Performance and Matrix Effect Observed in QuEChERS Extraction and Tandem Mass Spectrometry Analyses of Pesticide Residues in Different Target Crops

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Abstract

The method performance and matrix effect related to quantitative determination of pesticide residues was assessed after QuEChERS extraction and LC–MS–MS analysis. Dicloran, phosmet and phosmet-oxon, pirimiphos-methyl, and BNOA were analyzed in peach, apple, melon, cereals, tomato, and strawberry. The matrix effects, as well as recovery and process efficiencies, were determined for a fungicide, two insecticides, and a plant growth regulator. Crop samples were spiked either pre- or post-extraction, then the peak area was compared with the peak area in neat solvent. The mean recovery ranged from 73% to 98%, and repeatability (as RSD) was between 3% and 16%, depending on the compound and spiking level. The matrix effect occurred as ionic suppression and was found in the range of 5% to 22% depending on the compound. Recovery efficiencies were good and substantially comparable, being in the range of 93–96%. Although the suppression observed still appears to be acceptable considering the overall process efficiency, it seems evident that the matrix effect is important when a reliable quantitative method must be applied.

Introduction

In recent years, triple quadrupole tandem mass spectrometry (MS–MS) has become the best analytical technique to determine pesticide residues in difficult matrices, such as crop commodities. This technique provides an enhanced selectivity, leading to better sensitivity and specificity.

A common perception regarding liquid chromatography (LC–MS–MS) analysis of pesticide residues is that selectivity is guaranteed by the selective tandem MS reaction, and therefore sample purification and chromatographic separation could be simplified or even eliminated. Contrary to this common belief, the reliability of quantitative assays could be adversely affected by matrix effects (1–6). As described, the main limit of the tandem MS assay is the reduction or enhancement of ion intensity of the analytes, resulting from co-eluting of undetected matrix components. The ionic suppression or enhancement at the interface level affects accuracy because standards in pure solvent do not undergo this process.

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The exact mechanism of ion suppression is not known; it seems that it may be caused by non-volatile material (7) or by compounds of high surface activity (8). The chemical nature of the analyte plays an important role too: Taylor (9) observed that matrix effects of polar compounds are more relevant than those of less polar compounds. Some instrumental parameters such as the ionization source (7), ionization mode (10), and flow rate (11) have been found to influence the extent of the matrix effect.

Hence, the clean up of plant extracts is often recommended even if no interference can be observed in the MS–MS chromatogram in the analyte retention time window.

LC can be coupled to tandem MS with better solutions than in past years, due to the parallel improvement of instruments and interfaces such as electrospray ionisation (ESI) and atmospheric pressure chemical ionization (APCI). LC can provide a wider compound coverage rather than gas chromatography, and hence LC–MS–MS is becoming quite a popular analytical technique.

During recent years, a fast and effective clean up technique, the QuEChERS (12–14), has become a very interesting complement of MS–MS assay (15–24) to overcome ionic suppression or enhancement at the interface due to matrix interferences. QuEChERS is the acronym of Quick, Easy, Cheap, Effective, Rugged and Safe, a matrix dispersive solid-phase extraction mainly based on the PSA bulk adsorbent, which is carried out on the acetonitrile extract of the crop to be analyzed. The bulk adsorbent is dispersed after extraction, some salts are added (magnesium sulphate, sodium chloride, and a citrate buffer), and then the extract can be directly analyzed after centrifugation. The QuEChERS extraction followed by LC–MS–MS assay is, therefore, a valuable tool for pesticide residues analysis, allowing good analytical performance and high throughput (22,25–28).

The goal of the present work was to evaluate the suitability of QuEChERS extraction and clean up, followed by LC–MS–MS analysis, for a reliable quantitative determination of some pesticide residues in crops; our aim was to focus on ionic suppression or enhancement due to the matrix.

Two insecticides, a fungicide, and a plant growth regulator were determined according to the procedure mentioned above, and method fitness-for-purpose was assessed in each case on representative crops. The extraction and clean up procedure

were the same for each analytical method and the LC–MS–MS method for the analysis of each specific active substance in/on the representative crops was the only difference among the tests performed.

Materials and Methods

Dicloran, phosmet together with its metabolite phosmet oxon, pirimiphos-methyl, and BNOA (Figure 1) were chosen among fungicides, insecticides, and plant growth regulators. The compounds comprised analytes amenable either in electrospray or atmospheric pressure chemical ionization, under positive or negative ionization mode. As far as the LC–MS–MS apparatus is concerned, a 1200 series liquid chromatograph system, equipped with quaternary pump, atmospheric pressure chemical ionization and electrospray ionization systems, coupled to a G6410A triple quadrupole mass spectrometer detector (all from Agilent Technologies, Santa Clara, CA) was used.

The extraction followed by the dispersive SPE clean up procedure was the same for all the compounds considered, while the matrices were actual field target crops of the corresponding plant protection products. The choice of the matrices tested included low (cereals) and high (peach, melon, and strawberry) water content commodities, as well as those considered acid matrices (apple, tomato). To evaluate method performance and matrix effects, the homogenous crop materials were spiked at different steps of the analytical procedure (either before or after QuEChERS extraction) with the reference standard at a fortification level close to the method limit of quantification, and then analyzed. Two matrices were considered for each compound; although they were not representative of all possible matrix effects, assessments on a larger number of fruit or vegetable species would not have been satisfactory in any case, considering that the matrix effect is supposed to be not only plant species related, but also depending on the cultivar considered and the ripening grade.

In order to enhance matrix effects and therefore to highlight any possible limits of the extraction and clean up procedure, all the chromatographic separations were carried out on a short HPLC column (Zorbax SB-C_{18}, 2.1 mm I.D., 30 mm length, 3.5 µm dp) (Agilent Technologies), hence more co-eluting peaks were likely to arise from the matrix. For each target compound, the best selected reaction monitoring (SRM) based on collision induced decompositions were defined; both ionization and ion transport conditions were optimized by introducing single analyte standard solutions into the MS system. The acquisition parameters adopted for each compound are shown in detail in Table I. The chromatographic system was calibrated, for quantitation, by using a minimum of 5 concentration levels of standard solutions prepared in neat solvent and analysed in duplicate.

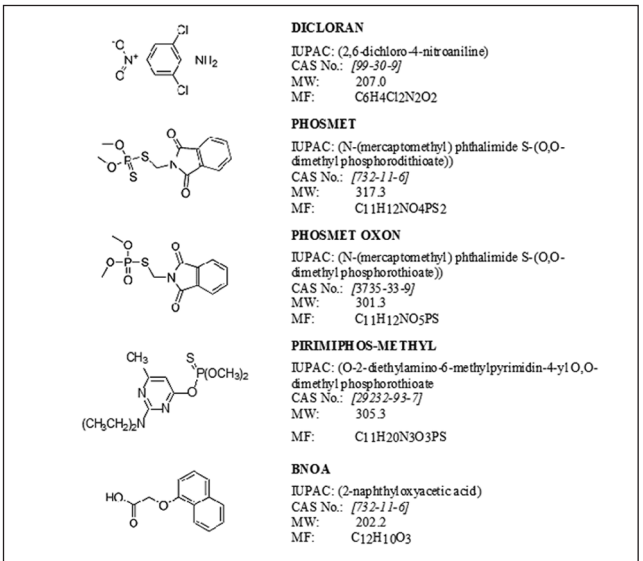

Figure 1. Name and structure of the analytes considered.

Chemicals and plant materials

The reference standards of phosmet, phosmet oxon, pirimiphos-methyl, dicloran, and BNOA were certified reference materials (all from Dr Ehrenstorfer GmbH, Augsburg, Germany). The salts used (magnesium sulphate, sodium chloride, trisodium citrate dehydrate, disodium hydrogencitrate sesquihydrate, ammonium formate, and potassium hydroxide) were analytical grade reagents all from Carlo Erba Reagenti (Milan, Italy). The bulk sorbent PSA was bondesil PSA (Varian Inc., Palo Alto, CA) and water was Milli-Q grade; lastly the solvents used (acetonitrile, methanol, and acetone) were for pesticide analysis and bought from VWR International Ltd. (Poole, England).

The peach, melon, apple, tomato, and strawberry samples came from a local supermarket. They were from organic agriculture and therefore devoid of any residue of the target analytes. Wheat and oat grains were supplied from the university's experimental farm; they were analyzed prior to use, to verify that residues of the target analytes were not detectable.

QuEChERS extraction

An accurate weight (10.0 g ± 0.05 g) of each crop sample was extracted in a centrifuge tube by adding 10 mL of acetonitrile, 4 g of magnesium sulphate, and 1 g of sodium chloride, followed by 1 g of trisodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquihydrate.

The extract was then shaken vigorously by hand for 1 min and then centrifuged (503 g for 5 min). An aliquot of the organic

Table I. Tandem MS SRM Acquisition Parameters for the Analytes Considered

Analyte	Ionization source / polarity	Precursor ion (*m/z*)	Product ions (*m/z*)	Collision energy (V)
Dicloran	APCI negative	204.9	168.8	28
			122.8	28
Phosmet	APCI negative	156.8	141.8	20
			111.8	20
Phosmet-oxon	APCI positive	160.0	133.0	25
			105.0	25
Pirimiphos-methyl	ESI positive	306.2	164.1	23
			136.1	23
BNOA	ESI negative	143.1	115.0	30
		144.1	116.0	30

phase supernatant (5 mL) was transferred to a second centrifuge tube, then 0.85 g of magnesium sulphate and 0.125 g PSA bulk sorbent were added. The tube was shaken vigorously for 30 s, centrifuged (503 g for 5 min), and an aliquot of the purified extract was analysed by LC–MS–MS according to the specific methods.

Analysis of phosmet and phosmet oxon

Phosmet is a cholinesterase inhibitor insecticide used on pome and stone fruits. The degradation pattern of this insecticide in plants leads to a main metabolite called phosmet oxon. As a method validation scheme, recovery tests were carried out at 0.01 mg/kg, 0.1 mg/kg, and 2.0 mg/kg spiking levels, while the matrices considered were peach and apple. Phosmet residues were determined with the APCI negative ionization mode; the mobile phase consisted of an acetonitrile–0.5 mM ammonium formate in water mixture (60/40, v/v) flowing at 0.3 mL/min and at a temperature of 35°C, and the injection volume was 10 µL.

A stock solution from each certified reference standard was obtained by weighing 10 mg of certified material in a 100-mL flask, then stoppering with methanol. Working standard solutions were then prepared daily by combining aliquots of the stock solutions and diluting with acetonitrile. Phosmet oxon residues were determined with the APCI positive ionisation mode and the LC conditions were the same as for phosmet determination.

The linear dynamic range was determined with 5 calibration points, according to a linear model and forcing the curve by the origin. The slope was 68.5 and 937.2 for phosmet and phosmet-oxon, respectively. The linear correlation coefficient was 0.999 for both compounds.

Analysis of dicloran

Dicloran is a protective dichloroaniline fungicide used on several crops. As a method validation scheme, recovery tests were carried out at 0.01 mg/kg, 0.1 mg/kg, and 1.0 mg/kg spiking levels and the matrices considered were melon (pulp), and lettuce. Dicloran residues were determined with APCI negative ionization mode. The LC mobile phase consisted of an acetonitrile (A) and 0.5 mM ammonium formate mixture (B) flowing at 0.3 mL/min and at a temperature of 35°C; the injection volume was 10 µL. The mobile phase gradient started from 65% A (v/v) until 3 min, then increased to 90% A from 3 to 3.5 min, and decreased to 65% A from 4 to 4.5 min.

A stock solution from a certified reference standard was prepared by accurately weighing about 10 mg of certified material in a 100-mL flask, then stoppering with acetone. Working standard solutions were then prepared daily by diluting with acetonitrile.

The linear dynamic range was determined with 5 calibration points, according to a linear model and forcing the curve by the origin. The slope was 46.8, and the linear correlation coefficient was 0.997.

Analysis of BNOA

BNOA is an auxin-like plant growth regulator used as fruit setting on tomatoes and strawberries. As a method validation scheme, recovery tests were carried out at 0.01 mg/kg and 0.05 mg/kg spiking levels on both tomato berries and strawberry fruits. BNOA residues were determined with ESI operating in the negative ionisation mode, and the mobile phase consisted of an acetonitrile–0.5 mM ammonium formate mixture (65/35, v/v) flowing at 0.3 mL/min and at a temperature of 35°C; the injection volume was 20 µL.

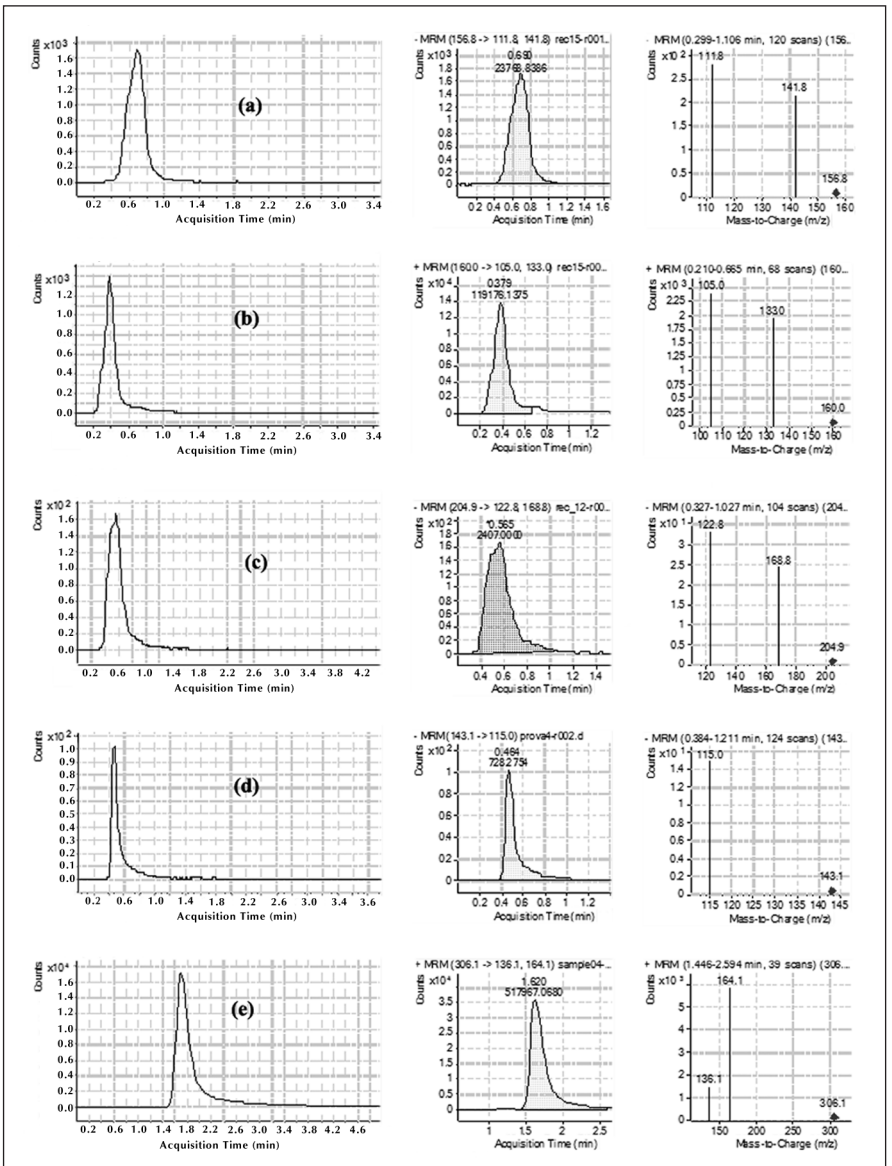

Figure 2. Representative LC–MS–MS chromatograms (TIC, MRM, and MS–MS spectra from left to right) of crop extracts spiked in post extraction by phosmet (a), phosmet oxon (b), dicloran (c), BNOA (d) and pirimiphos-methyl (e).

A stock solution from a certified reference standard was prepared by weighing 10 mg of certified material in a 100 mL flask, adding 500 µL KOH 1%, then stoppering with an acetonitrile–water (1:5, v/v) mixture. Working standard solutions were then prepared daily by diluting with water.

The linear dynamic range was determined with 5 calibration points, according to a linear model and forcing the curve by the origin. The slope was 33.8 and the linear correlation coefficient was 0.996.

Analysis of pirimihos-methyl

Pirimiphos-methyl is an organophosphate insecticide used on mainly for stored cereals. As a method validation scheme, recovery tests were carried out at 0.01 mg/kg, 0.1 mg/kg, and 1.0 mg/kg spiking levels and the matrices considered were bread-making wheat (hulled grains), and oat whole grains. Pirimiphos-methyl residues were determined with ESI positive ionisation mode. The LC mobile phase consisted of an acetonitrile (A) and 0.5 mM ammonium formate mixture (B) flowing at 0.2 mL/min and at a temperature of 35°C; the injection volume was 10 µL. The mobile phase consisted of 80% A for the whole chromatographic run.

A stock solution from a certified reference standard was prepared by accurately weighing ~10 mg of certified material in a 100 mL flask, then stoppering with methanol. Working standard solutions were then prepared daily by diluting with acetonitrile.

The linear dynamic range was determined with 5 calibration points, according to a linear model and forcing the curve by the origin. The slope was 337.4 and the linear correlation coefficient was 0.995.

Assessment of method performance and matrix effect

As far as the method performance is concerned, recovery tests were carried out at a minimum of two spiking levels and 5 replicate tests were done per level. Recovery data were used to assess accuracy while their RSD was the day-to-day precision (intermediate repeatability conditions: same operator, same laboratory, 3 different days of analysis). The limit of detection (LOD) was determined as the concentration that produces a peak with a height three times the level of the baseline noise. Standard solutions were used to evaluate the LOD and the linear dynamic range, while the limit of quantification (LOQ) was set to the lower spiking level at which precision as RSD was below 20% and accuracy (as mean recovery) was in the range of 70–110%. The target LOQ was 0.01 mg/kg for each pesticide considered in all the matrices tested.

The matrix effect was estimated according to Matuszewski et al (2): the comparison of the standard peak area in solvent (A), the standard spiked after (B) and the standard spiked before (C) extraction allowed the estimation of the recovery and matrix effect in terms of ion suppression or enhancement. In detail, the dataset obtained was elaborated to determine the matrix effect (ME), recovery efficiency (RE) of the extraction procedure and overall process efficiency (PE), as follows:

$$ME\ (\%) = B/A \times 100 \qquad \text{Eq. 1}$$

$$RE\ (\%) = C/B \times 100 \qquad \text{Eq. 2}$$

$$PE\ (\%) = C/A \times 100 = (ME \times RE) / 100 \qquad \text{Eq. 3}$$

The tests were done at the concentration level of 0.05 mg/kg (5 × LOQ), considering the two vegetable matrices on which recovery tests were done; three solutions per matrix were prepared by spiking either before or after extraction. The terms "process efficiency" and "recovery efficiency" were introduced by Buhrman et al (29) together with the concept of "ion suppression"; in their work, the authors did not consider the potential ionization enhancement, which is included in the matrix effect defined above. The ME calculated in this manner may be referred to as an absolute matrix effect; although this ME may cause concern, it should be more useful in the validation of pesticide residue methods to determine a "relative" matrix effect referring to ionic suppression or enhancement between different varieties of the agricultural commodity considered. The work in this paper aims to represent, therefore, an analytical approach which could be useful when a method for pesticide residue analysis based on MS or MS–MS detection is developed for or applied to a specific commodity.

Results and Discussion

Methods performance

The LC–MS–MS chromatographic profile of each sample was good and well reproducible, and no interferent peak could be observed in any of the cases. Therefore, the target analytes could be easily detected and quantified in the chromatogram. Five replicate recovery tests were performed at each spiking level and for each crop; the spiking levels were the target LOQ (0.01 mg/kg), 10–20 times the LOQ and a further level likely to be higher than the supposed highest pesticide residue. For BNOA, only two spiking levels were chosen, considering the fact that its residues are supposed to be below LOQ (30). Globally, 15 recovery tests were carried out for phosmet, phosmet oxon, pirimiphos-methyl, and dicloran, while 10 recoveries were done for BNOA, resulting in a dataset of 70 recovery tests, each of them analysed in duplicate. The accuracy (as % mean recovery) with the corresponding precision [as relative standard deviation (RSD)] achieved for each analyte from the recovery tests, are reported in Table II together with other method validation parameters. Both the accuracy and precision were easily within EU acceptability limits given for pesticide residue analysis, the accuracy being in the range of 70% to 110% and RSD well below 20%. In more detail, accuracy at different spiking levels ranged from 73% to 98%, and overall mean recovery (grouping all spiking levels for a single analyte) was between 87% and 96%. As far as concerns precision, RSD at different spiking levels ranged from 3% to 16%, and overall RSD was in the range of 6% to 16%.

Also the LOD and LOQ were satisfactory, in line with the corresponding EU maximum residue limit. Linear correlation coefficients, calculated through a linear regression model without any weighing, using five concentration levels for each analyte, were higher than 0.99 in all cases.

Evaluation of the matrix effects

The matrix effect and the possibility of ionization suppression or enhancement were evaluated by comparing the results gained as described in materials and methods. The first set of data (n = 6 for each compound) corresponded to the analyte peak in the neat solvent, at a concentration of 0.05 mg/kg. For the second dataset, 3 samples of each commodity were extracted and then spiked, after extraction, with the analyte to reach a concentration of 0.05 mg/kg. In the third set, analytes were spiked before extraction into vegetable samples at a level of 0.05 mg/kg; three replicates were done for each commodity. Hence 6 samples were generated for each analyte in each dataset, resulting in 90 solutions prepared in total; all these solutions were analyzed in duplicate by LC–MS–MS according to the method developed for each compound. The results on matrix effects are shown in Table III.

The matrix effect of the LC–MS–MS analysis following QuEChERS extraction occurred in all cases, in terms of ionization suppression, at a level statistically different (ANOVA, alpha 0.05) for each compound. Pirimiphos-methyl analysis was the most affected determination, for which suppression was found to be 23%, while phosmet-oxon analysis had the lower ion suppression (5% only). Recovery efficiency was good, however, and substantially comparable, being in the range of 93–96%. The pirimiphos-methyl matrix effect varied in the two matrices considered: an ionic suppression was observed in wheat (42%, n = 3) while an unexpected enhancement of response was observed in oat (22%, n = 3). Hence, an ionic suppression was reported by grouping all the data even if the response was very different in the two matrices considered.

On these bases, the differences in terms of overall process efficiency (found to be from 74% to 90%) of each compound can be ascribed to different matrix effects occurring as ion suppression, rather than to poor recoveries of the extraction and clean up procedure.

Albeit the ion suppression observed still seems to be acceptable considering that the overall process efficiency is adequate, it is evident that the ME is important when a quantitative method is applied and when it is critical to generate reliable data. However this effect is often overlooked in pesticide analysis, but the more recent EU validation standards (31) are now requiring this assessment at the method validation phase, proving that this effect is going to be taken into greater account. Considering that the matrix effect is quite poorly reproducible over different fruits (6), it could be appropriate to carry out method validation not only at different concentrations, but also using different fruits and vegetables. Due to the influence of crop variety (6), in addition to crop species, it would also seem important to calibrate the LC–MS–MS system using the specific matrix (the same species and variety) on which pesticide residues are to be measured. Unless there is a reliable definition of the matrix effect, the measurement of pesticide residues at a level close to the method limit of quantification could lead to false positive or false negative results. Furthermore, this assessment is very critical when pesticide residues are measured across the maximum residue limits allowed.

Table II. Validation Parameters of the Analytical Methods Considered

Analyte	Spiking level (mg/kg)	Process efficiency / accuracy (% recovery)	Precision (% RSD)	LOD (mg/kg)	LOQ (mg/kg)	Correlation coefficient (R^2)
Phosmet	0.01	90	10	0.003	0.01	0.999
	0.20	97	14			
	2.00	93	11			
	overall	93	13			
Phosmet-oxon	0.01	95	9	0.003	0.01	0.999
	0.20	96	13			
	2.00	92	13			
	overall	94	11			
Dicloran	0.01	98	14	0.003	0.01	0.997
	0.10	92	9			
	1.00	90	11			
	overall	93	12			
Pirimiphos-methyl	0.01	73	16	0.003	0.01	0.995
	0.10	92	11			
	2.00	97	13			
	overall	87	16			
BNOA	0.01	94	8	0.04	0.01	0.996
	0.05	96	3			
	overall	96	6			

Table III. Matrix Effect, Recovery Efficiency, and Overall Process Efficiency (Mean Values for Each Pesticide Considered in Two Representative Crops)

Analyte	Matrix	Mean peak area*			Matrix effect (ME, %)	Recovery efficiency (RE, %)	Process efficiency (PE, %)
		Standard in neat solvent	Matrix spiked post-extraction	Matrix spiked pre-extraction			
Phosmet	Peach and apple	25.80 ± 2.99	23.33 ± 2.94	21.70 ± 3.60	90	93	84
Phosmet oxon	Peach and apple	125.84 ± 13.97	119.86 ± 13.30	112.67 ± 20.05	95	94	90
Dicloran	Melon (pulp) and lettuce	28.76 ± 2.73	24.07 ± 2.60	22.39 ± 3.54	84	93	78
Pirimiphos-methyl	Wheat and oat	40.44 ± 4.17	31.31 ± 4.66	29.93 ± 5.43	77	96	74
BNOA	Tomato and strawberry	1.16 ± 0.10	0.91 ± 0.13	0.87 ± 0.12	78	96	75

* Mean ± standard deviation, in Arbitrary Units × 10^3 (n = 6)

Conclusions

The aim of this work was to investigate the matrix effect of the QuEChERS extraction and clean up followed by LC–MS–MS analysis of some pesticides in crop. The validation datasets proved that the analytical approach adopted was suitable for the determination of the considered pesticide residues in crops.

The difference between the overall process efficiencies observed among the different determinations should be ascribed to different matrix effects rather than to ineffective extraction efficiencies of the QuEChERS procedure. To obtain reliable results during validation, as well as to achieve accurate quantifications of pesticide residues, the potential matrix effect should therefore be studied.

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