted to pH 3 and filtered through a 0.7 μm glass fibre filter (Teknokroma, Spain), in order to remove particles that may interfere with the analysis. Before analysis, 100 μL of a 10 μg L^{-1} labeled standard of nicotine-d_3 in MeCN (surrogate standard) was added to 900 μL of water and the mixture was filtered directly into a vial using a 0.45 μm PTFE syringe filter (Millipore, USA).

3. Results and discussion

3.1. Optimization of SRM conditions

For the SRM method two transitions per compound were selected in order to comply with EU requirements for confirmatory analysis (Commission Decision 2002/657/EC) [22]. The less intense transition (SRM2) was used for the confirmation of each analyte, while for quantitative purposes the peak area of the most intense transition (SRM1) was considered. The ratio between the two SRM transitions (SRM2/SRM1) was calculated in order to be used as the identification criterion along with the retention time and the presence of both transitions according to Ref. [22]. The results are presented in Table 1 together with their corresponding coefficients of variation. As it is shown in Table 1 differences in intensity of up to ten times between the two monitored transitions were observed for almost 18 pesticides. This is a disadvantage when identifying these analytes, especially at low concentrations, were the signal-to-noise ratio for the confirmation must be higher than three.

Table 2
Number of data points per peak and the S/N at 0.5 μg L^{-1} obtained for Scheduled and Standard mode in water extract.

Pesticide name	No. data points per peak Standard mode	No. data points per peak Scheduled mode
Bensulfuron methyl	11	12
Diazinon	9	14
Edifenphos	9	12
Isoproturon	8	15
Malathion	8	13
Picoxystrobin	8	14
Tebuconazole	9	15
Teflubenzuron	10	11
Thiacloprid	10	15
Tricyclazole	9	17

3.2. Sample treatment and direct injection analysis

Direct injection of water samples is becoming an attractive procedure to traditional analytical techniques which in general include a preliminary pre-concentration step either with LLE or SPE [23–25]. This technique presents many advantages, such as no pre-concentration step and, as a consequence, minimum sample manipulation, less co-extractives compounds in the final extract, low consume of solvents, low cost and better reproducibility.

The excellent sensitivity of the equipment allowed us to study the performance of the direct injection of water samples for the determination of pesticide residues in paddy fields water.

Sample preparation was performed as it is described in Section 2.3. Based in our experience, the addition of MeCN to the water sample before the filtration step, improves the efficiency during the filtration process; otherwise some pesticides are partially lost during the filtration process [26–28].

For the selection of the working conditions, the effect of the pH of the samples was studied by comparing the response of each pesticide obtained during the analysis of a spiked water sample at pH 3, 5, 7 and 8. In general no significant differences were observed for almost all the pesticides. The responses at different pH were in the same order of magnitude except for some compounds such as malathion, temephos, propoxur, propaphos and metsulfuron methyl where the difference in the response between acidic or basic pH was around 20%, therefore pH 3 was selected for method validation [7].

3.3. Validation study

Validation studies were carried out using a real water sample. As no certified pesticide-free water sample could be obtained to be used as blank, paddy field water was used, which was previously analyzed and the presence of the target compounds considered. Nicotine-d_3 was used as surrogate standard in order to check the entire procedure.

3.3.1. LODs, LOQs and selection of the working method

In order to compare the sensitivity of Standard and MRM Scheduled™ modes, the limits of detection (LODs) were calculated using standard solutions prepared in pure solvent and in spiked paddy field water. The LODs were determined as the lowest pesticide concentration whose qualified transition (SRM2) presented a signal-to-noise ratio (S/N) ≥ 3. The quantification limits (LOQ) were determined also in pure sol-

Table 3
Main validation parameters: LOD, LOQ, coefficient of determination (R^2), reproducibility, repeatability and slope in matrix/slope in solvent ratio obtained for the developed method.

Pesticide	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)	Reproducibility (% RSD_{wR})		Repeatability (% RSD_r)		R^2	Slope in matrix/slope in solvent
			1 $\mu g\,L^{-1}$	50 $\mu g\,L^{-1}$	1 $\mu g\,L^{-1}$	50 $\mu g\,L^{-1}$		
Azimsulfuron	0.04	0.045	10.6	9.6	7.4	4.7	0.9978	1.11
Azoxystrobin	0.0004	0.003	10.1	11.8	8.4	5.0	0.9941	1.07
Bensulfuron methyl	0.003	0.006	10.1	3.2	8.6	13.9	0.9907	0.93
Bentazone	**0.008**	**0.03**	**16.5**	**16.8**	**11.5**	**10.3**	**0.9974**	**0.70**
Bispyribac sodium	0.06	0.060	18.1	15.7	7.9	7.9	0.9965	1.16
Carbaryl	0.03	0.050	16.3	12.2	5.8	5.6	0.9916	0.58
Carbendazim	0.009	0.009	17.8	13.1	5.2	2.4	0.9950	0.94
Carbofuran	0.002	0.004	16.5	14.9	6.1	4.9	0.9940	0.84
Chlorotoluron	0.07	0.075	12.2	10.5	6.8	4.7	0.9962	0.93
Clomazone	0.5	0.5000	18.3	14.6	11.6	3.3	0.9911	0.89
Cyproconazole	0.004	0.011	15.9	12.5	5.7	4.0	0.9922	1.00
Diazinon	0.001	0.002	11.0	3.2	1.5	2.5	0.9994	0.24
Diethofencarb	0.008	0.026	12.3	11.9	3.6	4.1	0.9949	0.89
Difenoconazole	0.006	0.010	16.8	9.5	1.2	3.6	0.9954	0.89
Difenoxuron	0.001	0.002	18.4	16.4	8.2	4.9	0.9940	1.01
Diflubenzuron	0.02	0.032	12.6	11.8	11.1	5.8	0.9987	0.82
Dimethoathe	0.008	0.008	17.0	14.7	3.7	3.4	0.9969	0.94
Diuron	0.01	0.015	13.5	10.8	6.4	6.8	0.9967	0.95
Edifenphos	0.005	0.011	16.6	10.1	7.4	5.2	0.9974	0.74
Ethiofencarb	0.002	0.007	11.1	10.5	5.6	4.4	0.9980	0.83
Fenobucarb	0.02	0.066	15.3	11.9	4.5	5.6	0.9972	0.90
Fenuron	0.03	0.030	13.3	9.5	5.8	1.8	0.9928	0.94
Fipronil	**0.05**	**0.2**	**10.4**	**11.8**	**8.1**	**7.0**	**0.9947**	**0.95**
Flufenoxuron	0.06	0.07	12.7	5.0	5.3	4.9	0.9938	0.70
Fluroxypir	0.02	0.04	17.6	15.0	4.2	4.7	0.9916	0.93
Flutolanil	0.004	0.005	10.0	9.1	11.6	5.5	0.9975	0.87
Imazamethabenz methyl	0.01	0.013	19.3	17.4	6.0	7.9	0.9901	0.94
Imazapic	0.01	0.090	7.5	1.9	4.1	3.4	0.9968	1.10
Imazapyr	0.007	0.017	16.2	12.1	3.5	3.0	0.9978	1.12
Imazaquin	0.005	0.005	9.3	9.0	13.2	6.4	0.9951	1.26
Imazosulfuron	0.01	0.012	14.2	12.0	7.0	7.3	0.9973	0.86
Imidacloprid	0.003	0.004	17.1	15.0	1.8	4.6	0.9959	1.10
Isoprocarb	0.03	0.032	13.9	13.7	3.7	8.8	0.9958	0.95
Isoproturon	0.06	0.060	16.3	14.7	6.2	3.6	0.9912	0.91
Kresoxim methyl	0.03	0.035	7.9	9.0	4.0	6.8	0.9973	0.92
Malathion	0.01	0.014	9.6	8.2	5.4	0.8	0.9945	0.95
Metsulfuron methyl	0.003	0.003	18.7	13.7	3.7	3.0	0.9941	1.00
Oxydemethon methyl	0.001	0.003	3.7	5.2	3.3	0.9	0.9954	0.91
Picoxystrobin	0.001	0.001	18.5	14.2	4.7	4.7	0.9951	1.00
Pirimicarb	0.001	0.001	16.3	4.9	2.1	1.6	0.9979	0.91
Prochloraz	0.04	0.055	6.4	9.0	6.9	7.8	0.9941	1.04
Promecarb	0.04	0.05	10.3	14.1	3.7	6.7	0.9935	0.92
Propanil	**0.08**	**0.08**	**9.5**	**12.6**	**12.3**	**10.2**	**0.9990**	**0.87**
Propaphos	0.003	0.005	18.4	14.2	7.4	6.3	0.998	0.92
Propaquizafop	0.03	0.03	12.0	10.3	6.2	0.6	0.9978	0.26
Propiconazole	0.04	0.04	10.1	11.0	5.9	3.1	0.9924	0.91
Propoxur	0.006	0.01	12.4	15.1	4.6	5.5	0.9973	0.82
Pyraclostrobin	0.003	0.004	10.1	12.0	3.8	0.8	0.9986	0.67
Pyrazosulfuron ethyl	0.03	0.005	14.9	7.6	5.9	10.7	0.9994	0.85
Pyridaphenthion	0.001	0.002	14.9	5.6	4.5	3.8	0.9974	0.95
Quinclorac	0.05	0.15	12.0	9.5	8.7	6.4	0.9985	1.03
Quinoxyfen	0.02	0.02	18.2	3.7	2.1	1.0	0.9980	0.83
Spiroxamine	0.02	0.04	17.3	2.6	3.5	2.9	0.9994	0.92
Tebuconazole	0.002	0.005	11.4	7.2	2.9	2.9	0.9982	0.97
Tebufenozide	0.001	0.002	14.3	8.9	7.8	4.0	0.9977	0.87
Teflubenzuron	**0.01**	**0.03**	**14.3**	**12.7**	**7.3**	**9.2**	**0.9971**	**0.18**
Temephos	0.03	0.03	11.4	12.7	2.5	3.9	0.9915	0.76
Tetraconazole	0.02	0.008	4.1	7.8	2.9	6.3	0.9978	1.03
Thiacloprid	0.002	0.001	16.6	14.2	2.7	2.1	0.9934	0.93
Thiamethoxam	0.03	0.03	3.9	4.0	3.3	1.8	0.9964	0.98
Thiodicarb	0.01	0.15	15.9	13.3	4.9	7.5	0.9968	0.80
Thiophanathe ethyl	0.008	0.02	15.7	10.1	12.6	9.3	0.9981	0.87
Triadimefon	0.02	0.02	13.1	10.0	9.1	11.8	0.9952	0.89
Triadimenol	0.03	0.03	15.2	10.9	4.2	3.0	0.9905	0.91
Triazophos	0.001	0.003	13.8	11.1	5.3	5.4	0.9954	0.89
Tricyclazole	0.001	0.002	15.4	13.8	3.7	3.8	0.9961	0.90
Trifloxystrobin	0.001	0.003	13.7	11.5	7.3	6.5	0.9985	0.96
Triflumizole	0.04	0.04	18.3	18.5	7.2	6.3	0.9987	0.90
Triflumuron	0.5	0.50	16.1	13.5	5.5	6.2	0.9985	0.86
2.4 D	**0.01**	**0.08**	**6.7**	**8.7**	**10.2**	**8.1**	**0.9997**	**0.91**

Pesticides in bold letter are those optimized in the negative mode.

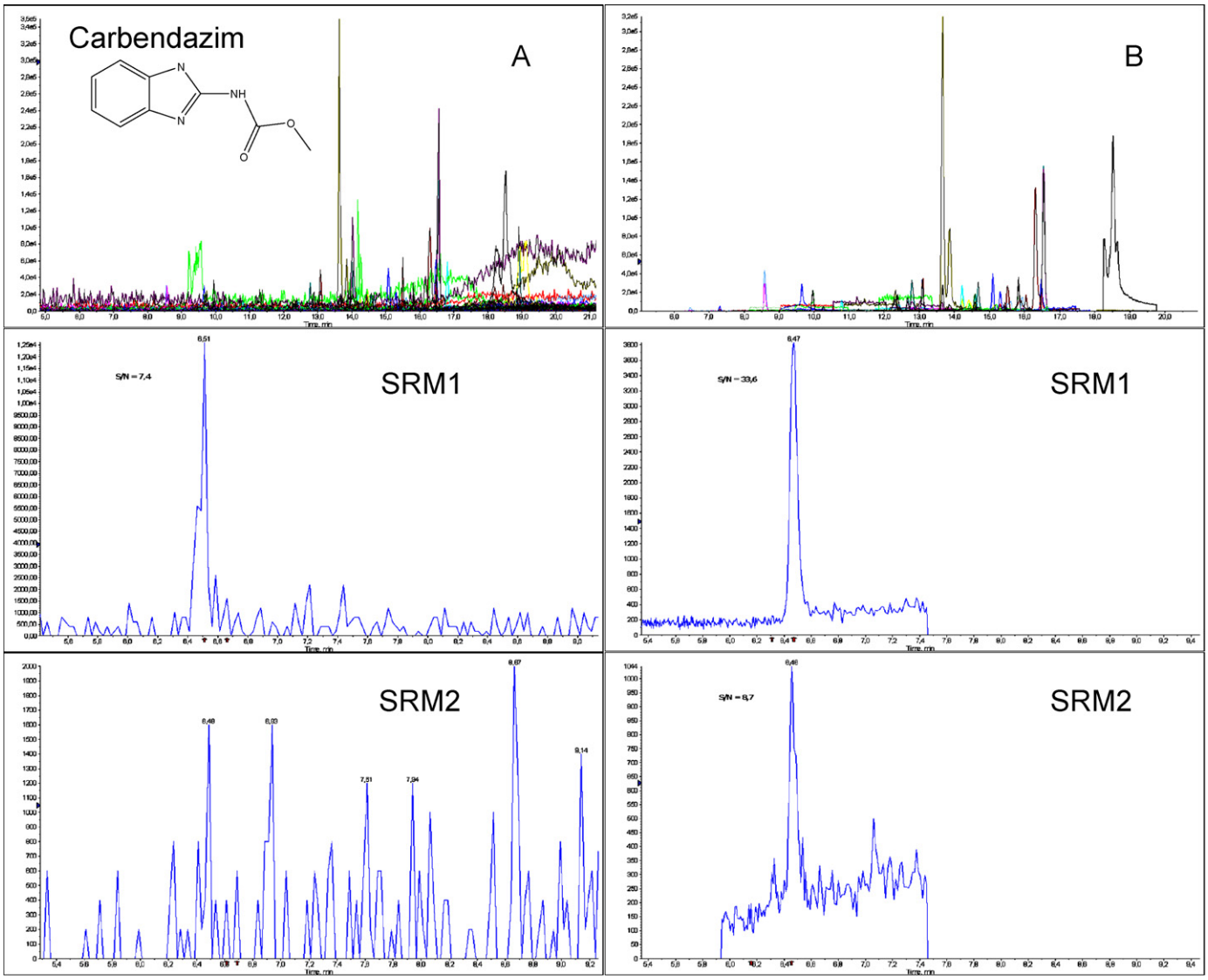

Fig. 1. Determination of carbendazim in paddy field water at 0.1 $\mu g\,L^{-1}$ by both modes (Standard mode: A and Scheduled mode: B).

vent and in paddy field water, as the minimum detectable amount of analyte with a $S/N \geq 10$ for the SRM1 transition. The LOD of quinclorac was 0.05 $\mu g\,L^{-1}$ calculated as the lowest pesticide concentration recognizable by library searching and presenting a fit value higher than 70% in the IDA experiments.

The criterion followed to select the working method (Scheduled or Standard) was the method with the highest number of com-

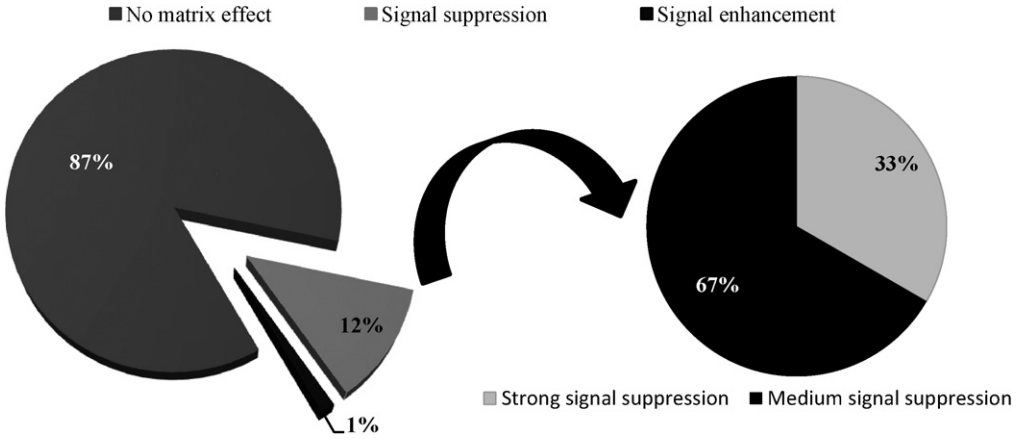

Fig. 2. Matrix effect discriminated by % of pesticides presenting strong, medium or no matrix effect.

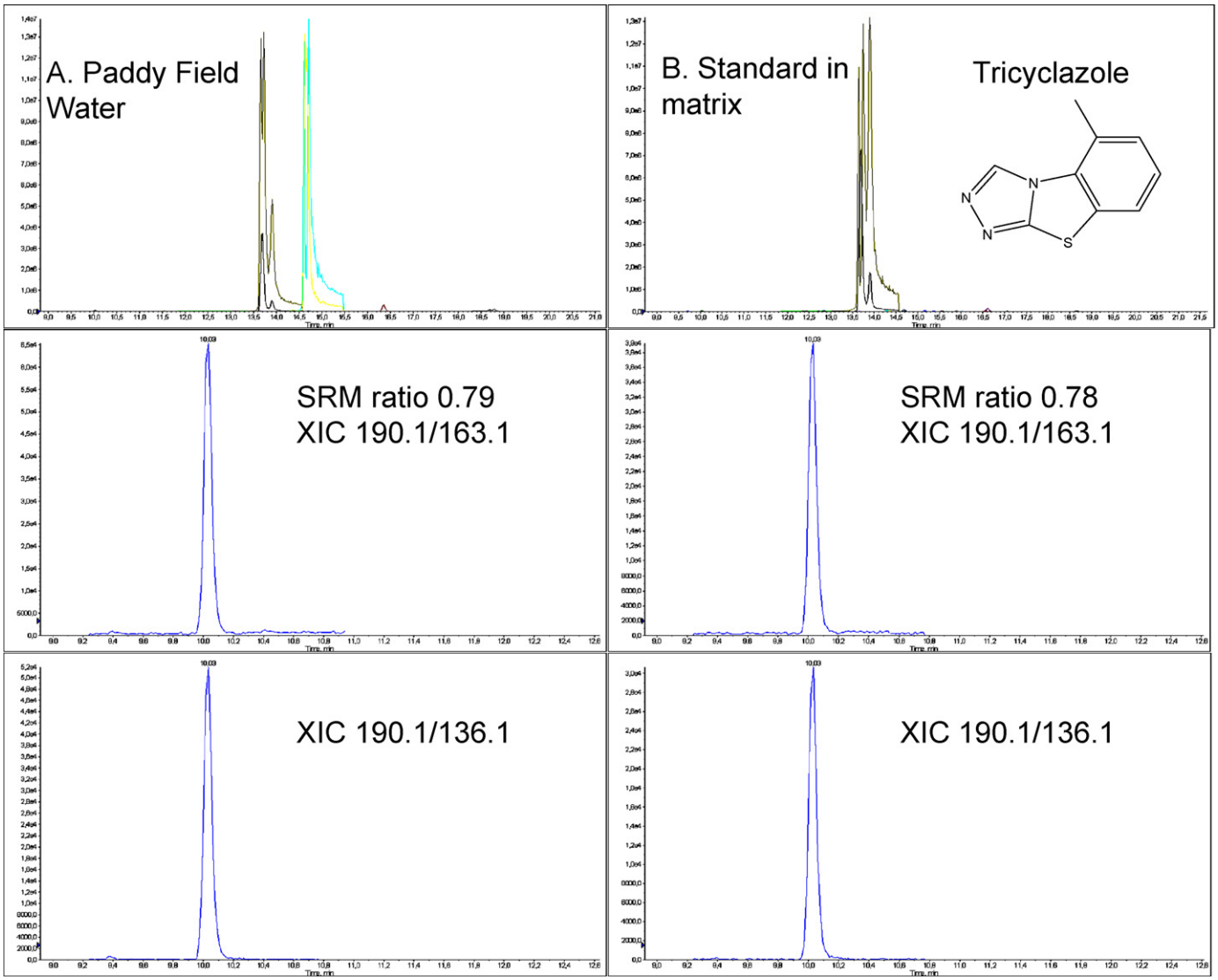

Fig. 3. Identification of tricyclazole in a paddy field water sample (A) at 1.90 μg L^{-1} and comparison with the standard in matrix (B).

pounds presenting a S/N ratio ≥ 3 at a concentration of 0.1 μg L^{-1} in matrix. The Scheduled method presented the best performance as 67 of the target pesticides presented a S/N of 3 or higher at 0.1 μg L^{-1} while the Standard method presented 41compounds which satisfied with these criteria. Fig. 1 shows the total ion chromatogram (TIC) and two extracted ion chromatograms for carbendazim. As it is shown in Fig. 1, the Scheduled method allows the identification and the quantitation of carbendazim, as both transitions presented a S/N higher than 3 and 10 corresponding to the LOD and LOQ, respectively, whereas in the chromatogram obtained with Standard method the second transition (SRM2) of carbendazim is missing and therefore this analyte cannot be confirmed using this method.

Overall, the LOQ values for the Scheduled method were in the range 2–150 ng L^{-1}.

Moreover the number of data points across a chromatographic peak was compared for both methods. The differences between the numbers of data points for both methods were not so pronounced as the LODs nevertheless for most of the pesticides the Scheduled method provided a higher number of data points per peak, 10–27 and the Standard method 7–14. In Table 2 are summarized the number of data points per peak for some of the target pesticides.

According with these results the best sensitivity in multiple reaction monitoring mode was achieved through the acquisition of the selected reaction monitoring transitions (SRM) with Scheduled mode, therefore the MRM Scheduled™ method was chosen for the validation study.

From the 78 pesticides included in the method, 7 compounds (bendiocarb, cyhalofop butyl, 3,4-dichloroaniline, epoxiconazole, hexaconazole, iprodione and molinate) presented LODs higher than 0.1 μg L^{-1} in the range 0.5–5 μg L^{-1},and pirimiphos-methyl presented linearity problems, therefore these 8 pesticides were excluded from the method and the validation studies were carried out with the other 70 pesticides (see Table 3).

3.3.2. *Linearity and matrix effect*

The linearity of the method was studied preparing a seven-point calibration curve in paddy field water in the range from 0.1 to 50 μg L^{-1} except for diazinon 0.1 to 25 μg L^{-1}. The linearity along the studied range was good, with correlation coefficients higher than 0.99 for all target compounds as it is shown in Table 3.

Matrix effects, either as signal suppression or enhancement, are a major drawback for quantitative trace analysis by LC–ESI/MS systems. Matrix co-extractives can compromise the quantitative analysis of the compounds at trace levels, as well as it

can greatly affect the method accuracy and reproducibility. Several proposals have been published to overcome this problem [29], but the most common one is the use of matrix-matched calibration standards for the quantification of the target analytes.

The analysis of water by solid phase extraction implies a pre-concentration step, thus matrix effect is generally observed. In this procedure, as there is not pre-concentration step we expected little or none matrix effect. However, in order to evaluate the extent of the matrix effects with the presented method, matrix-matched and solvent-based calibrations curves were prepared and the corresponding slope in matrix/slope in solvent ratio was calculated for each of the studied pesticides (see Table 3). Depending on the value (in percentage) different matrix effects could be observed. A percentage between −20% and 20% was considered as no matrix effect. Signal enhancement occurs if the % of the difference between the slopes is positive whereas a negative value is indicative of signal suppression.

A medium matrix effect was observed when the values ranged between −50% and −20% or 20–50% and a strong matrix effect would be below −50% or above 50%.

Eighty-seven percent of the pesticides under study did not present relevant matrix effect whereas 12% of the analytes (bentazone, carbaryl, diazinon, edifenphos, flufenoxuron, propaquizafop, pyraclostrobin and teflubenzuron) showed signal suppression. Imazaquin was the only pesticide presenting medium signal enhancement. Both strong and medium signal suppression were observed as it represented in Fig. 2. The high proportion of analytes showing little if any matrix effect, points out the advantage of working with high sensitivity equipment, which allows the analysis of the sample without performing a pre-concentration step.

Based on these results the quantification could be performed using solvent-based calibration curves for 61 of the studied analytes, avoiding the use of matrix-matched standards curves and hence reducing the uncertainty of the methodology.

3.3.3. *Reproducibility and repeatability*

The precision of the instrumental method, was estimated by determining the intra- and interday, % RSD, by the repeated analysis ($n = 5$) of a spiked paddy field water at 0.1 and 50 $\mu g\,L^{-1}$ level, from run-to-run, over 1 and 5 consecutive days. As it is shown in Table 3, the RSD ranged between 1–20% and below 14% for the reproducibility and repeatability, respectively.

3.3.4. *Specificity*

In order to achieve an unambiguous identification of the studied pesticides, the specificity of the method was evaluated via the SRM ratio of the target compounds. This ratio was calculated as the quotient between the qualifier and the quantifier transition. The maximum permitted tolerances for relative ion intensities is from 20 to 50% [22]. In order to validate this SRM ratio in water matrix, the SRM ratios in standard solution were also calculated. Afterwards the SRM ratios in matrix and in solvent were evaluated for each of the concentration levels of the calibration curves (0.1–50 $\mu g\,L^{-1}$) and the average was obtained. The identification criteria set for each of the presents compounds were very stable throughout the linearity range, with values of RSD below 20% (Table 1). Two other identification criteria were taken for the identification of the pesticides in the real matrices: the retention time and the presence of the two monitored transitions. In Fig. 3, the TICs and the extracted ion chromatograms (XIC) of the two monitored transitions of tricyclazole in real water and in standard are represented along with the three criteria used for the identification of the pesticide.

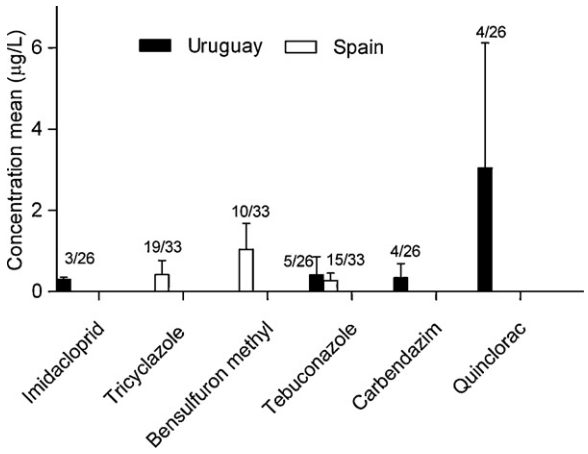

Fig. 4. Occurrence and concentrations mean in $\mu g\,L^{-1}$ of the pesticides detected in paddy fields waters.

4. Application to real samples

The proposed method was applied to the analysis of 59 paddy field water samples collected from different regions, 33 from Spain and 26 from Uruguay. Fig. 4 shows the occurrence and the mean concentrations found in the samples. From 59 analyzed samples, 31 presented pesticides. 10 samples presented 3 pesticides, 9 samples presented 2 pesticides and 12 other samples presented 1 pesticide.

Their occurrence follows the technological package applied in each country, as expected. The most frequently found pesticide in Uruguayan samples was the fungicide tebuconazole whereas the herbicide quinclorac was presented in the highest concentration, less often the antifungal carbendazim and the insecticide imidacloprid. In Uruguay, carbendazim is forbidden to use in rice, so its presence could be due to a high persistence in soils from where it can leach to the water. Tebuconazole, tricyclazole and bensulfuron methyl were found in the samples from Spain.

The compounds found in the samples are pesticides widely used in rice crops which are included in the Annex I of the EU legislation [30], but considering the standard of 0.1 $\mu g\,L^{-1}$ as the maximum concentration level allowed for individual pesticides in water, the concentration of the individual pesticides found in the analyzed samples were 1–70 times higher.

Quinclorac was present in four of the analyzed samples; this pesticide has been out of the Annex I since 2008, but is commonly used in Uruguay for rice crops. As it was discussed in Section 2.2.2, its confirmation was carried out by using linear ion trap in EPI mode, because it was not possible to obtained the two transitions required for it confirmation.

5. Conclusions

A quick, highly sensitive and “green” liquid chromatography ion trap spectrometric method for the separation and quantification of 70 pesticides in rice paddy field water was developed and optimized. The direct injection analysis of paddy fields water in the LC–MS/MS system is an attractive methodology as minimum sample manipulation is needed and non further clean-up is performed as in other conventional reported techniques for water analysis such as LLE and SPE. The LODs achieved with this methodology are within the range of $\mu g\,L^{-1}$ or $ng\,L^{-1}$ which are enough to fulfil the most restricted regulations. As most of the pes-

ticides did not present matrix effect, the quantification step can be performed using standard curves in solvent, avoiding the use of matrix-matched calibration curves. In this way, the precision and the analysis time are dramatically improved, which is an interesting issue for pesticide residue routine analysis.

The method usefulness was established through the confirmation of 6 different pesticides during the analysis of 59 real samples. 31 samples presented pesticides; being tricyclazole and tebuconazole the compounds most often found. Most of these samples presented a concentration of pesticides higher than 0.5 $\mu g\,L^{-1}$ which is the maximum concentration allowed by the EU legislation. These results highlight the consistent and good sensitivity that can be achieved using the developed method.

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