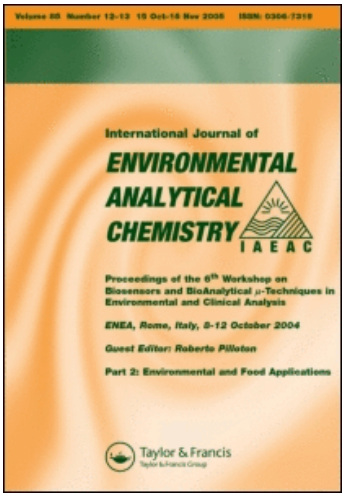

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Optimisation and validation of an analytical methodology for selected pesticides in waters by solid-phase extraction and liquid chromatography with ion-trap mass spectrometry detection

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Optimisation and validation of an analytical methodology for selected pesticides in waters by solid-phase extraction and liquid chromatography with ion-trap mass spectrometry detection

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Multiresidue analytical methodologies are being developed for several classes of pesticides, using either GC or LC techniques coupled with MS detection. However, to reach ultra-low levels, as imposed by recent European Directives, specific extraction methods must be applied. This work intends to present the optimisation steps followed to implement a method to analyse pesticides of different families, as diuron, bentazone and cymoxanil. Among three types of SPE sorbents tested (EnviCarb, Easy and HR-P), the best results were achieved with HR-P. Concerning LC/MS with electrospray ionisation, the ionisation modes (negative and positive in a single run), the individual mass fragmentation, the parent ions for subsequent fragmentation, the needle voltage and the RF loading, the excitation amplitude, as well as the determination of optimal conditions for temperature of the drying gas and the pressure values for the drying and nebulisation gases, were optimised. As quantification method, attending the relatively low extracting recoveries, matrix-matched calibration was used, in order to achieve detection limits at ng L^{-1} levels. However, because high uncertainties are achieved, it is proposed that the optimised direct injection in LC/MS is the best approach. In these conditions, analysis could be performed in less than 10 minutes and the limits of detection were 0.48, 0.13 and 4.26 μg L^{-1} for diuron, bentazone and cymoxanil, respectively.

Keywords: diuron; bentazone; cymoxanil; LC/MS

1. Introduction

The intensive use of pesticides on agricultural applications and the additional pollution caused by industrial discharges have resulted in the accumulation of these compounds and their transformation products in food, water and soil. The increasing awareness and concern about the human and environmental problems that arise from the improper use of pesticides have led to the endorsement of the Integrated Pest Management (IPM) Programme by the Commission of the European Communities. IPM should manage to find an appropriated regulation and control for pesticides that allow proper purchasing and marketing procedures, good technical quality, and the safe handling and disposal of pesticides [1].

The control and determination of pesticides in water resources is an emergent issue, especially due to their bioaccumulation and their transformation products on the

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environment, as a consequence of industrial and agricultural uses. Another aspect that makes the process difficult is the difference of properties presented by the pests that belong to several groups of action and consequently, have various mechanisms of action and distribution in the environmental matrices [2].

Although there are several methodologies developed for pesticides analysis of a certain group, the validation of a multiresidue method that makes possible the correct identification and the quantification of a large number of pesticides from different groups, at low concentrations, in due time, is still a difficult and promising matter.

In June 2007, the Registration, Evaluation and Authorisation of Chemicals (REACH) programme was created [3]. This new European regulation was launched to protect human health and the environment by avoiding any harmful consequences of the production, use and disposal of hazardous industrial chemicals. Consequently REACH needs to find solid analytical methods in order to identify and permit the progressive elimination of the most harmful compounds, so that the environment can be preserved. Most of the pesticides are produced at high amounts and will be thus subjected to the REACH programme. However, substances used only in plant protection products and included either in Annex I to Directive 91/414/EEC [4,5], Regulation (EEC) No. 3600/92 [6,7], Regulation (EC) No. 703/2001 [8], Regulation (EC) No. 1490/2002 [9,10], or Decision 2003/565/EC [11] are regarded as being registered and are therefore not subjected to re-registration under the REACH programme.

To face the environmental contamination, the European Commission (EC) has adopted several specific directives and decisions, mainly the Water Framework Directive (WFD) 2000/60/EC [12], according to which all inland and coastal waters within defined river basin districts must reach at least good status by 2015. The WFD established environmental objectives and ecological targets and how they should be achieved. The list of widely used pesticides is very long, but only some of them are regulated in water by the European Union (EU) [12–18]. The Decision No. 2455/2001/EC [19], which amends the Directive 2000/60/EC [11], has established a list of 33 priority substances in the field of water by the water policy, the third part of which are pesticides. Previously another Directive, 98/83/EC [14], had set limits for pesticides in water intended for human consumption ($100\,ng\,L^{-1}$ for individual pesticides and $500\,ng\,L^{-1}$ for the sum of all pesticides). Depending on the method of water treatment applied, the water intended for drinking water production is also subjected to a maximum limit that varies between 1 and $5\,\mu g\,L^{-1}$ for pesticides (Directive 75/440/EEC) [15]. More recently, the Directive 2006/118/EC [16] on the protection of groundwater against pollution and deterioration has set a maximum of $0.1\,\mu g\,L^{-1}$ for individual pesticides and $0.5\,\mu g\,L^{-1}$ for total pesticides (including the active substances and their relevant metabolites, degradation and reaction products).

Finally, environmental quality standards (EQS), both annual average (AA) and maximum allowable concentrations (MAC) have been proposed for a number of pesticides and other contaminates in inland and other surface waters These EQS are very low for some compounds such as endosulfan and less restrictive for other compounds such alachlor, atrazine, diuron or simazine, with AA concentrations of 0.3, 0.6, 0.2 and $1\,\mu g\,L^{-1}$, respectively, in both inland and other surface waters [17].

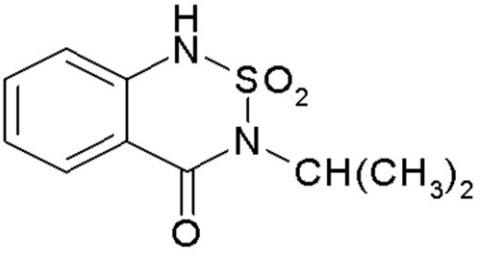
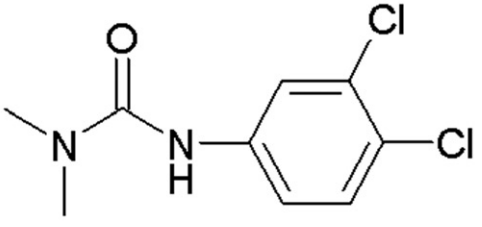
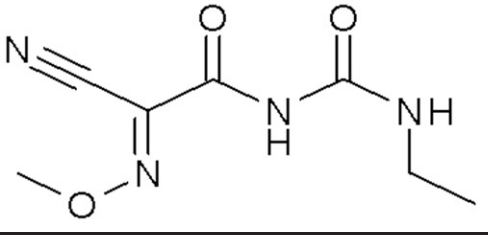
The levels established are, in some cases, extremely low and, for the majority of them, there are not analytical methodologies of reference that enable their determination at those levels. On the other hand, the member states of the European Union have to establish monitoring plans for pollutants in water, according to the contamination products expected (e.g. kind of industries, pesticides that are used in agriculture, among other

factors) in order to accomplish the demanding of quality for residual surface and underground water defined in the Directive. In Portugal, the monitoring plans used and the sampling sites are determined by the Commissions of Coordination and Regional Development. In the case of agricultural pesticides, there are methods developed for several classes. However, only few of them are multiresidue methodologies, involving different classes of pesticides in a single run.

The selected pesticides (Table 1), from different families, are mostly used for agricultural purposes. Concerning the REACH programme, the E.C. directives and the EQS recommendations, only diuron, from the selected pesticides, is object of maximum legal limits (referred above).

The need to analyse modern pesticides that are more polar, thermolabile and volatile made crucial the appearance of new techniques. Consequently the liquid chromatography coupled with mass spectrometry via atmospheric pressure ionisation has been the elected methodology. There are innumerable studies concerning extraction and preconcentration of pesticides using solid-phase microextraction (SPME) [20], solid-phase extraction (SPE) [21], and other techniques such in-tube-solid-phase extraction, matrix-solid-phase dispersion (MSPD) and stir-bar sorption extraction (SBSE), to refer to only some of them [22]. For the study of bentazone, derivatisation prior to gas chromatography detection is

Table 1. Name, structure, formula and class of each pesticide in study.

Name	Structure formula	Activity
Bentazone		Benzotiodizin herbicide
Diuron		Phenylurea herbicide
Cymoxanil		Aliphatic nitrogen fungicide

one of the most used, in order to improve sensibility [23,24]. On the other hand, there are studies that present only one of the pesticides in study, such as diuron [25,26].

Concerning the available extraction procedures to analyse cymoxanil, bentazone and diuron if the objective is to implement LC/MS technique, it seems that SPE extraction is the best approach, because SPME works better with GC methods, MSPD is advisable for solid matrices and few studies report SBSE for the selected pesticides. Therefore Table 2 includes some published papers that use SPE extraction to analyse some of the selected pesticides by LC/MS.

Considering the methodologies described in Table 2, it should be emphasised that the lower limits of detection are achieved with on-line SPE and/or LC/MS equipped with triple quadrupole [28,29,30,31]. This fact configures a rather expensive kind of analysis, difficultly available at most of the water quality control laboratories.

The multiresidue methodologies that use SPE previous to LC/MS [32–34] achieve limits of detection at levels of $\mu g\,L^{-1}$.

Rodrigues *et al*. [32], included diuron and cymoxanil in their study, where several SPE columns are used and the samples were drinking, ground and surface water. Kampioti *et al*. [31] analysed 20 pesticides of different groups in river waters and the sample volume extracted by SPE was optimised, testing also different kinds of columns. Borba da Cunha *et al*. [35] analysed among other pesticides bentazone and diuron, using different LC columns.

From the above description, it can be concluded that, as far as the authors know, no methodology comprising simultaneously the three selected pesticides of this study is available. Besides that fact, few papers are concerned with the uncertainty measurement associated to the results, mainly in the vicinity of the limits of the detection, where the sources of uncertainty are most significant. Finally, this paper describes the optimisation approach applied to LC/MS that may be extended in future to other pesticides in the same chromatographic run.

2. Experimental

2.1 *Reagents and standards*

The solvents used for extractions and standards preparation were: acetone (HPLC grade, Carlo Erba), methanol (HPLC grade, Carlo Erba), ethanol (HPLC-gradient grade, Panreac), Dicloromethane (SupraSolv®, for gas chromatography, Merck), chloridric acid (37% for analysis, ACS Merck) and formic acid (89–91%, GR for analysis, ACS Merck). The solvents used for LC/MS were: acetonitrile (Lichrosolv® hypergrade for liquid chromatography (LC/MS), Merck) and water (Lichrosolv® for chromatography, Merck).

The pesticide standards were: cymoxanil (PESTANAL®, analytical standard, 99.1%, Riedel-de-Häen), bentazone (PESTANAL®, analytical standard, 99.1%, Riedel-de-Häen), and diuron (p.a., 99.5%, Chem Service).

The solid-phase extraction (SPE) columns were: HR-P (3 mL/200 mg) from Macherey-Nagel, Easy (3 mL/200 mg) from Macherey-Nagel and Supelclean™ ENVI™-carb SPE tubes (3 mL/0.25 g) from Supelco.

2.2 *Standards preparation*

Individual stock solutions of each pesticide at $500\,mg\,L^{-1}$ were prepared in ethanol, for bentazone and diuron and in acetonitrile, for cymoxanil. Calibration standards of

Table 2. Number of pesticides presented on each study, selected pesticides, extraction technique employed, type of column, recovery of the extraction methodology, detection technique used and the respective limits of detection (LOD).

No. of pesticides	Sample volume (mL)	Extraction technique	SPE column	Recovery (%)	Detection technique	LOD (ng L^{-1})	Refs.
14 (DIU and CYM)*	400	SPE	MN HR-P	99.7	LC-ESI-MS/MS QqQ MRM	21000	[32]
	400		MN C8	99.9		20200	
20 (BEN and DIU)*	20	On-line SPE	GP For 20 mL:	69.0	LC-ESI-MS/MS Q-QqQ	0.140	[31]
	20		C18 EC or PLRP-s	97.0 or 96.0	SRM (2 transitions)	0.590	
20 (BEN and DIU)*	–	–	–	–	LC-ESI-MS/MS	53.0	[35]
	–				MRM (2 transitions)	613	
18 (DIU)*	18	On-line SPE	Oasis HLB	For sufrace water: 101	LC-ESI-MS/MS QqQ MRM	0.500	[30]
14 (DIU)*	4	On-line SPE	C18/PLRP-s	n.a	LC-APCI-MS/MS QqQ MRM	10.0	[28]
35 (DIU)*	1.3	On-line SPE	C18	95.0	LC-ESI-MS/MS QqQ	5.00	[29]
12 (DIU)*	50	SPE	Supelco LC-18	87.4	LC-ESI-MS/MS Single Quadrupole SIM	250	[33]
6 (BEN and DIU)*	400	SPE	Oasis HLB	–	LC-ESI-MS/MS QqQ MRM 200	100	[34]

MN – Macherey Nagel.
n.a. – not available.
*Pesticides presented on this case study.

mixtures including all the compounds were prepared in acetonitrile at concentrations from 1 to 500 $\mu g\,L^{-1}$. For the matrix-matched calibration curve, spiked solutions at 0.1, 0.4, 0.6, 1, 5 and 10 $\mu g\,L^{-1}$ were prepared in water, from an intermediate standard solution of 1 $mg\,L^{-1}$. For the tests with the different SPE columns, a standard mixture of pesticides was prepared in water at individual concentration of 30 $\mu g\,L^{-1}$.

2.3 *Solid-phase extraction*

For SPE, three types of columns were tested (ENViCarb, Easy and HR-P). Different washing and elution procedures were applied, accordingly to dedicated recommendations, but for all, 750 mL of the sample or the standard mixture containing 30 $\mu g\,L^{-1}$ of each pesticide and 45 mL of methanol (phase exchanger) were extracted.

Two different methods were developed for the Envi-Carb columns. In the first method, the conditioning step used 6 mL of acetone plus 6 mL of acidified water with 0.2% (v/v) HCl, while 1 mL of methanol and 6 mL of acetone was applied in the elution stage. In the second one, the conditioning was performed with 10 mL of water (LC/MS grade) and the elution solvents were 6 mL of dicloromethane and 1.5 mL of methanol with 0.01% of formic acid.

Using Easy columns the conditioning was done with 2×2 mL of acetone and 2×2 mL of water (LC/MS grade) and, on the elution step, methanol: acetone (1:1) was used in two steps (4 mL first, elution is performed and afterwards 3.5 mL).

The conditioning of HR-P columns was done with 3 mL of methanol and 3 mL of water LC/MS grade and, on the elution step, methanol : acetone (3 : 2) was used in two steps (4 mL first, elution is performed and afterwards 3.5 mL).

2.4 *Matrix matched calibration*

The standards were prepared as described on the previous section. The SPE procedure was performed using a sample volume of 750 mL and HR-P columns. The extracts were evaporated and recovered in 1 mL of acetonitrile prior to LC/MS analysis.

2.5 *LC/MS analyses*

The LC/MS analyses were performed using a Varian 500-MS LC Ion Trap Mass Spectrometer equipped with an electrospray ionisation source (ESI). For the chromatographic separation, a Pursuit UPS C18 (50 mm × 2 mm i.d., 2.4 μm) column was used. A gradient elution was performed with a mobile phase of water (A) and acetonitrile (B), at a flow rate of 0.2 $mL\,min^{-1}$, and the gradient programme: 0–1.0 min 100% B, 1.0–3.5 min linear gradient to 50% B, and kept at 50% B for 1.5 min. The injection volume was 10 μL.

The MS conditions were optimised during the experimental work, and the final conditions were: Voltages: Capilar: 40 V, Needle: 5300 V, Shield: 600 V; $T_{drying\ gas}$: 350°C; Pressures: $P_{nebulisation}$: 45 psi, $P_{drying\ gas}$: 15 psi; $RF_{\ loading}$: 70%.

3. Results and discussion

3.1 *Solvent selection*

In order to avoid the matrix effect, due to the interference of the solvent in the mass spectrometric behaviour of analytes, a mixed standard was prepared in different solvents

and directly injected in LC/MS. The MS operational conditions were those defined in Material and Methods. These assays were important because afterwards, the extraction methodologies will be compared and the elution solvents after SPE were different. Therefore one has to conclude whether response is better due to the efficiency of extraction or simply due to solvent effect on MS behaviour. The results obtained for each pesticide using the different solvents, the same used after SPE, are showed in Figure 1.

From the results obtained, it is possible to conclude that the solvent that enables a better response for diuron is water, bentazone is better detected when using acetonitrile and cymoxanil revealed an higher peak area when using diclorometh ane/methanol. That fact results from the different chemical properties (solubility, electronegativity, polarity) of the different analytes. In order to analyse all the pesticides in a single run, a compromise situation was selected and acetonitrile was used in subsequent experiments, also because stabilisation on LC/MS was better achieved (acetonitrile is present in mobile phase). The calibration standards were prepared owing to these results.

3.2 *Optimisation procedures*

Optimising chromatographic separations, using a liquid chromatograph system coupled to mass spectrometry, is somewhat different from the conventional HPLC procedure of optimisation when other detectors, as UV or fluorescence, are present. In the latter, the chromatographic separation is firstly optimised and only the detector response afterwards. Considering the capabilities of the MS detector, the first step is to obtain the best operational parameters for the individual analytes, by direct-infusion of a standard solution in the electrospray mass spectrometer. This procedure will allow obtaining the mass fragmentation pattern of the individual components, as well as the parent ions if a second fragmentation needs to be set. In this work, the mass optimisation was determined by the best response, varying one-by-one, the capillary voltage, then the needle voltage and the RF loading. Finally, for the best individual conditions the excitation amplitude CID was set.

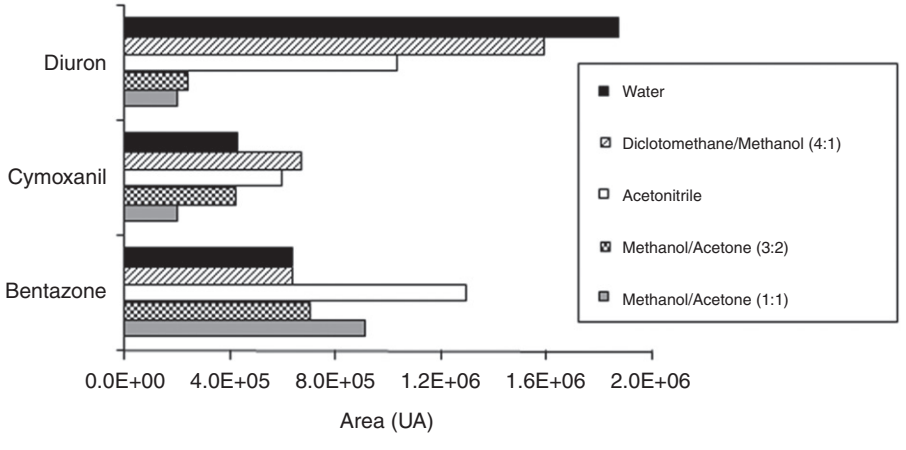


Figure 1. Response obtained by LC/MS direct injection of standard solutions with concentrations of $30\,\mu g\,L^{-1}$ on the pesticides analysed using different solvents.

After MS optimisation by direct infusion, in combination with the mobile phase, direct injection will allow the ion source optimisation, which includes the determination of optimal conditions for the temperature of the drying gas, as well as the best drying and nebulisation gas pressures. The shield voltage was set to 600 V, according to the manufacturer. The conditions obtained in this first step of the optimisation are described in Table 3.

The second step of the optimisation was the injection of the individual standard solutions (100 $\mu g\,L^{-1}$) at the flow rate of 0.2 $mL\,min^{-1}$, using the conditions described above for the MS, in order to verify the peak response and the retention time for the individual components. ESI operated in the negative ionisation mode for bentazone and positive one for cymoxanil and diuron.

The third step was directed towards the multiresidue separation. By using a highly efficient C18 short column (50 mm × 2 mm internal diameter), separations could be achieved in less than 10 minutes for these case-study compounds. A standard mixture with the concentration of 500 $\mu g\,L^{-1}$ for each analyte was injected and the previous MS conditions were adjusted to obtain the best peak response. Table 4 presents the final compromise conditions.

3.3 *Quantification by direct injection*

Quantification was performed in full scan mode (m/z 50–300) with the ions defined in Table 3. In Figure 2 the calibration curves, obtained at two electron multiplier offset voltages 150 and 300 V are represented.

By direct injection, using a multiplier offset of 150 V, the linearity interval was from 5 to 500 $\mu g\,L^{-1}$ for bentazone and diuron and from 5 to 1000 $\mu g\,L^{-1}$ for cymoxanil. The detection limits were 31, 89 and 116 $\mu g\,L^{-1}$, for bentazone, diuron and cymoxanil, respectively. On the other hand, for 300 V multiplier offset, the linearity interval was from 1 to 100 $\mu g\,L^{-1}$ for bentazone and cymoxanil and from 35 to 100 $\mu g\,L^{-1}$ for diuron. The detection limits were 0.13, 4.26 and 0.48 $\mu g\,L^{-1}$, for bentazone, cymoxanil and diuron, respectively.

Although the detection limits achieved with higher multiplier offset were much lower than the other obtained with lower offset, the legal limits imposed by legislation (up to 0.1 $\mu g\,L^{-1}$) could not be attained by direct injection. Therefore, if lower limits of detection need to be set for compliance with legislation, or if the matrices are too complex, posing significant interfering problems, an extraction step is advisable. In this work, three different types of columns were tested – Envi-Carb, Easy and HR-P. Figure 3 presents the results obtained with a standard mixture of concentration 30 $\mu g\,L^{-1}$.

Table 3. Optimal mass spectrometry conditions for the individual analytes directly-infused.

Analyte	Ionization mode	Capillary voltage (V)	Needle voltage (V)	RF loading (%)	$T_{drying\ gas}$ (°C)	$P_{drying\ gas}$ (psi)	$P_{nebulisation\ gas}$ (psi)	Excitation amplitude CID (V)
Cymoxanil	Positive	34	5300	70	250	10	45	0.88
Bentazone	Negative	70	3536	70	400	20	60	0.96
Diuron	Positive	56	5213	76	350	40	35	0.61

Table 4. Final conditions used to determined cymoxanil, bentazone and diuron by LC/MS.

		LC parameters		MS parameters				
Analyte	Flow rate	Mobile phase	Gradient elution	Quantification ion (m/z)	Temp.	Pressures	Voltages	RF loading
Cymoxanil				199				
Bentazone	0.2 mL min^{-1}	Water (A)	0.0 min–100% B 1.0 min–100% B		350°C for drying gas (He)	45 psi for nebulization gas (N_2); 15 psi for drying gas	Capillary – 40 V	
		Acetonitrile (B)	3.5 min–50% B 5.0 min–50% B	239			Needle – 5300 V Shield – 600 V	70%
Diuron				233				

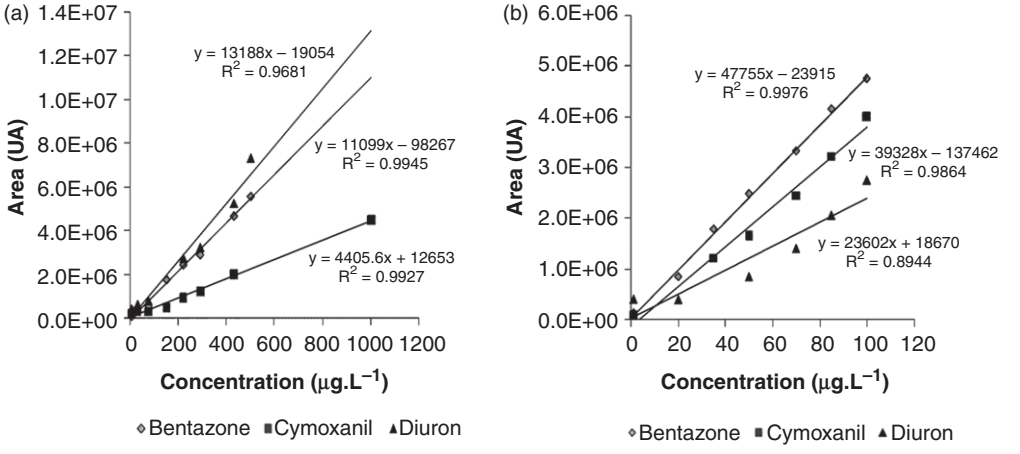


Figure 2. Calibration curves obtained by injection of standard mixtures, using an electron multiplier offset of 150 V(a) and 300 V (b).

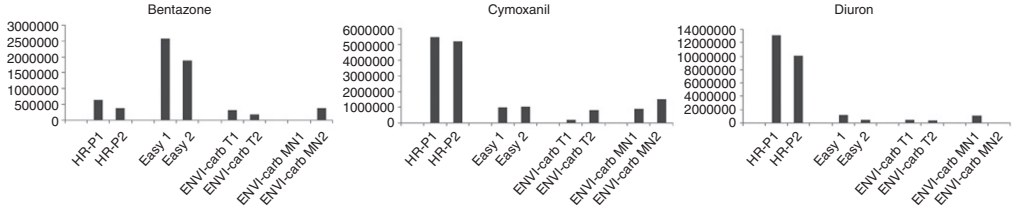


Figure 3. Comparative performance of the three different sorbents used to extract a standard mixture of bentazone, cymoxanil and diuron with concentration of 30 μg L^{-1}.

In the tested conditions of elution, HR-P1 represented the best compromise and was selected for the subsequent validation studies, which included the linearity and detection limits obtained by matrix-matched calibration.

3.4 *Quantification by matrix-matched calibration after SPE*

Spiking mixtures of the three pesticides, at concentrations between 0.1 and 10 ppb, in aqueous solutions were extracted with the SPE methodology described for HR-P columns. Figure 4 describes the relationship between the detector response and the level of concentration, where the greater amount of results in the lower part of the graph is explained by the fact that those should be the levels expected to appear in real samples. The higher standards concentrations were used to obtain a better correlation coefficient.

The limits of detection obtained for each pesticide were determined according to a signal-to-noise ratio of 3 and were 1.52 μg L^{-1} for cymoxanil, 0.37 μg L^{-1} for bentazone

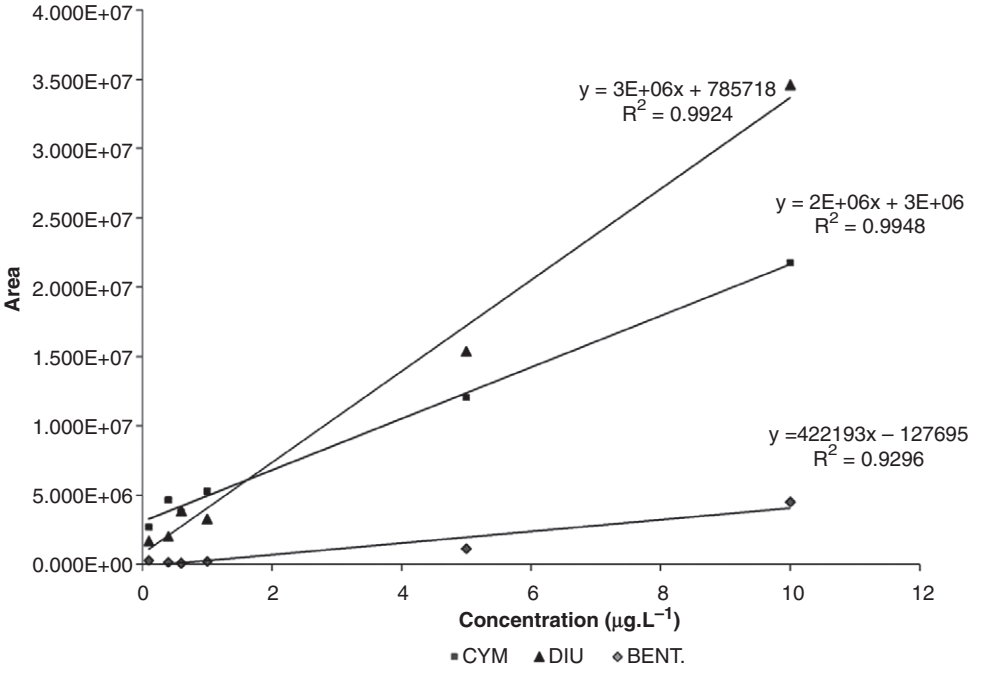


Figure 4. Matrix-matched calibration curves obtained for bentazone (BEN), cymoxanil (CYM) and diuron (DIU) using the SPE procedure for HR-P to extract mixture standards with concentrations between 0.1 and 10 $\mu g\,L^{-1}$, recovered in acetonitrile prior to LC/MS analyses.

and 0.27 $\mu g\,L^{-1}$ for diuron. Those detection limits are only slightly lower than those obtained by direct injection of water samples, meaning probably that, although reproducible, an extremely low efficiency of extraction was achieved.

In order to quantify the global uncertainty associated to the matrix-matched calibration, precision and recovery assays were performed. For precision evaluation, in terms of coefficient of variation (CV%), four independent extractions of the standard mixture at the intermediate and the lower level of concentration were assayed. For recovery evaluation the results of the matrix-matched calibration were compared against the expected result owing to the prepared standards.

The following four main sources of uncertainty, accordingly to Eurachem Citac Guide [36] and the methodology of uncertainty measurement, used by Ratola *et al.* [37] were considered:

- U1 – uncertainty associated to standard preparation.
- U2 – uncertainty associated to the calibration curve.
- U3 – uncertainty associated to precision.
- U4 – uncertainty associated to accuracy.

Results of Table 5 clearly show that extremely high uncertainties are achieved, but it must emphasised that the results highlighted are not detectable and therefore should be excluded. For higher concentrations however, global uncertainties varied from 200

Table 5. Uncertainty values and global uncertainty obtained for each pesticide and respective the levels of concentration.

	C μ L^{-1}	Ul	U2	U3	U4	U (%)
CYM	0.10	0.10	4.18	0.09	0.18	418.50
	0.40	0.02	1.00	0.31	0.19	106.73
	0.60	0.01	0.68	0.26	0.10	73.53
	1.00	0.02	0.40	0.13	0.05	42.12
	5.00	0.00	0.08	0.00	0.08	11.17
	10.00	0.00	0.05	0.02	0.06	7.92
BEN	0.10	0.10	10.23	0.01	1.29	1031.06
	0.40	0.02	2.59	0.06	0.75	269.27
	0.60	0.01	1.73	0.22	0.15	175.24
	1.00	0.02	1.03	0.06	0.28	106.72
	5.00	0.00	0.20	0.28	0.74	81.47
	10.00	0.00	0.15	0.07	0.09	18.98
DIU	0.10	0.10	6.04	0.03	0.37	604.75
	0.40	0.02	1.50	0.05	0.04	150.24
	0.60	0.01	0.98	0.11	0.33	103.67
	1.00	0.02	0.59	0.20	0.15	64.06
	5.00	0.00	0.12	0.01	0.03	11.88
	10.00	0.00	0.08	0.08	0.11	16.10

Ul – uncertainty associated to standard preparation, U2 – uncertainty associated to the calibration curve, U3 – uncertainty associated to precision, U4 – uncertainty associated to accuracy. U – global uncertainty calculate according to Eurachem.

to 19%, for bentazone, from 400 to 8% for cymoxanil and from 150 to 16%, for diuron. Those extremely high uncertainties are, mainly due to the uncertainty arising from the calibration curve and probably, the SPE extraction methodology is the main responsible. Because of this results and considering that the limits of detection are not significantly diminished using SPE extraction, it seems that the best approach to quantify those pesticides is direct injection in LC/MS in the optimised conditions.

4. Conclusions

A quick (less than 10 minutes) and highly sensitive liquid chromatographic/ion-trap mass spectrometric method for the separation and simultaneous quantification of three selected pesticides (diuron, bentazone and cymoxanil) was developed and optimised, using a Pursuit C18 column and a mobile phase composed by water and acetonitrile, in gradient elution mode.

Using direct injection and a multiplier offset of 300 V, the limits of detection were of 0.13, 4.26 and 0.48 μg L^{-1}, for bentazone, cymoxanil and diuron, respectively. When using a matrix–matched calibration, the limits of detection were 1.52 μg L^{-1} for cymoxanil, 0.37 μg L^{-1} bentazone and 0.27 μg L^{-1} for diuron, but owing to the rather high uncertainties obtained in the vicinity of the detection limits, it seems that direct injection is preferable to this approach.

The described methodology of optimisation can be in future enlarged to other pesticides in the same run.

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