Comparison of matrix solid-phase dispersion and modified QuEChERS methods for extraction of pesticide residues from onion

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Received 10th August 2011, Accepted 28th March 2012
DOI: 10.1039/c2ay05491d

Matrix solid-phase dispersion (MSPD) and modified QuEChERS methods were compared for the extraction of pesticide residues from onion followed by determination by liquid chromatography with electrospray ionization tandem mass spectrometry. The efficiency of the methods was statistically compared using recovery and precision data, matrix effects and other extraction characteristics such as the sample mass, solvent volume, extraction time and limits of detection and quantification (LOQ). In general, faster extractions and lower LOQ values (0.0005 to 0.05 mg kg$^{-1}$) were achieved with QuEChERS, whereas greater ruggedness and lower matrix effects were obtained with MSPD. For both methods, high extraction yields were achieved (61.8–120%) with relative standard deviations lower than 20%. Matrix effects were observed for both methods, and were compensated by using matrix-matched calibration.

A. Introduction

Onion (Allium Cepa L.) is one of the most consumed vegetables in the world. It is considered a functional food due to its antioxidant and anticancer properties which are a consequence of the presence of bioactive compounds such as anthocyanins and quercetin.1,2

Onion can be affected by different diseases of fungal, bacterial, viral and nematode origin. This contamination can occur in the field, post-harvest, and during transportation, storage and marketing and, as a consequence, it can cause losses of up to 100% in the production of marketable bulbs.3,4

The high productivity of food is achieved only by the use of pesticides in all stages of the production chain. However, non-compliance with good farming practices can affect food security and result in the occurrence of pesticide residues in onion. In 2008, in Brazil, this culture was included in the Program of Analyses of Pesticide Residues in Food (PARA) where one hundred and three onion samples were analysed, and three were considered unsatisfactory due to the use of acephate, which is a pesticide not authorized for this crop.5

Pesticides residues in food can be extracted using efficient analytical procedures through sample preparation. An efficient sample preparation method depends on the matrix, as well as on the properties and analyte concentration, and it is a fundamental step to ensure the efficiency of analytical procedures, especially for the analysis of trace compounds in foods.7 Accelerated solvent extraction (ASE),8 liquid–liquid extraction (LLE),9 ultrasound-assisted extraction (UAE),10 supercritical fluid extraction (SFE),11,12 matrix solid-phase dispersion (MSPD),13–20 and QuEChERS (from quick, easy, cheap, effective, rugged and safe),21–23 are among the most widely used methods for the extraction of pesticides in food.

The MSPD and QuEChERS methods were developed as an alternative to the more traditional sample preparation methods which employ high volumes of organic solvents and are lengthy and laborious.17,23

MSPD was introduced in 1989 by Barker24 and it has presented acceptable recoveries for a wide range of analytes with the advantages of low cost and moderate consumption of organic solvents.13,23 Since its introduction, MSPD has been cited as an extraction method in over 250 publications. The analytes normally extracted are drugs, pesticides, constituents of natural occurrence in a wide variety of matrices.14–20,26

In 2003, Anastassiades and co-workers27 introduced the QuEChERS method which has been widely used in the determination of pesticides in complex matrices. The method was called QuEChERS because it is quick, easy, cheap, effective, rugged and safe. The method was adopted by the Association of Official Analytical Chemists (AOAC, USA) for the determination of pesticide residues in food in 2001.29 The European...
Committee for Standardization also considers QuEChERS as an official method.  

Liquid chromatography (LC) and gas chromatography (GC) are well established chromatographic techniques used for the determination of pesticide residues in food. These techniques can be coupled to different detectors and high selectivity and sensitivity are obtained by coupling with mass spectrometry. 

This paper compares two sample preparation methods MSPD and QuEChERS for the extraction of dimethoate, methalaxyl-M, tebuconazole, azoxystrobin and difenoconazole in onion samples with determination by liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). A comparison of methods previously developed and validated by Rodrigues and co-workers in 2010 (ref. 34) and 2011 (ref. 35) taking into account the extraction time, sample mass, solvent volume consumption, recovery (RSD%), precision (RSD%), matrix effect (ME) and limit of detection (LOD) and of quantification (LOQ) was carried out.

B. Experimental

Instrumentation

Pesticides separation was performed in a liquid chromatograph Waters Alliance 2695 Separations Module (Waters, Milford, USA) fitted with an autosampler, a membrane degasser and a quaternary pump. Mass spectrometry detection was performed on a Micromass Quattro Micro API (Waters, Milford, USA) with an ESI interface. The LC column was an XTerra 3.5 µm particle size (50 × 3 mm i.d.) (Waters, Milford, MA, USA). The drying gas, as well as the nebulization gas, was nitrogen. The nebulization and desolvation gas flow rate were set at 50 and 350–550 L h⁻¹, respectively. Analytical instrument control, data acquisition and data treatment were performed by the software MassLynx (Micromass, Manchester, UK), version 4.1. Mobile phase components were ultrapure water and acetonitrile, both containing 0.1% (v/v) formic acid, in the proportion 52 : 48 (v/v), with elution in the isocratic mode at a flow rate of 0.4 mL min⁻¹.

For sample preparation the following equipment was used: a food processor model Mega Master Plus R1 3170 500 W (Walita, São Paulo, Brazil); a vortex model Certomat® MV-B Braun (Bioteck Internacional, Alemar, Brazil) and a micro-processed centrifuge model Quimis® Q222T (QUIMIS, Brazil).

Reagents and pesticide standards

Analytical standards of dimethoate (purity ≥ 99.4%), methalaxyl-M (purity ≥ 99.0%), tebuconazole (purity ≥ 99.6%), azoxystrobin (purity ≥ 97%) and difenoconazole (purity ≥ 99%) were purchased from Sigma-Aldrich (São Paulo, Brazil). Formic acid (purity 98%), analytical grade was supplied by Merck (Darmstadt, Germany). Acetonitrile, chromatographic grade, anhydrous magnesium sulfate and sodium chloride were purchased from J.T. Baker (Mallinckrodt, Phillisburg, NJ, USA). Water was purified with a Direct-Q UV3® (resistivity 18.2 MΩ cm) water purification system (Millipore, Bedford, MA, USA). Bondesil-PSA (40 µm) was supplied by Varian (Palo Alto, CA, USA).

Preparation of analytical standards

Pesticide stock solutions containing 100 and 1000 mg L⁻¹ of the target compounds were prepared in methanol and stored at −18 °C. These solutions were used for preparing the pesticide working solutions, which were used for sample spiking and for preparation of calibration curves. Working standard solutions were prepared monthly, while the dilutions were prepared daily.

Samples

All experiments for the method validation were carried out using onion samples with no detectable pesticide residues (blank). A representative portion of onion (250 g) was purchased from a local market, and chopped and homogenized in a food chopper. All parameters were optimized to a 1.0 mg kg⁻¹ spike level, which corresponds to the intermediate concentration of the method linear range. Three replicates of the extracts were analyzed and were injected three times each.

MSPD procedure

0.5 g sample was fortified with a working standard solution containing the five pesticides. After one hour (the required time to provide the interaction between the analytes and matrix), 1.0 g of C18 was added to the fortified sample and the sample and the sorbent were gently blended for 5 min to obtain an homogeneous mixture. The new phase formed was transferred to an MSPD column (SPE empty tube) and eluted with 10 mL of acetonitrile. The eluate was collected in a graduated conical tube (15 mL capacity) and 10 µL was injected into the LC-ESI-MS/MS according to the procedure described by Rodrigues and co-workers.

QuEChERS procedure

An aliquot of 10.0 g of processed sample was weighed into a polypropylene tube (50 mL capacity) followed by the fortification step at 1.0 mg kg⁻¹. Then, 10 mL of acetonitrile was added followed by manual agitation for 15 s and mechanical agitation for 1 min in a vortex mixer. This was followed by the addition of 4.0 g of anhydrous magnesium sulfate and then the agitation stage was repeated. Finally, the tube was centrifuged at 5000 rpm for 3 min. An aliquot of extract was removed and injected into the chromatographic system, according to the procedure described by Rodrigues and co-workers.

Method validation

Method validation targeted the following parameters: analytical curve, linearity, LOD, LOQ, accuracy (recovery) and precision (repeatability and intermediate precision) as recommended by SANCO, the National Institute of Metrology, Standardization and Industrial Quality (INMETRO) and the Brazilian National Agency of Sanitary Vigilance (ANVISA).

Evaluation of matrix effect using MSPD and modified QuEChERS

The study of matrix effect (ME) was performed according to Matuszewski et al. The peak area of the standard solution...
diluted with the blank matrix extract was compared with the peak area of the standard solution diluted in solvent. The results of ME equal to 100% indicate that there is no matrix effect, while values higher or lower than 100% indicate suppression or enrichment of ionization by the matrix components.

**Statistical calculations**

All statistical calculations, including one-way analysis of variance (ANOVA), were performed using GraphPad InStat (GraphPad InStat Software Inc, Version 3.00, 1997) software. A significance level of 95% was adopted for all comparisons.

**C. Results and discussion**

**MSPD and modified QuEChERS: sample extraction characteristics**

Fig. 1 compares the recovery ($R\%$) and relative standard deviation (RSD%) for the compounds under study using MSPD and modified QuEChERS at a 1.0 mg kg$^{-1}$ spike level. Pesticides quantification was performed with matrix-matched calibration for both methods. $R\%$ values of between 85.5 and 107.1% and RSD% < 9.8% were obtained for all the compounds using both methods. The QuEChERS method shows higher recoveries for metalaxil-M and tebuconazole ($t$ test, $P < 0.05$). For dimethoate, azoxyystrobin and difenoconazole, no significant difference ($t$ test, $P > 0.05$) was found between MSPD and QuEChERS methods.

Table 1 shows the parameters for the extraction of pesticides by MSPD and QuEChERS. The QuEChERS method uses a larger sample amount, about 20 times more than MSPD. Moreover, the extraction process is 3 times faster for the QuEChERS. The average values of $R\%$ and RSD% for the methods are suitable for the determination of compounds in onion samples.

The MSPD technique presented high ruggedness during the optimization process, showing small variations for $R\%$ for most of the compounds when significant changes (e.g. C18 amount, different interaction times after fortification, different elution solvents and different sorbents) in the extraction procedure were performed.

**Table 1** Characteristics of MSPD and modified QuEChERS methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MSPD</th>
<th>QuEChERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample mass/g</td>
<td>0.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Solvent volume/mL</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Extraction time/min</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>78.3–120.0</td>
<td>61.8–120.0</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.2–17.1</td>
<td>3.3–20.0</td>
</tr>
<tr>
<td>LOQ/mg kg$^{-1}$</td>
<td>0.01–0.1</td>
<td>0.0005–0.05</td>
</tr>
<tr>
<td>LOD/mg kg$^{-1}$</td>
<td>0.003–0.03</td>
<td>0.00015–0.015</td>
</tr>
</tbody>
</table>

The modified QuEChERS method presented small variations in the accuracy values without the use of a clean-up step and NaCl addition, according to the procedure described by Rodrigo and co-workers.35

**Matrix effect**

From Fig. 2, it can be seen that when the QuEChERS method is used, the matrix effect is more pronounced than when the MSPD method is used. This is due to the larger sample amount employed with QuEChERS. For the QuEChERS method, the relation is 1.0 g of sample to 1.0 mL of solvent, whereas for the MSPD method, the relation is 0.5 g of sample to 10.0 mL of solvent.

A modified QuEChERS method was established without a clean-up step, using matrix matched calibration to compensate for the matrix interference.35 Both methods generate strong ionization suppression for dimethoate. This was also observed by Lehotay and co-workers40 for dimethoate in a lime matrix. In addition, the suppression was approximately 2 times higher for the modified QuEChERS method. For methalaxyl-M, the matrix effect by MSPD is hardly observed. On the other hand, with the modified QuEChERS method, the matrix effect is approximately 50%. The higher matrix effect for these compounds seems to be related to their polarity. Dimethoate (log $P = 0.704$) and methalaxyl (log $P = 1.71$) eluted in the first 2 minutes of the chromatographic run, the region where, normally, most of the interferents are eluting together and affecting the ionization of the analytes. For the pesticides azoxyystrobin, difenoconazole and tebuconazole, the matrix effect found was considered low (<20%) using both methods, and for difenoconazole no
significant difference (t-test, \( P > 0.05 \)) was found when MSPD and QuEChERS methods were used.

Method validation

Analytical curve and linearity. Linearity parameters obtained with matrix-matched calibration from 0.005 to 1.0 mg L\(^{-1}\) using MSPD or QuEChERS are shown in Table 2.

Through the data obtained by the analytical curves it is possible to conclude that the linear regression model is suitable for the analytical determinations under study. The correlation coefficients \((r)\) were higher than 0.99 and are consistent with the validation guidelines.\(^{36-38}\)

Limits of detection (LOD) and limits of quantification (LOQ) of the methods. The LOD and LOQ, for the studied pesticides obtained according to the procedure described by Rodrigues and co-workers\(^{34,35}\) for the methods using MSPD and modified QuEChERS are presented in Table 3.

The LOQs reached by both methods were lower than the maximum residue limits (MRLs) established by Brazilian legislation (ANVISA) for the compounds methalaxyl-M, tebuconazole, azoxystrobin and difenoconazole. Lower LOQs were obtained with the QuEChERS method than with the MSPD method because the QuEChERS method uses a larger sample mass (sample : acetonitrile ratio, 1 : 1) than the MSPD method (sample : acetonitrile ratio, 1 : 20).

Accuracy and precision. The literature for the validation of chromatographic methods indicates that the intervals of acceptable recoveries for the determination of residues should be between 70 and 120%, with a precision lower than 20%.\(^{36-38}\)

Method accuracy was evaluated by recovery tests and precision was evaluated by repeatability (RSD\(_r\)) and intermediate precision (RSD\(_{pi}\)).\(^{34,35}\) When employing the MSPD technique the levels of fortification were 0.01, 0.1 and 1.0 mg kg\(^{-1}\) for tebuconazole, azoxystrobin and difenoconazole, respectively, and 0.1 and 1.0 mg kg\(^{-1}\) for dimethoate and metalaxyl-M, respectively. For the QuEChERS method the levels of fortification were 0.0005, 0.005, 0.05 and 1.0 mg kg\(^{-1}\). Recovery values (\(R\%\)) ranged from 78.3 to 120.0% and from 61.8 to 120.0% for MSPD and modified QuEChERS methods, respectively. The RSD\(_r\) values for RSD\(_r\) and RSD\(_{pi}\) studies ranged from 1.2 to 17.1% and 1.0 to 20.0% using the MSPD method and from 3.3 to 20.0% and 2.0 to 20.0% using the modified QuEChERS method, respectively. The individual results of accuracy and precision for both methods were published in the studies developed by Rodrigues and co-workers.\(^{34,35}\)

\[ y = 7951.9x - 3.59037 \quad r = 0.997 \]
\[ y = 7951.9x + 3.59037 \quad r = 0.997 \]

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>LOD</th>
<th>LOQ</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethoate</td>
<td>0.033</td>
<td>0.1</td>
<td>0.016</td>
<td>0.05</td>
</tr>
<tr>
<td>Methalaxyl-M</td>
<td>0.033</td>
<td>0.1</td>
<td>0.0016</td>
<td>0.005</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>0.0033</td>
<td>0.01</td>
<td>0.00016</td>
<td>0.0005</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>0.0033</td>
<td>0.01</td>
<td>0.00016</td>
<td>0.0005</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>0.0033</td>
<td>0.01</td>
<td>0.00016</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Statistically significant differences (one-way ANOVA, \( P < 0.05 \)) for RSD\(_r\) were found at all levels for two pesticides (dimethoate and metalaxil-M) using the MSPD method and for one pesticide (dimethoate) using the QuEChERS method, although the accuracy and precision of the methods are within the acceptable ranges for the chromatographic method validation. The RSD\(_{pi}\) for azoxystrobin and dimethoate using MSPD and QuEChERS methods, shows no significant difference (one-way ANOVA, \( P > 0.05 \)) for all levels.

D. Conclusions

The results show that both methods, MSPD and modified QuEChERS, are efficient, fast, precise and accurate for the determination of dimethoate, metalaxyl-M, tebuconazole, azoxystrobin and difenoconazole in onion by LC-ESI-MS/MS. In general, the QuEChERS method provided lower LOQ values and is faster, whereas MSPD requires less mass of sample and was shown to be more environmentally friendly.

MSPD and QuEChERS can be considered powerful extraction methods for the extraction of pesticides from onion samples with good repeatability and low quantification limits; lower than the requirements of Brazilian and European Union legislation. This fact is extremely important because since 2008 Brazil assumed global leadership of pesticide consumption, according to datum from the National Pesticide Industry Association (SINDAG).\(^{41}\)

Both methods do not require a clean-up step for the purification of onion extracts, however, compared with MSPD, QuEChERS presents advantages related to analysis time and presented lower LOQs than MSPD.

The matrix components using both methods affected the ionization of some analytes by LC-ESI-MS/MS, highlighting the importance of studying the matrix effect for different extraction procedures for pesticide determination in food. In this study, external calibration through matrix-matched calibration was used to compensate for the influence of matrix effects in the quantification of compounds such as dimethoate, metalaxyl-M and azoxystrobin.

Table 2 Analytical curves parameters prepared in onion extract obtained with MSPD and with modified QuEChERS followed by LC-ESI-MS/MS analysis

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>MSPD</th>
<th>QuEChERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethoate</td>
<td>( y = 7951.9x - 3.59037 )</td>
<td>( y = 7951.9x + 3.59037 )</td>
</tr>
<tr>
<td>Methalaxyl-M</td>
<td>( y = 67411.2x + 88.9374 )</td>
<td>( y = 43794.5x - 9.50831 )</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>( y = 83143x + 43.0249 )</td>
<td>( y = 159082x + 409.68 )</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>( y = 194101x + 84.8929 )</td>
<td>( y = 124042x + 19.2466 )</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>( y = 62332.2x + 48.9934 )</td>
<td>( y = 124487x + 183.681 )</td>
</tr>
</tbody>
</table>
Acknowledgements

We thank CAPES, FINEP and CNPq for the financial support.

Notes and references

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38 Agência Nacional de Vigilância Sanitária (ANVISA), Guía para Validação de Métodos Analíticos e Bioanalíticos, RE no. 889, Brazil, 2003.