Journal of Chromatography A, 1218 (2011) 1477–1485

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# Simultaneous determination of pesticides, biopesticides and mycotoxins in organic products applying a quick, easy, cheap, effective, rugged and safe extraction procedure and ultra-high performance liquid chromatography–tandem mass spectrometry

R. Romero-González[a,*], A. Garrido Frenich[a], J.L. Martínez Vidal[a], O.D. Prestes[b], S.L. Grio[c]

[a] Group "Analytical Chemistry of Contaminants", Department of Analytical Chemistry, Almeria University, E-04071 Almeria, Spain
[b] Chemistry Department, Laboratory of Pesticide Residues Analysis, Federal University of Santa Maria, RS, Brazil
[c] Faculty of Science and Technology, National Autonomous University of Nicaragua from León, Nicaragua

ARTICLE INFO

*Article history:*
Received 5 December 2010
Received in revised form 10 January 2011
Accepted 12 January 2011
Available online 19 January 2011

*Keywords:*
Pesticides
Biopesticides
Mycotoxins
Simultaneous extraction
UHPLC–MS/MS

ABSTRACT

A method for the simultaneous determination of pesticides, biopesticides and mycotoxins from organic products was developed. Extraction of more than 90 compounds was evaluated and performed by using a modified QuEChERS-based (acronym of Quick, Easy, Cheap, Effective, Rugged, and Safe) sample preparation procedure. The method was based on a single extraction with acidified acetonitrile, followed by partitioning with salts, avoiding any clean-up step prior the determination by ultra-high performance liquid chromatography/tandem quadrupole mass spectrometry (UHPLC–MS/MS). Validation studies were carried out in wheat, cucumber and red wine as representative matrixes. Recoveries of the spiked samples were in the range between 70 and 120% (with intra-day precision, expressed as relative standard deviation, lower than 20%) for most of the analysed compounds, except picloram and quinmerac. Inter-day precision, expressed as relative standard deviation, was lower than 24%. Limits of quantification were lower than 10 $\mu g\,kg^{-1}$ and the developed method was successfully applied to the analysis of organic food products, detecting analytes belonging to the three types of compounds.



## 1. Introduction

Safety and quality of food supplies is an integral part of food security and consumer protection. One of the most important aims in high quality food is the control of residues and contaminants. These compounds can either occur during production (e.g. pesticides) [1] or can be formed during storage or food processing (e.g. mycotoxins) [2]. In order to assure human food safety, European Union has established maximum residue limits (MRLs) of pesticides [3] and mycotoxins [4] permitted in products of animal or vegetable origin that are intended for human or animal consumption.

Bearing in mind the negative effects of pesticides [5], there is an increasing demand for organic products [6]. Organic farming is allowed to use biopesticides in pest controls, but scarce scientific literature is available on the use and environmental impact of natural products in organic agriculture. Furthermore, the use of organic practices is thought to reduce the risk of plant infection by pathogens, but there are some evidences that the reduced use of fungicides may lead to a greater contamination by mycotoxins in organic food [7]. Besides, pesticides can be detected in organic food samples, and this can readily be explained as the environmental contamination by past pesticide use, or by "drift" (sprays blown in from adjacent non-organic farms) [8].

Multiresidue methods are a logical choice in the analysis of residues and contaminants and are the most adequate strategy for monitoring purposes [9,10]. These methods try to cover the high demand for residue analysis providing an increase in the productivity of laboratories, as well as decreasing the cost of analyses [11,12], and liquid chromatography (LC) coupled to mass spectrometry (MS) has been widely used [13,14]. Furthermore, ultra-high performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC–MS/MS) reduces the analysis time and limits of detection, increasing sample throughput, and it has been successfully applied for the analysis of residues and contaminants in foodstuffs in last years [15–17].

Although contaminated food by pesticides, biopesticides and mycotoxins, have different sources, they have similar physicochemical properties. Nevertheless, the analysis of pesticides [18–20], biopesticides [21,22] and mycotoxins [11,23,24] is routinely carried out applying different chromatographic methods

* Corresponding author. Tel.: +34 950015823; fax: +34 950015483.
*E-mail address:* rromero@ual.es (R. Romero-González).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.01.034

[25,26]. However, it could be desirable to have a comprehensive method for the analysis of these types of compounds [27]. For that, it is necessary to develop generic extraction procedures, providing less-time consuming, harmful, and cost-effective methodologies, assuring suitable recoveries, precision and ruggedness. In this sense, in the last few years, several approaches such as the well-known QuEChERS procedure (acronym name for Quick, Easy, Cheap, Effective, Rugged, and Safe), and "dilute and shoot" methodologies [11] have been developed. For instance, QuEChERS has been developed by Anastassiades et al. [28] for the extraction of a wide polarity range of pesticides [29] and other compounds such as mycotoxins from food matrixes [30].

In this study, a modified QuEChERS sample preparation method coupled with UHPLC–MS/MS has been developed for the simultaneous determination of more than 90 compounds, including pesticides, biopesticides (such as pyrethrins, rotenone, azadirachtin, veratridine, cevadine and deguelin) and mycotoxins (including aflatoxins B1, B2, G1, G2, ochratoxin A, T-2 toxin and HT-2 toxin) in organic food samples. The developed approach is adequate to determine these compounds in such samples due to the effectiveness of the extraction procedure and the fast chromatographic analysis, providing adequate performance characteristics.

## 2. Experimental

### 2.1. Reagents and chemicals

Pesticide reference standards (purity higher than 99%) were purchased from Dr. Ehrenstofer (Augsburg, Germany) and Riedel-de-Haën (Seelze-Hannover, Germany). Ochratoxin A and T-2 toxin were obtained from Biopure (Tulln, Austria). Aflatoxins B1, B2, G1, G2 and stock standard solution of HT-2 toxin (in acetonitrile) were purchased from Sigma–Aldrich (Madrid, Spain). Nicotine and rotenone were obtained from Fluka (Steinheim, Germany). A technical mixture of the naturally occurring pyrethrins (pyrethrin I, 11.10%; pyrethrin II, 7.80%; cinerin I, 1.60%; cinerin II, 1.50%; jasmolin I, 0,70%; jasmolin II, 0.60%), veratridine, cevadine and deguelin were purchased from Sigma–Aldrich. Azadirachtin was obtained from Supelco (St. Louis, MO, USA).

Stock standard solutions of individual compounds (with concentrations ranged from 200 and 300 mg $L^{-1}$), were prepared by exact weighing of the compound followed by dissolution in 50 mL of methanol, acetonitrile or acetone, and stored at −18 °C in the dark. A multi-compound working standard solution (2 mg $L^{-1}$ concentration of each compound) was prepared by appropriate dilutions of the stock solutions with methanol and stored under refrigeration at 4 °C.

Methanol and acetonitrile (HPLC-grade) were purchased from J.T. Baker (Deventer, Netherlands). Ultrapure water was obtained from a Milli-Q Gradient water system (Millipore, Bedford, MA, USA). Acetic acid, ammonium acetate and anhydrous magnesium sulphate were supplied by Panreac (Barcelona, Spain) and ammonium formate was obtained from Fluka.

### 2.2. Instruments and apparatus

Chromatographic analyses were performed in an ACQUITY UPLC™ system (Waters, Milford, MA, USA) equipped with a binary solvent delivery system, degasser, autosampler and column heater. Chromatographic separation was performed using an Acquity UPLC BEH $C_{18}$ column (100 mm × 2.1 mm), with 1.7 μm particle size, from Waters. MS/MS detection was performed using an Acquity TQD tandem quadrupole mass spectrometer (Waters, Manchester, UK), equipped with an electrospray ionization interface (ESI) operating in positive ion mode.

A vortex mixer IKA (Wilmington, USA) model MS1, PB 602-S balance (Mettler Toledo, Greinfesee, Switzerland), a Reax 2 rotary agitator from Heidolph (Schwabach, Germany) and a Braun MX 32 kitchen blender (Barcelona, Spain) were used to process samples. Centrifugation was performed in a Centronic BL II centrifuge (J.P. Selecta, Barcelona, Spain).

### 2.3. Chromatographic conditions

A gradient program was used consisting of methanol (eluent A) and an aqueous solution of ammonium formate (5 mM) (eluent B). The gradient profile started at 20% of eluent A and increased linearly up to 95% in 11 min, keeping constant for 0.5 min before being returned to the initial conditions in 0.5 min. The column was re-equilibrated for 1.0 min at the initial mobile phase composition, obtaining a total run-time of 13.0 min. The flow-rate was set at 0.45 mL $min^{-1}$, and column temperature was kept at 30 °C. The injection volume was 5 μL and, the autosampler was flushed with a methanol/water solution (1:9 v/v) before sample injection to avoid carry-over.

For MS/MS detection, source parameters were as follows: capillary voltage, 3.0 kV; extractor voltage, 3 V; source temperature, 120 °C and desolvation gas temperature, 350 °C. Desolvation gas and cone gas (both nitrogen) flow-rates were set at 50 and 600 L $h^{-1}$, respectively. Collision-induced dissociation was performed using argon as collision gas at a pressure of $4.0 \times 10^{-3}$ mbar in the collision cell. For instrument control, data acquisition and processing, MassLynx and QuanLynx software version 4.1 (Waters) were used.

### 2.4. Sample preparation

Several procedures were evaluated during the optimization of the extraction method:

*Method A*: *QuEChERS method using acetate buffer* [29]. For cucumber and red wine samples, 10 g of sample was weighed in a 50-mL polypropylene centrifuge tube. For wheat, 5 g of homogenised sample was weighed and 5 mL of water was added, soaking for 1 h. Subsequently, 10 mL of 1% acetic acid in acetonitrile (v/v) was added, and the tubes were shaken for 1 min with a vortex. Then, 4 g of anhydrous magnesium sulphate and 1.5 g of sodium acetate were added and the tubes were shaken immediately for 1 min. After centrifugation at 5000 rpm (4136 × *g*) for 5 min, the supernatant was taken and filtered, through a Millex-GN nylon filter (0.20 μm, Millipore, Carrightwohill, Ireland) prior UHPLC–MS/MS analysis.

*Method B: Sonication extraction*. 5 g of sample was weighed into a 50-mL polypropylene centrifuge tube and 10 mL of a mixture of acetonitrile/water 80:20 (v/v) was added. The mixture was vortexed for 2 min and after that, the tube was kept in an ultrasonic bath for 30 min. Then, the mixture was centrifuged for 10 min at 5000 rpm (4136 × *g*), and the supernatant was filtered through a Millex-GN nylon filter and transferred into an auto-sampler vial prior UHPLC–MS/MS analysis.

*Method C: Generic extraction procedure*. Analytes were extracted using an extraction method based on the procedure previously described by Mol et al. [11]: 2.5 g of sample was weighed into a 50-mL polypropylene centrifuge tube and 5 mL of water was added. The mixture was shaken with a vortex during 1 min. If wheat matrix was studied, the mixture was allowed to soak for 1 h. Then 15 mL of acetonitrile (1% formic acid, v/v) was added, and the sample was extracted by end-over-end shaking for 1 h at 50 rpm. After that, the mixture was centrifuged for 10 min at

5000 rpm (4136 × g), and the supernatant was filtered through a Millex-GN nylon filter and transferred into an auto-sampler vial prior UHPLC–MS/MS analysis.

### 2.5. Method validation study

Three representative matrixes were selected for validation purposes. Cucumber was selected as a representative commodity with high water content, wheat as low water content and high starch and/or protein content [31] and red wine as a representative commodity of alcoholic beverages. Therefore, three validation data sets were carried out for each type of matrix, according to the European SANCO guideline [31]. Linearity was studied using matrix-matched calibration by analysing samples of cucumber, wheat and red wine at five concentration levels between 5 and 100 $\mu g\,kg^{-1}$. Trueness and precision (intra-day and inter-day) were evaluated with spiked samples of each commodity. Recovery and intra-day precision were studied at three concentration levels (10, 25 and 50 $\mu g\,kg^{-1}$ for wheat and cucumber and 10, 25 and 50 $\mu g\,L^{-1}$ for red wine), analysing five replicates at each concentration. Inter-day precision was evaluated at 25 $\mu g\,kg^{-1}$ (25 $\mu g\,L^{-1}$ for red wine) and five spiked samples were analysed daily for a period of one week. Limits of quantification (LOQs) were determined as the amount of analyte for which signal-to-noise ratios (S/N) were equal than 10.

### 2.6. Analysis of samples

A variety of 40 different organic samples (18 cereals or cereals based processed food, 13 vegetables and 9 wines) obtained from local markets were analysed with the proposed method.

## 3. Results and discussion

### 3.1. UHPLC–MS/MS analysis

LC coupled to MS detection is the most useful technique for the simultaneous detection of multiclass analytes because it allows the robust analysis of the compounds at low levels in complex matrixes such as foodstuffs.

In this study, the UHPLC–MS/MS procedure was based on previous studies based on the multiresidue determination of mycotoxins [23] and pesticides [15]. Although both methods used methanol as organic solvent, the aqueous phases involved in the separation of the target compounds were different. Thus, ammonium formate 5 mM was applied for the separation of mycotoxins, whereas an aqueous solution of formic acid (0.01%, v/v) was utilized for the determination of pesticides. Better results were obtained when ammonium formate was used and it was selected for the simultaneous determination of the selected compounds. Furthermore, the gradient was optimized in order to provide a good separation of the selected compounds in less than 13 min. The analytes were sorted in 14 overlapping functions, using a maximum of 12 compounds (24 transitions) per acquisition function, centring the chromatographic peak in the time window, minimizing the risk of peak loss due to unexpected slight changes in retention time. Dwell times ranging from 0.015 to 0.050 s were used to obtained satisfactory peak shape and number of points per peak for all the analytes. Table S-1 (see supplementary data) shows the retention time windows (RTWs) and MS/MS parameters per each compound, observing that the current procedure can determine the selected compounds in less than 13 min with reproducible results for determination and confirmation.

### 3.2. Optimization of the extraction procedure

In order to reduce sample handling and increase sample throughput, generic [11] or well-known methodologies such as QuEChERS procedure [28] were tested, bearing in mind that they allow the extraction of a wide range of compounds. Thus, the generic extraction developed by Mol et al. [11], was checked considering that it allows the simultaneous extraction of pesticides, mycotoxins, plant toxins, and veterinary drugs from different types of matrixes. QuEChERS-based methodologies have been applied for the extraction of compounds with a wide range of physico-chemical properties from different samples [32]. Furthermore, a sonication procedure, using a mixture of acetonitrile/water (80:20, v/v) was tested, bearing in mind that this mixture has been widely used for the extraction of mycotoxins from foodstuffs [23]. In order to evaluate the performance of the three selected methods, wheat blank samples spiked at 50 $\mu g\,kg^{-1}$ were treated applying the three procedures described in Section 2.4, showing the obtained results in Fig. S-1 (see supplementary data).

It can be observed that the best results were obtained when QuEChERS procedure was used, allowing the extraction of more than 80 compounds with suitable recoveries (70–120%) and relative standard deviation (RSD) lower than 20%. When the ultrasound method was applied more than 80 compounds were extracted, but only 36 compounds, including all the mycotoxins and biopesticides assayed in this study, were quantitatively extracted, whereas this approach was not suitable for most of the selected pesticides. Finally, an intermediate situation was obtained when the procedure described by Mol et al. was applied. More than 50 compounds were extracted with recoveries ranging from 70 to 120% and RSD values lower than 20%.

Bearing in mind these results the QuEChERS method was tested for the three matrixes evaluated. It can be indicated that all the selected compounds were extracted from the three matrixes, except picloram and quinmerac, with recoveries lower than 20 and 45% respectively. Furthermore, 3 mycotoxins, ochratoxin A, T-2 toxin and HT-2 toxin were not extracted from cucumber, although good quantitative recoveries were obtained when wheat and red wine were studied. This can be due to the recovery of these mycotoxins strongly depends on the matrix type, and acetonitrile extract is efficient only for some matrixes [33]. Despite these exceptions, it must be emphasized that the proposed extraction procedure allows the reliable extraction of a wide range of compounds from different matrixes.

Finally, Fig. 1 shows an UHPLC–MS/MS chromatogram of wheat fortified with the target compounds at 50.0 $\mu g\,kg^{-1}$, showing in Fig. 1a a combined MRM (quantification transition) chromatogram from a total standard mixture and in Fig. 1b, it can be observed a chromatogram with representative compounds from each function. In both cases it can be noted that clean chromatograms without interferences are obtained.

### 3.3. Validation study

The selected analytical method was validated in terms of matrix effect, linearity, trueness, intra-day precision, inter-day precision and limits of quantification (LOQs).

It is well known that the presence of matrix components can affect the ionization of the target compounds when ESI is used. This may be due to competition between the analyte and a coeluting component for the available charge, which mostly results in signal suppression, or due to the influence of a matrix component on the release of ions from the electrospray droplets to the gas phase. In this work, three matrixes such as wheat, cucumber and red wine were selected for the evaluation of matrix effect, analysing standards at different concentrations in pure solvents

**Table 1**
Matrix effect, determination coefficients ($R^2$), limit of quantification (LOQ) and recoveries obtained for the target compounds in the three matrixes evaluated.

| Compound | Wheat | | | | Cucumber | | | | Red wine | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Matrix effect[a] | $R^2$ | LOQ ($\mu g\,kg^{-1}$)[b] | Recovery[c] | Matrix effect[a] | $R^2$ | LOQ ($\mu g\,kg^{-1}$)[b] | Recovery[c] | Matrix effect[a] | $R^2$ | LOQ ($\mu g\,kg^{-1}$)[b] | Recovery[c] |
| Nicotine | 0.5 | 0.9972 | 5.1 | 79 (12) | 0.6 | 0.9993 | 3.3 | 72 (18) | 0.2 | 0.9983 | 10.0 | 71 (25) |
| Propamocarb | 1.5 | 0.9990 | 4.2 | 70 (9) | 0.6 | 0.9979 | 5.4 | 75 (20) | 1.2 | 0.9970 | 4.2 | 97 (3) |
| Pymetrozine | 1.3 | 0.9999 | 4.2 | 79 (11) | 0.9 | 0.9892 | 3.9 | 104 (14) | 1.2 | 0.9982 | 3.3 | 71 (3) |
| Methomyl | 1.4 | 0.9750 | 4.2 | 74 (19) | 0.7 | 0.9944 | 4.8 | 75 (7) | 0.5 | 0.9961 | 3.0 | 105 (6) |
| Thiamethoxam | 1.7 | 0.9970 | 5.4 | 95 (11) | 0.6 | 0.9941 | 5.1 | 93 (16) | 0.5 | 0.9938 | 3.6 | 109 (12) |
| Monocrotophos | 1.5 | 0.9950 | 4.8 | 97 (10) | 1.0 | 0.9905 | 3.9 | 80 (12) | 0.6 | 0.9987 | 3.0 | 74 (10) |
| 2,6 Dichlorobenzamide | 1.5 | 0.9999 | 3.9 | 103 (13) | 0.7 | 0.9954 | 3.9 | 98 (7) | 0.7 | 0.9961 | 5.1 | 90 (18) |
| Atrazine desisoproyl | 1.5 | 0.9990 | 3.0 | 105 (13) | 0.9 | 0.9939 | 4.5 | 114 (9) | 0.8 | 0.9982 | 3.0 | 83 (10) |
| Imidacloprid | 1.6 | 0.9890 | 4.5 | 85 (19) | 0.5 | 0.9973 | 5.1 | 105 (3) | 0.6 | 0.9986 | 3.6 | 73 (18) |
| Metamitron | 2.1 | 0.9990 | 7.5 | 97 (18) | 0.4 | 0.9950 | 5.7 | 75 (8) | 0.5 | 0.9991 | 5.7 | 76 (10) |
| Chlorsulfuron | 1.5 | 0.9977 | 3.3 | 107 (13) | 0.1 | 0.9932 | 3.6 | 95 (7) | 1.2 | 0.9902 | 5.6 | 75 (18) |
| Vamidothion | 2.2 | 0.9990 | 3.6 | 102 (9) | 0.4 | 0.9951 | 4.5 | 104 (3) | 0.3 | 0.9952 | 5.4 | 71 (16) |
| Chloridazon | 2.4 | 0.9998 | 8.4 | 103 (8) | 0.2 | 0.9837 | 6.0 | 88 (4) | 0.1 | 0.9975 | 6.0 | 73 (17) |
| Acetamiprid | 1.6 | 0.9900 | 5.1 | 113 (8) | 0.8 | 0.9989 | 3.6 | 100 (5) | 0.7 | 0.9983 | 4.8 | 78 (13) |
| Triasulfuron | 1.4 | 0.9990 | 4.5 | 99 (18) | 0.5 | 0.9837 | 3.3 | 84 (9) | 0.6 | 0.9964 | 5.4 | 93 (8) |
| Carbendazim | 1.6 | 0.9990 | 5.7 | 97 (7) | 0.6 | 0.9986 | 8.7 | 81 (8) | 0.7 | 0.9978 | 3.6 | 72 (9) |
| Cinosulfuron | 2.0 | 0.9901 | 5.7 | 99 (10) | 0.2 | 0.9829 | 4.2 | 103 (4) | 0.2 | 0.9962 | 4.8 | 91 (20) |
| Atrazine desethyl | 1.4 | 0.9990 | 7.5 | 110 (11) | 0.9 | 0.9950 | 3.3 | 103 (3) | 0.8 | 0.9992 | 4.9 | 108 (18) |
| Thifensulfuron-methyl | 1.6 | 0.9990 | 3.6 | 70 (19) | 0.8 | 0.9875 | 3.0 | 75 (10) | 0.7 | 0.9897 | 5.1 | 70 (19) |
| Thiacloprid | 1.8 | 0.9992 | 5.4 | 114 (6) | 0.6 | 0.9968 | 5.1 | 103 (2) | 0.6 | 0.9939 | 5.4 | 71 (16) |
| Aldicarb | 0.8 | 0.9973 | 4.2 | 103 (8 | 0.5 | 0.9970 | 4.8 | 103 (6) | 0.5 | 0.9977 | 5.2 | 73 (18) |
| Aflatoxin G2 | 2.0 | 0.9910 | 4.8 | 106 (20) | 0.6 | 0.9981 | 3.3 | 92 (14) | 0.7 | 0.9965 | 3.6 | 85 (18) |
| Metoxuron | 1.9 | 0.9884 | 6.6 | 92 (15) | 0.5 | 0.9887 | 3.6 | 101 (7) | 0.4 | 0.9962 | 3.7 | 74 (12) |
| Thiabendazole | 1.4 | 0.9991 | 5.7 | 82 (15) | 0.8 | 0.9926 | 5.7 | 110 (6) | 0.5 | 0.9990 | 5.4 | 70 (15) |
| Aflatoxin G1 | 1.8 | 0.9960 | 3.9 | 93 (17) | 0.5 | 0.9941 | 3.9 | 101 (13) | 0.7 | 0.9966 | 3.6 | 92 (8) |
| Iodosulfuron methyl | 1.3 | 0.9980 | 5.7 | 104 (16) | 0.4 | 0.9938 | 4.8 | 110 (11) | 0.5 | 0.9897 | 3.3 | 74 (13) |
| Simazine | 1.7 | 0.9860 | 8.1 | 95 (18) | 0.6 | 0.9914 | 3.9 | 92 (9) | 0.8 | 0.9962 | 3.6 | 73 (9) |
| Aflatoxin B2 | 2.3 | 0.9997 | 4.2 | 110 (6) | 0.7 | 0.9874 | 3.0 | 98 (3) | 0.6 | 0.9974 | 5.7 | 71 (17) |
| Metribuzin | 0.7 | 0.9940 | 3.0 | 108 (13) | 0.9 | 0.9946 | 5.7 | 101 (5) | 1.0 | 0.9982 | 3.9 | 72 (16) |
| Aflatoxin B1 | 0.8 | 0.9890 | 5.7 | 101 (11) | 0.8 | 0.9965 | 5.6 | 98 (12) | 0.8 | 0.9969 | 4.8 | 88 (13) |
| Thiophanate methyl | 1.2 | 0.9972 | 3.9 | 108 (11) | 0.5 | 0.9982 | 4.2 | 92 (9) | 0.6 | 0.9977 | 3.9 | 100 (12) |
| Carbofuran | 1.0 | 0.9814 | 4.8 | 107 (4) | 0.5 | 0.9984 | 3.9 | 103 (2) | 0.5 | 0.9968 | 5.7 | 70 (8) |
| Bendiocarb | 0.9 | 0.9992 | 4.5 | 98 (7) | 0.6 | 0.9966 | 5.1 | 110 (5) | 0.5 | 0.9970 | 5.1 | 77 (14) |
| Ofurace | 1.3 | 0.9983 | 5.4 | 75 (17) | 0.6 | 0.9981 | 5.2 | 103 (5) | 0.6 | 0.9940 | 4.2 | 74 (20) |
| Terbuthylazine desethyl | 1.5 | 0.9940 | 4.2 | 103 (7) | 0.6 | 0.9892 | 4.9 | 95 (3) | 0.7 | 0.9978 | 5.4 | 75 (16) |
| Tepraloxydim | 1.8 | 0.9834 | 3.3 | 93 (5) | 0.5 | 0.9912 | 4.5 | 81 (4) | 0.5 | 0.9988 | 5.1 | 89 (12) |
| Carbaryl | 0.7 | 0.9912 | 5.7 | 98 (4) | 0.6 | 0.9923 | 3.0 | 102 (3) | 0.6 | 0.9955 | 5.7 | 75 (5) |
| Monolinuron | 1.2 | 0.9833 | 4.2 | 96 (11) | 0.7 | 0.9943 | 4.8 | 108 (8) | 0.7 | 0.9966 | 5.1 | 75 (8) |
| Veratridine | 0.9 | 0.9950 | 4.8 | 112 (7) | 0.7 | 0.9908 | 5.1 | 97 (12) | 0.4 | 0.9984 | 4.5 | 78 (14) |
| Lenacil | 1.0 | 0.9992 | 10.0 | 99 (11) | 0.4 | 0.9877 | 6.6 | 101 (5) | 0.2 | 0.9901 | 6.3 | 74 (16) |
| Metobromuron | 0.7 | 0.9971 | 3.9 | 102 (10) | 0.6 | 0.9924 | 3.6 | 94 (18) | 0.8 | 0.9990 | 4.8 | 70 (18) |
| Azadirachtin | 0.9 | 0.9962 | 4.8 | 109 (12) | 0.5 | 0.9982 | 3.5 | 98 (6) | 0.6 | 0.9983 | 5.1 | 93 (6) |
| Chlorotoluron | 1.0 | 0.9980 | 4.2 | 74 (10) | 0.9 | 0.9894 | 3.7 | 112 (2) | 0.8 | 0.9981 | 4.5 | 74. (7) |
| Atrazine | 1.1 | 0.9983 | 5.7 | 102 (12) | 0.6 | 0.9942 | 3.9 | 118 (5) | 0.4 | 0.9973 | 3.0 | 78 (12) |
| Metazachlor | 1.1 | 0.9930 | 5.1 | 109 (5) | 0.7 | 0.9954 | 5.4 | 107 (12) | 0.6 | 0.9994 | 3.2 | 86 (13) |
| HT-2 toxin | 1.1 | 0.9934 | 3.3 | 80 (18) | –[d] | – | – | < 20 | 2.5 | 0.9905 | 3.5 | 106 (4) |
| Ochratoxin A | 1.2 | 0.9891 | 3.4 | 103 (13) | – | – | – | 24 (4) | 0.6 | 0.9965 | 2.0 | 77 (18) |
| Isoproturon | 1.1 | 0.9952 | 9.8 | 103 (19) | 0.6 | 0.9907 | 7.5 | 101 (5) | 0.5 | 0.9989 | 3.9 | 70 (7) |
| Fensulfothion | 1.1 | 0.9995 | 3.3 | 70 (10) | 0.5 | 0.9977 | 3.6 | 101 (11) | 0.5 | 0.9998 | 8.1 | 102 (12) |
| Cevadine | 0.7 | 0.9960 | 4.2 | 109 (14) | 0.6 | 0.9867 | 5.7 | 76 (4) | 1.0 | 0.9989 | 3.9 | 104 (18) |
| Diuron | 1.0 | 0.9963 | 9.7 | 103 (4) | 0.6 | 0.9934 | 8.7 | 99 (10) | 0.5 | 0.9693 | 7.2 | 72 (8) |
| Azaconazole | 5.8 | 0.9996 | 3.0 | 106 (19) | 0.5 | 0.9996 | 5.1 | 95 (6) | 0.5 | 0.9975 | 3.9 | 79 (10) |
| Linuron | 1.2 | 0.9991 | 3.6 | 120 (10) | 0.4 | 0.9915 | 5.4 | 102 (9) | 0.4 | 0.9981 | 5.4 | 108 (8) |

Table 1 (*Continued*)

| Compound | Wheat | | | | Cucumber | | | | Red wine | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Matrix effect[a] | $R^2$ | LOQ ($\mu g\,kg^{-1}$)[b] | Recovery[c] | Matrix effect[a] | $R^2$ | LOQ ($\mu g\,kg^{-1}$)[b] | Recovery[c] | Matrix effect[a] | $R^2$ | LOQ ($\mu g\,kg^{-1}$)[b] | Recovery[c] |
| Propazine | 0.9 | 0.9994 | 3.3 | 101 (17) | 0.8 | 0.9932 | 5.1 | 99 (9) | 1.1 | 0.9955 | 4.8 | 84 (18) |
| Diethofencarb | 1.0 | 0.9993 | 5.1 | 93 (10) | 0.6 | 0.9905 | 3.0 | 100 (8) | 0.7 | 0.9908 | 4.5 | 102 (15) |
| T-2 toxin | 1.0 | 0.9996 | 4.8 | 74 (15) | – | – | – | < 20 | 1.6 | 0.9829 | 4.2 | 89 (17) |
| Sebuthylazine | 0.7 | 0.9980 | 4.2 | 105 (7) | 0.7 | 0.9901 | 4.8 | 99 (7) | 0.6 | 0.9971 | 4.2 | 75 (11) |
| Terbuthylazine | 0.6 | 0.9932 | 4.5 | 77 (15) | 0.5 | 0.9982 | 4.2 | 104 (8) | 0.4 | 0.9996 | 5.1 | 93 (4) |
| Terbumeton | 0.8 | 0.9996 | 8.7 | 75 (16) | 0.4 | 0.9956 | 3.0 | 107 (3) | 0.4 | 0.9985 | 5.4 | 72 (21) |
| Methiocarb | 0.7 | 0.9982 | 4.2 | 73 (20) | 0.6 | 0.9824 | 5.7 | 109 (5) | 0.4 | 0.9994 | 3.9 | 105 (15) |
| Propyzamide | 1.7 | 0.9820 | 3.9 | 106 (15) | 0.6 | 0.9990 | 3.3 | 96 (3) | 0.5 | 0.9966 | 4.9 | 70 (13) |
| Promecarb | 1.2 | 0.9850 | 6.3 | 107 (7) | 0.5 | 0.9844 | 3.3 | 108 (7) | 0.5 | 0.9854 | 3.0 | 103 (8) |
| Paclobutrazol | 0.8 | 0.9992 | 3.9 | 93 (9) | 0.8 | 0.9979 | 3.9 | 103 (5) | 0.6 | 0.9985 | 5.4 | 76 (12) |
| Cycloxydim | 1.5 | 0.9982 | 3.3 | 93 (14) | 0.6 | 0.9936 | 3.0 | 80 (10) | 0.9 | 0.9949 | 4.5 | 78 (19) |
| Flutalonil | 1.4 | 0.9983 | 5.4 | 93 (7) | 0.7 | 0.9966 | 4.5 | 104 (9) | 0.7 | 0.9965 | 5.4 | 88 (11) |
| Prometryn | 1.1 | 0.9990 | 4.2 | 108 (8) | 0.9 | 0.9959 | 5.4 | 107 (6) | 0.6 | 0.9998 | 4.8 | 73 (10) |
| Iprovalicarb | 0.7 | 0.9950 | 4.5 | 105 (14) | 0.7 | 0.9821 | 7.5 | 102 (5) | 0.7 | 0.9968 | 5.7 | 87 (9) |
| Triadimenol | 0.3 | 0.9982 | 6.3 | 86 (8) | 1.0 | 0.9979 | 5.1 | 109 (8) | 0.6 | 0.9996 | 3.9 | 79 (22) |
| Triazophos | 0.6 | 0.9981 | 3.0 | 109 (12) | 0.6 | 0.9918 | 4.2 | 107 (3) | 0.6 | 0.9999 | 3.6 | 72 (19) |
| Sethoxydim | 0.6 | 0.9980 | 5.4 | 84 (15) | 0.1 | 0.9955 | 3.9 | 93 (10) | 1.2 | 0.9645 | 7.5 | 97 (10) |
| Tebutam | 1.1 | 0.9995 | 3.0 | 98 (8) | 0.7 | 0.9943 | 5.7 | 109 (6) | 0.4 | 0.9988 | 4.8 | 80 (17) |
| Fenhexamide | 0.9 | 0.9999 | 5.4 | 99 (10) | 0.8 | 0.9975 | 3.0 | 106 (7) | 0.7 | 0.9986 | 5.4 | 72 (14) |
| Metholachlor | 1.1 | 0.9902 | 3.6 | 103 (8) | 0.6 | 0.9895 | 4.2 | 96 (11) | 0.6 | 0.9810 | 5.7 | 75 (16) |
| Spiroxamine | 1.7 | 0.9994 | 5.1 | 73 (10) | 0.5 | 0.9961 | 5.7 | 107 (5) | 0.3 | 0.9991 | 3.6 | 93 (3) |
| Triflumizole | 1.1 | 0.9922 | 6.3 | 70 (20) | 2.1 | 0.9939 | 4.5 | 109 (16) | 1.7 | 0.9869 | 6.6 | 81 (20) |
| Epoxiconazole | 1.5 | 0.9924 | 3.6 | 105 (15) | 0.8 | 0.9986 | 3.9 | 106 (4) | 0.6 | 0.9968 | 3.9 | 72 (6) |
| Fenbuconazole | 0.6 | 0.9974 | 5.7 | 107 (10) | 0.6 | 0.9980 | 3.6 | 94 (4) | 0.7 | 0.9962 | 3.6 | 93 (7) |
| Imazalil | 0.9 | 0.9993 | 5.1 | 110 (12) | 0.8 | 0.9994 | 3.3 | 98 (3) | 0.6 | 0.9987 | 5.4 | 71 (9) |
| Rotenone | 1.0 | 0.9960 | 3.3 | 104 (15) | 0.7 | 0.9990 | 5.4 | 93 (9) | 1.0 | 0.9991 | 4.8 | 70 (14) |
| Degueline | 0.9 | 0.9980 | 3.0 | 90 (16) | 0.8 | 0.9851 | 5.1 | 104 (9) | 0.9 | 0.9976 | 3.0 | 79 (16) |
| Diflubenzuron | 2.2 | 0.9950 | 3.6 | 109 (16) | 0.3 | 0.9953 | 3.9 | 104 (3) | 0.3 | 0.9977 | 4.5 | 95 (13) |
| Furmecyclox | 0.8 | 0.9902 | 4.5 | 71 (15) | 0.9 | 0.9960 | 3.3 | 80 (6) | 0.6 | 0.9869 | 6.3 | 78 (11) |
| Thiazopyr | 1.0 | 0.9953 | 4.5 | 78 (9) | 0.5 | 0.9961 | 4.2 | 103 (16) | 0.1 | 0.9992 | 3.6 | 94 (13) |
| Bitertanol | 1.3 | 0.9829 | 8.4 | 99 (14) | 0.5 | 0.9875 | 5.4 | 95 (5) | 0.7 | 0.9937 | 3.0 | 72 (16) |
| Pencycuron | 1.7 | 0.9924 | 6.9 | 107 (17) | 0.6 | 0.9905 | 3.9 | 101 (3) | 0.5 | 0.9952 | 5.1 | 71 (12) |
| Trifloxystrobin | 0.9 | 0.9993 | 7.8 | 93 (17) | 0.7 | 0.9901 | 3.6 | 103 (5) | 0.5 | 0.9929 | 5.7 | 73 (7) |
| Hexythiazox | 1.3 | 0.9835 | 4.8 | 73 (20) | 0.8 | 0.9979 | 3.6 | 103 (8) | 1.0 | 0.9885 | 4.5 | 76 (16) |
| Fluazifop-P-butyl | 1.0 | 0.9983 | 4.8 | 107 (13) | 0.7 | 0.9995 | 4.2 | 106 (4) | 0.6 | 0.9996 | 3.0 | 71 (16) |
| Piperonyl butoxide | 0.5 | 0.9971 | 3.6 | 107 (13) | 0.8 | 0.9970 | 4.1 | 100 (4) | 0.7 | 0.9978 | 4.5 | 88 (8) |
| Spinosad | 0.8 | 0.9993 | 4.5 | 106 (14) | 0.5 | 0.9996 | 5.1 | 80 (4) | 0.6 | 0.9964 | 3.0 | 83 (7) |
| Fenazaquin | 0.5 | 0.9983 | 3.9 | 103 (7) | 0.6 | 0.9997 | 3.6 | 101 (6) | 0.5 | 0.9931 | 5.4 | 70 (17) |
| Fenpropimorph | 1.0 | 0.9992 | 4.2 | 105 (10) | 0.5 | 0.9980 | 4.8 | 106 (6) | 0.4 | 0.9967 | 4.5 | 77 (17) |
| Pyrethrins | 2.6 | 0.9974 | 7.0 | 95 (20) | 1.8 | 0.9917 | 9.8 | 97 (6) | 1.7 | 0.9861 | 7.5 | 76 (15) |

[a] Matrix effects are expressed as the ratio between the calibration curve slopes of matrix-matched standards and solvent-based standards.

[b] Limit of quantification (LOQ) was determined as the concentration at which S/N = 10.

[c] Recovery values (%) obtained at 10 $\mu g\,kg^{-1}$ or 10 $\mu g\,L^{-1}$. Intraday precision, expressed as RSD, is given in brackets ($n = 4$).

[d] Not quantitative extraction in this matrix.

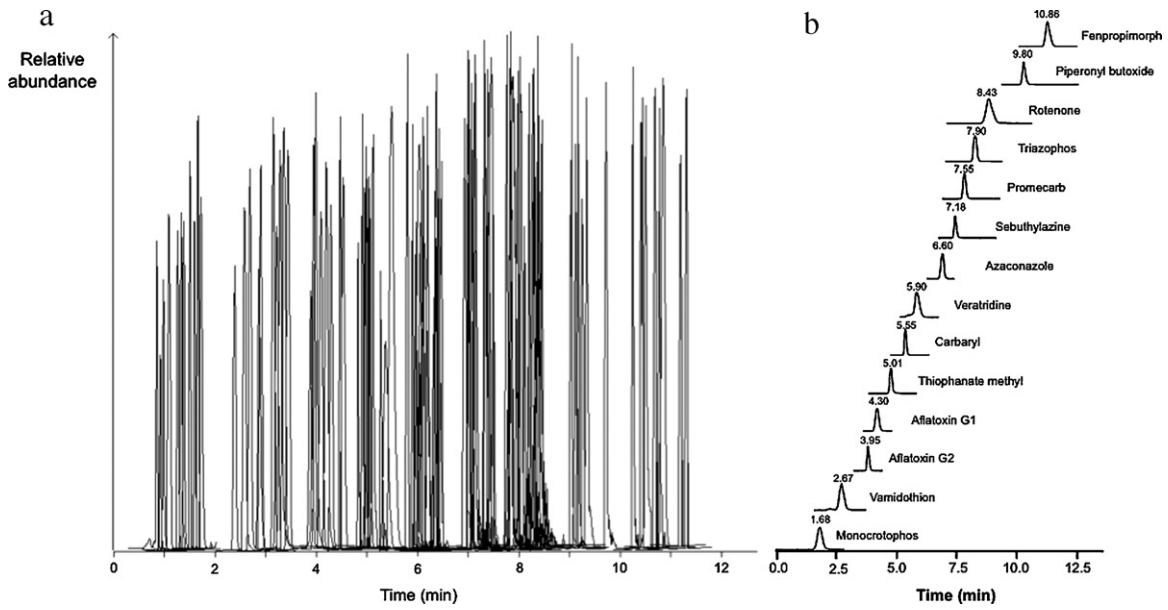


**Fig. 1.** (a) Combined UHPLC–MS/MS chromatogram from a blank wheat sample spiked at 50 μg kg$^{-1}$, based on quantifying MS/MS transitions, and (b) selected UHPLC–MS/MS chromatograms.

and in the three matrixes indicated. The slopes obtained in the calibration with matrix matched-standards were compared with those obtained with solvents standards. Table 1 shows slope ratio matrix/solvent for each compound and matrix effect was observed for most of the selected compounds in the three matrixes evaluated. In general it can be observed that a signal enhancement was observed when wheat matrix was evaluated, whereas for cucumber and red wine, signal suppression was noted. However, it can be noted that for two compounds, chlortoluron and propazine, no matrix effect was observed in the three matrixes evaluated.

Therefore, matrix-matched calibration was used for quantification purposes, prepared as described in Section 2.5 for every type of food commodity, to counteract this matrix effect. Peak area was used as response and good linearity was obtained for all the selected compounds with determination coefficients higher than 0.98 for the target compounds (see Table 1), and deviations of the individual points from the calibration curve lower than 20%.

The obtained results of LOQs are shown in Table 1, observing that LOQs were always lower than 10.0 μg kg$^{-1}$ in wheat (lenacil), cucumber (pyrethrins) and wine (nicotine). In order to simplify the subsequent routine quality controls, the LOQ was established at 10 μg kg$^{-1}$.

Trueness was estimated through recovery studies. The obtained results are shown in Table 1 for the lowest assayed concentration and Table S-2 (see supplementary data), which indicated the results for the highest levels evaluated (25 and 50 μg kg$^{-1}$ for wheat and cucumber, and 25 and 50 μg L$^{-1}$ for wine). In general, most of the analysed compounds showed satisfactory recoveries, with mean values ranging from 70 to 120% at all three levels of fortification (Tables 1 and S-2). Two pesticides were not extracted in the three matrixes evaluated (picloram and quinmerac) and poor recoveries were obtained for ochratoxin A, HT-2 toxin and T-2 toxin in cucumber (recoveries lower than 20%). This illustrates the difficulties of development a

**Table 2**
Concentrations of compounds found in the organic samples analysed.

| Compound | Cereals ($n$ = 18) | | Vegetables ($n$ = 13) | | Alcoholic beverages ($n$ = 9) | |
|---|---|---|---|---|---|---|
| | Concentration range (μg kg$^{-1}$) | No. positive samples | Concentration range (μg kg$^{-1}$) | No. positive samples | Concentration range (μg L$^{-1}$) | No. positive samples |
| Nicotine | 13.8–28.4 | 8 | 28.1 | 1 | | |
| Triasulfuron | 29.8 | 1 | | | | |
| Atrazine desethyl | 32.6–74.0 | 2 | | | | |
| Aflatoxin G1 | 7.4 | 1 | | | | |
| Aflatoxin B1 | 10.0–26.0 | 2 | | | | |
| Veratridine | 46.2 | 1 | | | | |
| Azadirachtin | | | | | 11.1 | 1 |
| Fenhexamide | 10.4–14.8 | 2 | | | | |
| Triflumizole | 28.2 | 1 | | | 18.9–43.4 | 2 |
| Epoxiconazole | 15.4–178.2 | 3 | 10.4–47.0 | 3 | | |
| Rotenone | 10.6–13.6 | 4 | | | | |
| Bitertanol | | | | | 10.1 | 1 |
| Pyrethrin II | 10.4–23.2 | 2 | | | | |
| Piperonyl butoxide | 14.2–31.4 | 3 | 20.7–25.8 | 2 | | |
| Cinerine II | 20.4–63.6 | 3 | | | | |
| Cinerine I | 17.8 | 1 | | | 10.6–28.6 | 2 |
| Pyrethrin I | 11.0–26.4 | 3 | | | 34.1 | 1 |
| Jasmoline I | 16.6–94.8 | 9 | | | | |
| ΣPyrethrins | 16.6–94.8 | 12 | | | 10.6–34.1 | 3 |

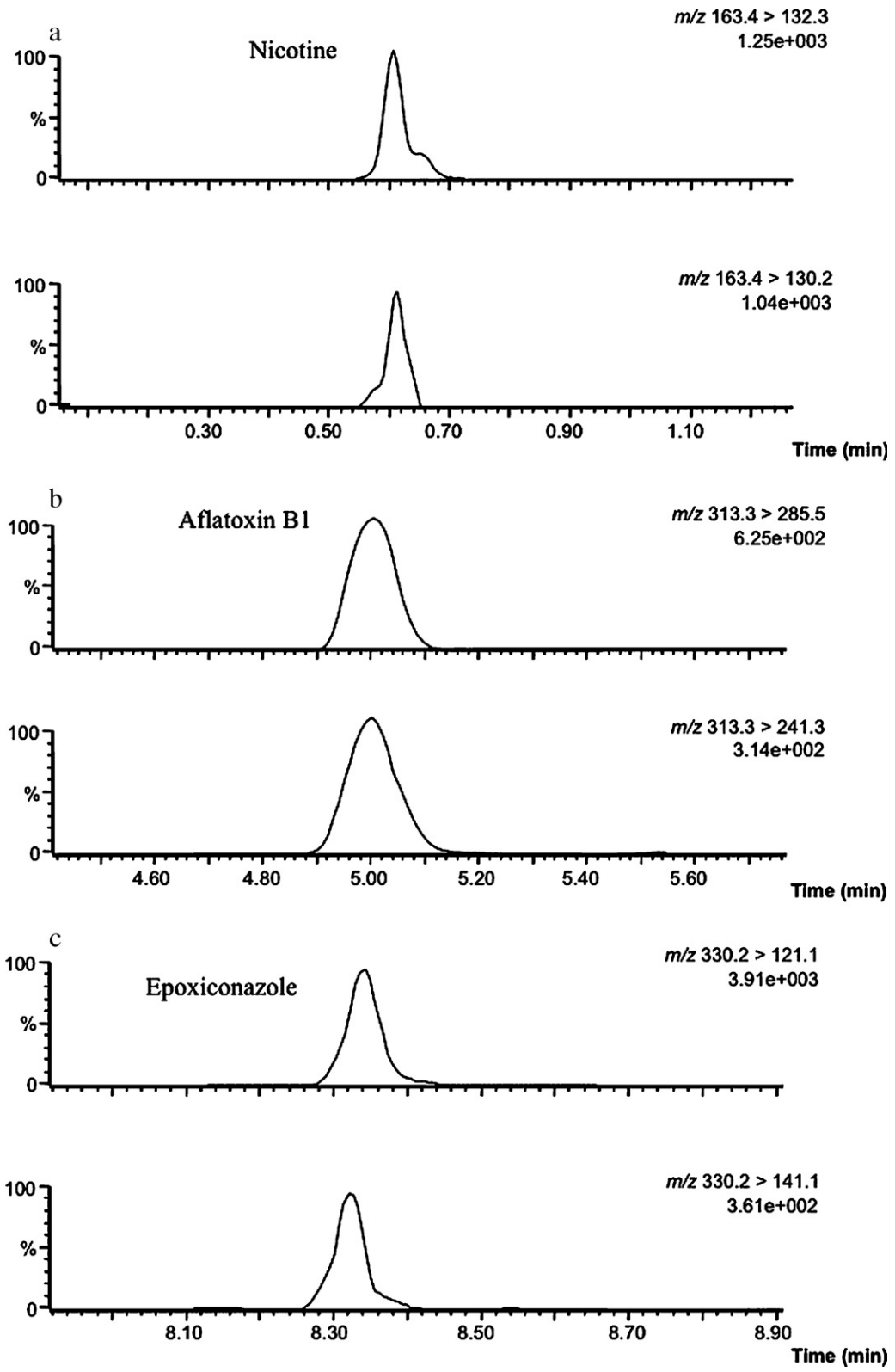


**Fig. 2.** UHPLC–MS/MS chromatograms for: (a) dried mushrooms containing nicotine at 28.1 μg kg$^{-1}$, (b) wheat sample containing aflatoxin B1 at 10.0 μg kg$^{-1}$ and (c) eggplant sample containing epoxiconazol at 47.0 μg kg$^{-1}$. Quantification and confirmation transitions are shown for the three positive samples.

unique multiresidue method for the determination of a number of compounds with a wide range of physical–chemical properties. Finally it must be indicated that recoveries did not depend on the matrix evaluated and no significant differences were observed.

Precision of the overall method was calculated in terms of intra-day and inter-day precision, expressed as RSD, showing the obtained results in Tables 1 and S-2. It can be observed that RSDs values were always lower than 20% for all the compounds and concentration levels assayed, except for nicotine and metamitron

in red wine and cucumber respectively, which presented values higher than 20%, at the lower concentration level evaluated. In relation to inter-day precision, it can be noted that these values were similar or slightly higher than the repeatability ones (precision intra-day), ranging from 4% to 24% for most of the analysed compounds, indicating the stability of the developed method.

Finally, the selectivity of the method was tested analysing blank samples. The absence of any chromatographic peak in every matrix, at the same retention time as target compounds, indicated there were no matrix compounds that might give a false positive signal in these samples.

### 3.4. Analysis of real samples

A total of 40 organic samples were collected from organic markets in Almeria (Spain) and they were analysed following the described method, showing the obtained results in Table 2. Biopesticides levels were below MRLs, except nicotine in dried mushrooms, which was detected at concentration of 28.0 $\mu g\,kg^{-1}$. This value is higher than the MRL (10 $\mu g\,kg^{-1}$) set by Regulation n 396/2005 [3]. It must be indicated that in 2009, the European Food Safety Authority (EFSA) has been informed by food business operators that dried mushrooms may contain levels of nicotine higher than MRL. No clear reason has been established for this unexpected presence of nicotine in dried mushrooms, although EU was forced set a guideline with temporary limits for nicotine levels in fresh and dried mushrooms (0.04 $mg\,kg^{-1}$ in fresh wild mushrooms, 1.2 $mg\,kg^{-1}$ in dried mushrooms) [34]. Other biopesticides such as veratridine, azadiracthin, rotenone and pyrethrins were detected. It must be indicated that the levels of rotenone in crops (10.6–13.6 $\mu g\,kg^{-1}$) were higher than the MRL established by EU for this compound in wheat (10 $\mu g\,kg^{-1}$).

Furthermore, the detected levels of aflatoxins (G1 and B1) in corn, were higher than the level set by EU [4] of 2 and 4 $\mu g\,kg^{-1}$ for aflatoxin B1 and sum of aflatoxins respectively. This fact is in accordance with Ref. [7], which indicates that higher concentrations of mycotoxins can be detected in organic food products.

The positive results for synthetic pesticides (e.g. the values range between 10.0 and 33.0 $\mu g\,kg^{-1}$ for cereals, between 10.0 and 47.0 $\mu g\,kg^{-1}$ for fruits, vegetables and beverages) in organic samples are in accordance with report published in 2009 by EFSA [35]. It must be highlighted that only traces of few compounds were detected. Despite no specific MRLs for organic products are set by EU, none of the assayed samples have values higher than the values established for conventional products.

Finally, Fig. 2 shows the UHPLC–MS/MS chromatograms for three positives results of nicotine (28.1 $\mu g\,kg^{-1}$), aflatoxin B1 (10.0 $\mu g\,kg^{-1}$) and epoxiconazole (47.0 $\mu g\,kg^{-1}$) in dried mushrooms, wheat and eggplant, respectively.

## 4. Conclusions

QuEChERS based method combined with UHPLC–MS/MS provides a sensitive and selective method for the simultaneous determination of more than 90 compounds belonging to several families such as pesticides, biopesticides and mycotoxins in different organic food products. Good recovery and precision were obtained indicating the reliability of the data obtained when the developed procedure was applied. MS/MS detection increased confidence in compound identification when matrixes such as crops, vegetables and beverages were analysed. The simultaneous detection of the three types of compounds in the matrixes evaluated indicate that this method can be used for the routine analysis of pesticides, biopesticides and mycotoxins at trace levels in organic samples, considering the extraction procedure is fast and easy to perform and the running analysis time is less than 13 min.

## Acknowledgments

The authors gratefully acknowledge Spanish Ministry of Science and Innovation-FEDER (Project Ref: AGL2006-12127-C02-01 and CTQ2009-07686) for financial support. RRG is also grateful for personal funding through the Ramon y Cajal program (Spanish Ministry of Education and Science-EFS). ODP is grateful to the CAPES-Brazil for a postgraduate scholarship (1849-0903). SLG also wishes to thank the postgraduate scholarship from the Spanish Agency for International Development Cooperation (AECID) (Spanish Ministry of Foreign Affairs and Cooperation).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.01.034.

## References

[1] H.F. De Brabandera, H. Noppea, K. Verheydena, J.V. Bussche, K. Wille, L. Okermana, L. Vanhaeckea, W. Reybroeckb, S. Oogheb, S. Croubels, J. Chromatogr. A 1216 (2009) 7964.
[2] P. Villa, P. Markaki, Food Control 20 (2009) 455.
[3] Regulation (EC) 396/2005 of the European parliament and of the council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Off. J. Eur. Commun. L70/1, 16.3.2005. Consolidated version obtained from http://ec.europa.eu/sanco_pesticides/public/index.cfm (lass accessed December 2010).
[4] Commision Regulation (EC) No. 472/2002/EC of 12 March 2002 amending Regulation (EC) No. 466/2001 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Commun. L75/18, 16.3.2002.
[5] A. Hercegová, M. Dömötörová, D. Kruzlicová, E. Matisová, J. Sep. Sci. 29 (2006) 1102.
[6] A.M. Aldanondo-Ochoa, C. Almansa-Sáez, Land Use Policy 26 (2009) 669.
[7] C. Juan, J.C. Moltó, C.M. Lino, J. Mañes, Food Chem. 107 (2008) 525.
[8] E.G. Amvrazi, T.A. Albanis, Food Chem. 113 (2009) 253.
[9] P. Payá, M. Anastassiades, D. Mack, I. Sigalova, B. Tasdelen, J. Oliva, A. Barba, Anal. Bioanal. Chem. 389 (2007) 1697.
[10] J.F. García-Reyes, C. Ferrer, M.J. Gómez-Ramos, A. Molina-Díaz, A.R. Fernández-Alba, Trends Anal. Chem. 26 (2007) 239.
[11] H.G.J. Mol, P. Plaza-Bolaños, P. Zomer, T.C. de Rijk, A.A.M. Stolker, P.P.J. Mulder, Anal. Chem. 80 (2008) 9450.
[12] C. Díez, W.A. Traag, P. Zommer, P. Marinero, J. Atienza, J. Chromatogr. A 1131 (2006) 11.
[13] Y. Picó, D. Barceló, Trac-Trends Anal. Chem. 27 (2008) 821.
[14] K. Malik, C. Blasco, Y. Picó, J. Chromatogr. A 1217 (2010) 4018.
[15] A. Garrido Frenich, J.L. Martínez Vidal, E. Pastor-Montoro, R. Romero-González, Anal. Bioanal. Chem. 390 (2008) 947.
[16] C.C. Leandro, P. Hancock, R.J. Fussell, B.J. Keely, J. Chromatogr. A 1144 (2007) 161.
[17] J. Wang, D. Leung, J. Agric. Food Chem. 57 (2009) 2162.
[18] P. Plaza-Bolaños, A. Garrido Frenich, J.L. Martínez Vidal, J. Chromatogr. A 1167 (2007) 9.
[19] I.R. Pizzutti, A. Kok, M. Hiemstra, C. Wickert, O.D. Prestes, J. Chromatogr. A 1216 (2009) 4539.
[20] I.R. Pizzutti, A. Kok, R. Zanella, M.B. Adaime, M. Hiemstra, C. Wickert, O.D. Prestes, J. Chromatogr. A 1142 (2007) 123.
[21] P. Caboni, G. Sarais, A. Angioni, V.L. Garau, P. Cabras, J. Agric. Food Chem. 53 (2005) 8644.
[22] D. Drożdżyński, J. Kowalska, Anal. Bioanal. Chem. 394 (2009) 2241.
[23] A. Garrido Frenich, J.L. Martínez Vidal, R. Romero-González, M.M. Aguilera-Luiz, Food Chem. 117 (2009) 705.
[24] R. Romero-González, J.L. Martínez Vidal, M.M. Aguilera-Luiz, A. Garrido Frenich, J. Agric. Food Chem. 57 (2009) 9385.
[25] R.M. González-Rodríguez, R. Rial-Otero, B. Cancho-Grande, J. Simal-Gándara, J. Chromatogr. A 1196 (2008) 100.
[26] M. Dömötörová, E. Matisová, J. Chromatogr. A 1207 (2008) 1.
[27] O. Lacina, J. Urbanová, A. Krplová, J. Hajšlová, Chem. Listy 102 (2008) 404.
[28] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412.
[29] S.J. Lehotay, K. Mastovska, A.R. Lightfield, J. AOAC Int. 88 (2005) 615.

[30] A. Desmarchelier, J.M. Oberson, P. Tella, E. Gremaud, W. Seefelder, P. Mottier, J. Agric. Food Chem. 50 (2010) 7510.
[31] European Commission Directorate General Health and Consumer Protection, Guidance Document on Method Validation and Quality Control Procedures for Pesticide Residues Analyses in Food and Feed, SANCO/10684/2009, 01 January 2010.
[32] A. Wilkowska, M. Biziuk, Food Chem. 125 (2011) 803.
[33] A. Trebstein, W. Seefelder, U. Lauber, H.U. Humf, J. Agric. Food Chem. 56 (2008) 4968.
[34] European Food Safety Authority (EFSA) Statement Q-2009-00527 of 07 May 2009. EFSA J. RN286 (2009) 1.
[35] European Food Safety Authority (EFSA) Statement Q-2008-714 of 07 June 2009. EFSA J. 305 (2009) 1.