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Analytical Methods

Multifamily determination of pesticide residues in soya-based nutraceutical products by GC/MS–MS

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ABSTRACT

An analytical method based on a modified QuEChERS extraction coupled with gas chromatography–tandem mass spectrometry (GC–MS/MS) was evaluated for the determination of 177 pesticides in soya-based nutraceutical products. The QuEChERS method was optimised and different extraction solvents and clean-up approaches were tested, obtaining the most efficient conditions with a mixture of sorbents (PSA, C18, GBC and Zr-Sep$^+$). Recoveries were evaluated at 10, 50 and 100 μg/kg and ranged between 70% and 120%. Precision was expressed as relative standard deviation (RSD), and it was evaluated for more than 160 pesticides as intra and inter-day precision, with values always below 20% and 25%, respectively. Limits of detection (LODs) ranged from 0.1 to 10 μg/kg, whereas limits of quantification (LOQs) from 0.5 to 20 μg/kg. The applicability of the method was proved by analysing soya-based nutraceuticals. Two pesticides were found in these samples, malathion and pyriproxyfen, at 11.1 and 1.5 μg/kg respectively.

1. Introduction

Soya bean (*Glycine max*) is one of the most important agricultural crops (Pizzutti et al., 2007). The advantages and the importance of this legume and related products have been very well known (Pizzutti, de Kok, Hiemstra, Wickert, & Prestes, 2009). Soya is one of the main protein sources and nowadays, they represent a significant part of vegetarian diets and they are also used for baby food formulas (Schollenberger et al., 2007). Therefore, the demand for soya and food supplements from soya is strongly increasing (Krenn & Pötsch, 2006).

Nowadays large amounts of medicine and food dual-purpose herbs are used throughout the world (Du et al., 2012). Nutraceutical products fall under the wide definition of functional foods i.e., those foods and food components that are believed to improve overall health and well-being, reduce the risk of specific diseases, or minimise the effects of other health concerns (Kapsak, Rahavi, Childs, & White, 2011). The great popularity of these products has resulted in elevated scrutiny from consumers, health professionals, and regulators about the quality and levels of active ingredients in these products (Sullivan & Crowley, 2006).

In general, various kinds of microbial contaminants could be found in medicinal plants and herbal materials (Łozowicka et al., 2014). However, pesticides are frequently applied during herbs growing process in agriculture for demolishing or controlling any pest. Therefore, nutraceutical products are liable to contain pesticide residues, which can be accumulated from agricultural practices and storage periods (Du et al., 2012).

In 2003, due to public and industry concerns, the U.S. Food and Drug Administration (FDA) proposed requiring dietary supplement manufactures to adhere to current Good Manufacturing Practices (cGMP) standards (U.S. Food and Drug Administration). The final rule was issued in full effect in June 2010 (Chen, Al-Taher, et al., 2012). Because many dietary supplements are largely derived from botanical sources, they must be tested for pesticide contaminants to satisfy such cGMP regulations (Chen, Al-Taher, et al., 2012).

Nutraceutical products and related raw materials typically represent very complex matrices for pesticide residue analysis, with a wide range of biochemical composition, water and fat content. Therefore, they exhibit different polarities, solubility and pK_a values (Dai, Ren, He, & Huo, 2011). Most of these products are dried and concentrated, which creates a greater challenge during the

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development of analytical methods and sensitive instrumentation is required for detecting trace levels of pesticides (Chen, Al-Taher, et al., 2012). The use of gas and liquid chromatography coupled to tandem mass spectrometry (GC–MS/MS and LC–MS/MS) is very helpful (Mastovska & Wylie, 2012).

Some methods have been developed for screening pesticides in nutraceutical products, medicinal plants and herbals. Most of them are based on GC coupled with several detectors, such as flame photometric detector (FPD) (Wan, Mao, Yan, Shen, & Wu, 2010; Wong et al., 2007), electron capture detection (ECD) (Quian et al., 2010; Rao, Meena, & Galib, 2011; Xu et al., 2011) or mass spectrometry (MS) (Chen, Al-Taher, et al., 2012; Dai et al., 2011; Du, Song, & Wang, 2011; Ganzera, Aberham, & Stuppner, 2006; Ho, Tsoi, & Leung, 2013; Mao, Wana, Yan, Shen, & Wei, 2012; Nguyen, Lee, Lee, & Lee, 2010; Sadowska-Rociek, Surma, & Cieślik, 2013; Tagami et al., 2009; Tusa, Moldovan, & Vlassa, 2009; Wang, Xiao, & Cheng, 2011; Wong et al., 2007; Łozowicka et al., 2014). However, these procedures have been improved with the use of GC–MS/MS (Hayward et al., 2013; Mastovska & Wylie, 2012) and LC–MS/MS (Chen, Al-Taher, et al., 2012, Chen, Song, et al., 2012; Ganzera et al., 2006).

In order to determine pesticide residues, a proper sample preparation technique is required to isolate and concentrate the target compounds. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method has been applied for the multiresidue pesticide analysis of herbs (Chen, Al-Taher, et al., 2012; Dai et al., 2011; Mastovska & Wylie, 2012; Chen, Song, et al., 2012; Du et al., 2011; Nguyen et al., 2010; Sadowska-Rociek et al., 2013; Wong et al., 2007; Xu et al., 2011). Nowadays, new sorbent materials, Zr-Sep and Zr-Sep$^+$ have been used for complex matrices. Zr-Sep is a mixture of C18 and silica coated with zirconium-dioxide and Zr-Sep$^+$ is silica coated with zirconium-dioxide and octadecylsiloxan carrier groups, and they have been utilised for samples with higher lipid content (Lozano et al., 2014; Rajski, Lozano, Uclés, Ferrer, & Fernández-Alba, 2013).

Only a few analytical methods for the determination of pesticide residues in soya products have been described in the recent bibliography (Pizzutti et al., 2007, 2009). In these studies, pesticide residues have been analysed in the raw material but not in the nutraceutical products, which are more complex matrices. It means that the sample preparation is a crucial step in pesticide residue analysis in complex nutraceutical products.

To the best of our knowledge, no analytical method has been developed to the simultaneous determination of multiclass pesticide residues in soya-based nutraceuticals. The main objective of this work has been the development of an analytical method based on QuEChERS extraction procedure for qualitative and quantitative analysis of more than 170 pesticides by GC–MS/MS.

2. Materials and methods

2.1. Chemicals and reagents

Pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel-de-Haën (Seelze-Hannover, Germany) with purity >99%. Stock standard solution of each pesticide (with concentration ranging from 200 to 300 mg/L) was prepared by weighing of the pesticides and dissolving with 50 mL of methanol, acetone or acetonitrile. A working standard solution, containing a mixture of all the studied pesticides at 1 mg/L, was prepared by dilution in acetone and stored at 4 °C. Internal standard (I.S.) isotopically labelled parathion ethyl-d10 (20 mg/L) was prepared in acetone.

Anhydrous magnesium sulphate was purchased from Panreac (Barcelona, Spain). Sodium acetate (NaOAc) was obtained from J.T. Baker (Deventer, The Netherlands). Octadecyl silica (C18) was obtained from Agilent Technologies (Avondale, PA, USA). Zirconia-coated silica (Zr-Sep$^+$) was obtained from Supelco (Bellefonte, PA, USA). Primary secondary amine (PSA), graphitised black carbon (GBC) and Florisil cartridges (500 mg, 3 mL) were obtained from Scharlab (Barcelona, Spain). Ethyl acetate was received from Sigma–Aldrich (Madrid, Spain). Acetonitrile and methanol were also purchased from Scharlab. Acetone was obtained from Carlo Erba (Milan, Italy). Every solvent used was pesticide residue grade.

2.2. Instrument and apparatus

High-volume centrifuge equipped with a bucket rotor (4 × 250 mL) from Orto Alresa, Mod. Consul (Madrid, Spain) was used for the centrifugation.

Chromatographic analyses were carried out in a Scion GC system (Bruker Corporation, Freemont, CA, USA) equipped with an autosampler from the same company. Capillary column GC 30 m x 0.25 mm i.d. × 0.25 μm film thickness VF-5MS (Varian) was utilised for GC separation. Helium was used as carrier gas with a constant flow rate of 1 mL/min. The glass liner was fitted with a carbofrit plug, from Restek (Bellefonte, PA, USA). A fused silica untreated capillary column (2 m × 0.25 mm) from Supelco (Bellefonte, Pennsylvania, USA) was used as a pre-column.

A Scion QqQ-MS/MS (Bruker) was used for mass spectrometric detection operating in electron ionisation mode (EI, 70 eV).

2.3. Samples

Pesticide free soya nutraceutical products obtained from a local bio store (Almería, Spain) were used as blank matrix to prepare matrix-matched standard solutions for the calibration and fortified samples for the recovery studies. During the analysis of samples, 11 soya-based nutraceuticals were obtained from local supermarkets (Almería, Spain). Several capsules were stored at 5 °C until the moment of analysis.

2.4. Sample preparation

The soya nutraceutical capsules were chopped with a blender. Sample preparation was based on a modified QuEChERS procedure (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003).

Two grams of homogenised sample were weighed into a 50 mL centrifuge tube. Eight mL of water were added to the sample and shaken 30 s by vortex, and the sample was left to hydrate for 15 min. Then, 10 mL of ethyl acetate (A) or acetonitrile (B) were added to the mixture and shaken by vortex during 1 min. After that, 4 g of $MgSO_4$ and 1 g of anhydrous NaOAc were added and the mixture was shaken vigorously by hand for 1 min. Subsequently, the mixture was centrifuged at 3700 rpm for 10 min.

Different sorbents were tested for the clean-up process, including PSA, GBC, C18, Zr-Sep$^+$, Florisil and the mixture of these sorbents. The final extract obtained after the cleaning step was diluted with ethyl acetate (1:1, v/v) prior GC analysis.

2.4.1. Cleanup with PSA, GBC, Zr-Sep$^+$ and C18 (individually)

1.5 mL of the organic phase was transferred to an Eppendorf vial which contains 100 mg of PSA, GBC, Zr-Sep$^+$ or C18. The vial was shaken 1 min by vortex and subsequently centrifuged at 3700 rpm for 10 min. For solvent A, 975 μL of the organic phase was transferred to a vial and 25 μL of the IS solution were added prior GC–QqQ-MS/MS analysis. For solvent B, 1 mL of extract was transferred to a glass tube and heated to dryness under a nitrogen stream. 975 μL of ethyl acetate were added and transferred to a vial with 25 μL of the solution of the IS for GC–QqQ-MS/MS analysis.

2.4.2. Cleanup with Florisil

First, 2 mL of the organic phase were slowly transferred through a Florisil cartridge. Then, 975 μL of the extract with solvent A were transferred to a vial and 25 μL of the IS solution were added for GC–QqQ-MS/MS analysis, while for solvent B, 1 mL of extract was collected and heated to dryness under a nitrogen stream. Then, 975 μL of ethyl acetate were used for the quantitative transfer of the residue obtained after evaporation to a vial with 25 μL of the solution of the IS for GC analysis.

2.4.3. Cleanup with a mixture of sorbents (PSA + GBC + C18 + Zr-Sep$^+$)

This process was only tested with ethyl acetate as organic solvent. For that, 1.5 mL of the organic phase were transferred to an Eppendorf vial, which contains 50 mg of each sorbent (PSA, GBC, Zr-Sep$^+$ and C18). The vial was shaken 1 min by vortex and subsequently centrifuged at 3700 rpm for 10 min. Then, 975 μL of the organic phase were transferred to a vial and 25 μL of the solution of the IS were added for GC–QqQ-MS/MS analysis.

2.5. GC–QqQ-MS/MS analysis

Aliquots of 3 μL of the final extract were injected into the chromatographic system at a syringe injection flow rate of 5 μL/s. The injector temperature program started at 70 °C (hold for 5 min), and then it was increased with a rate of 200 °C/min until 300 °C (hold for 20 min). The injector split ratio was initially set at 20:1. Splitless mode was switched on at 0.5 min until 3.5 min. At the beginning of the injection, the column temperature was set at 70 °C (hold for 3.5 min), and the temperature was increased until 180 °C at a 25 °C/min rate, and then until 325 °C (hold 5 min) at a rate of 15 °C/min. A cryogenic cooling with CO_2 was used when the injector temperature was at 250 °C in order to reach the initial injector temperature as fast as possible for the next injection. The total run time was 23 min.

The QqQ mass spectrometer was operated in the selected reaction monitoring (SRM) mode. The temperatures of the transfer line, manifold, and ionisation source were set at 300, 40, and 280 °C, respectively. The analysis was performed with a filament-multiplier delay of 4.5 min in order to prevent instrument damage. The electron multiplier voltage was set at 1600 V (+200 V offset above the value obtained in the auto-tuning process). Mass peak widths set in the first and third quadrupole were of 1.5 and 2.0 *m/z*, respectively.

2.6. Validation process

Linearity was evaluated using matrix-matched standard solutions at eight concentration levels (0, 1, 2, 5, 10, 25, 50 and 100 μg/kg). Linear least square regression analysis was applied using relative peak area as analytical signal. Zero point has been included into calibration curve to make sure that the blank samples are pesticide free. The IS was added to the matrix matched standards at 500 μg/L.

For the recovery studies, samples were spiked at three different concentrations (10, 50 and 100 μg/kg), and five replicates were used for each level. Blank soya-based nutraceuticals were fortified with pesticides before the extraction. Spiked samples were left to stand for 30 min prior to their extraction. Relative peak areas of pesticides after modified QuEChERS method were compared with relative peak areas of matrix-matched standards.

Precision was studied as intra and inter-day precision, expressed as relative standard deviation (RSD). For intra-day precision, spiked samples at 10, 50 and 100 μg/kg were analysed (five replicates). Inter-day precision was studied at the same concentration levels by processing spiked samples in five different days.

Finally, limits of detection (LODs) and quantification (LOQs) were calculated by injecting six fortified samples at lower concentration levels, being 0.1, 0.5, 1, 2, 5 and 10 μg/kg. The limits were determined for the quantification transition by the signal-to-noise ratio (S/N) criteria, and limits were established as the lowest concentration of the analyte yielding a S/N of 3 (LODs) or 10 (LOQs).

3. Results and discussion

In this study, 177 pesticides were investigated using the modified QuEChERS procedure. Table 1 summarises the studied pesticides with retention time, precursor ions, product ions and the ion ratio. Because several families of pesticides, with different physical and chemical properties were studied, the development of a simple multiresidual analytical method for the determination of pesticide residues in complex soya-based nutraceutical matrix was a challenge, and special attention has been paid to the extraction and clean-up procedure, in order to minimise matrix effect.

3.1. Extraction and clean up procedure

For the optimisation of QuEChERS method, fortified samples at 100 μg/kg were used. Bearing in mind the complexity of the matrix, the nature of the sorbent used during the clean-up step was also evaluated.

Acetonitrile and ethyl acetate have usually been used in multiresidual analytical methods as extraction solvents. To test their extraction capability for soya-based nutraceuticals, the effect of the extraction solvent was evaluated. Acetonitrile possesses many advantages in extraction; however, it is seldom used in GC analysis for its large solvent expansion volume during GC vaporisation, high toxicity and low volatility (Li et al., 2008). Therefore, acetonitrile extracts were evaporated under a soft nitrogen stream and reconstituted with ethyl acetate for GC–QqQ-MS/MS analysis, as it has been described in Section 2.4.

Fig. 1 shows the obtained results for the solvent selection experiments with different sorbents (Florisil, GBC, PSA and Zr-Sep$^+$) used during the clean-up procedure. It can be seen that 78–92% of the analysed pesticides were satisfactorily recovered (recovery ratios between 70% and 120%) using ethyl acetate as extraction solvent and the four sorbents checked at this stage. On the other hand, when acetonitrile was used for the extraction of the target compounds, only 3–28% of the pesticides were satisfactorily recovered with the same cleaning sorbents used previously. It was possibly caused by analyte loss during the evaporation of acetonitrile and reconstitution of the evaporation residues prior to the GC–QqQ-MS/MS analysis, as well as coextraction of interferent compounds. Therefore, ethyl acetate was used for further experiments.

Soya-based nutraceuticals are very complicated matrices, including fatty acids, pigments and saccharides. As it was observed previously, the cleanup could be a critical step of the analytical method for the determination of pesticide residues in these complex matrices. Various sorbents, such as C18, PSA, GBC, Florisil and Zr-Sep$^+$ were tested, bearing in mind that each sorbent can be used for specific purposes. Thus, PSA can effectively remove saccharides, polar organic acids and lipids, while Florisil is applied for isolation of polar and low-fat compounds. For the adsorption of pigments, GBC is widely used. On the other hand, Zr-Sep$^+$ and C18 are applied to remove lipid components (Du et al., 2012). Fig. 2 shows the results when 100 mg of each sorbent (C18, Florisil, GBC, PSA, Zr-Sep$^+$) or a mixture of PSA, GBC, Zr-Sep$^+$ and C18 (50 mg/each) were used. It can be observed that the worst results (only 34% of compounds were suitably extracted) were obtained when C18 was used. However, with Florisil, GBC, PSA or Zr-Sep$^+$,

Table 1
Retention time windows (RTWs) and MS/MS parameters of the selected pesticides.

Compound	RTW (min)	Precursor ion (*m/z*)	Product ions (collision energy, eV)[a]	Ion ratio (%)
2,4,6-Trichlorophenol	8.66 ± 0.04	196	97 (30); **132 (15)**	20.0
2,4-DDD	14.29 ± 0.02	235	**165 (25)**; 199 (15)	20.0
2,4-DDT	14.73 ± 0.02	235	**165 (25)**; 199 (15)	47.2
4,4-DDD	14.75 ± 0.02	235	**165 (25)**; 199 (15)	47.1
4,4-DDE	13.72 ± 0.02	318	176 (50); **246 (20)**	61.8
4,4-DDT	14.70 ± 0.02	235	**165 (25)**; 199 (15)	46.8
4,4′-Dichlorobenzophenone	12.72 ± 0.04	250	**139 (15)**; 215 (10)	88.7
Acephate	9.66 ± 0.01	136	42 (5); **94 (10)**	49.9
Aclonifen	14.38 ± 0.04	264	182 (25); **194 (15)**	65.3
Acrinathrin	15.86 ± 0.02	181	127 (30)	71.7
		289	**93 (10)**	
Alachlor	11.95 ± 0.02	269	160 (20); **188 (10)**	39.9
Aldrin	12.58 ± 0.01	263	**193 (35)**; 228 (20)	71.8
Alpha-HCH	11.26 ± 0.09	219	109 (35); **183 (10)**	44.3
Azinphos Ethyl	16.25 ± 0.04	160	105 (10); **132 (5)**	68.8
Azinphos Methyl	15.91 ± 0.05	160	**105 (10)**; 132 (5)	64.8
Azoxystrobin	18.29 ± 0.04	344	**156 (40)**; 172 (45)	85.8
Benalaxyl	14.54 ± 0.02	266	**148 (15)**	36.8
		325	148 (25)	
Benfluralin	10.37 ± 0.01	292	160 (25); **264 (10)**	45.2
Beta-HCH	12.11 ± 0.01	219	109 (35); **183 (10)**	43.5
Bifenyl	8.80 ± 0.03	154	128 (25); **153 (10)**	42.0
Bifenox	15.54 ± 0.04	341	**189 (20)**; 281 (15)	65.1
Bifenthrin	15.16 ± 0.01	181	115 (50); **165 (25)**	28.2
Boscalid	17.19 ± 0.05	204	169 (15)	34.8
		342	**140 (15)**	
Bromacil	12.52 ± 0.08	205	162 (15); **188 (15)**	52.4
Bromophos ethyl	13.22 ± 0.02	359	**303 (12)**; 331 (10)	69.4
Bromophos methyl	12.74 ± 0.02	331	286 (30); **316 (20)**	76.8
Bromopropylate	15.32 ± 0.02	341	157 (40); **183 (20)**	63.5
Buprofezin	13.78 ± 0.02	249	106 (25); **193 (10)**	63.1
Bupirimate	14.26 ± 0.01	273	150 (10); **193 (10)**	68.7
Butralin	12.61 ± 0.02	266	74 (20); **190 (15)**	73.1
Cadusafos	11.19 ± 0.12	213	73 (10); **89 (15)**	63.6
Captan	13.20 ± 0.03	117	82 (30)	62.7
		149	**70 (20)**	
Carbophenothion	14.57 ± 0.02	157	**45 (10)**	54.1
		342	157 (15)	
Chlordane	13.52 ± 0.02	373	**266 (22)**; 301 (10)	62.3
Chlorbenside	13.36 ± 0.04	268	89 (40); **125 (15)**	29.5
Chlorfenapyr	13.88 ± 0.02	247	200 (30); **227 (15)**	49.8
Chlorfenson	13.65 ± 0.04	175	75 (30); **111 (10)**	44.8
Chlorfenvinphos	13.00 ± 0.02	267	**159 (15)**	66.4
		323	267 (15)	
Chlormefos	9.07 ± 0.03	234	**121 (15)**; 154 (5)	48.4
Chloropropylate	14.10 ± 0.02	251	111 (30); **139 (10)**	20.0
Chlorothalonil	11.53 ± 0.06	266	**168 (28)**; 231 (20)	63.5
Chlorpyrifos ethyl	12.42 ± 0.02	314	**258 (15)**; 286 (10)	73.6
Chlorpyrifos methyl	11.87 ± 0.02	286	136 (25); **241 (30)**	44.5
Chlorthal-dimethyl	12.51 ± 0.02	301	**223 (25)**; 273 (15)	41.2
Chlozolinate	12.92 ± 0.02	331	186 (15); **259 (10)**	49.6
Clodinafop propargyl	14.64 ± 0.03	349	238 (15); **266 (10)**	20.0
Cyanofenphos	14.61 ± 0.03	185	**157 (10)**	76.1
		157	110 (15)	
Cycloate	10.32 ± 0.02	154	72 (10); **83 (5)**	20.0
Cyfluthrin	16.88 ± 0.02	163	**127 (10)**	65.0
		226	206 (20)	
Cynidon ethyl	18.99 ± 0.05	358	302 (30); **330 (10)**	20.0
Cypermethrin	16.88 ± 0.02	163	**127 (10)**	48.3
		181	127 (30)	
Cyproconazole	14.08 ± 0.03	222	**125 (20)**; 153 (10)	37.6
		185	**157 (10)**	
Delta-HCH	12.11 ± 0.01	219	109 (35); **183 (10)**	42.3
Deltamethrin	18.12 ± 0.03	172	**93 (10)**	44.3
		253	93 (20)	
Diazinon	11.16 ± 0.01	304	137 (35); **179 (15)**	36.1
Dichlorvos	8.03 ± 0.03	185	**93 (15)**; 109 (20)	20.0
Dichlobenil	8.59 ± 0.05	171	100 (25); **136 (15)**	68.1
Dichlofenthion	11.75 ± 0.02	279	**222 (15)**; 251 (5)	41.6
Dicloran	11.06 ± 0.06	206	148 (20); **176 (10)**	52.1
Dicofol o,p	14.10 ± 0.02	251	111 (35); **139 (20)**	35.7
Dicofol p,p	15.50 ± 0.03	251	111 (35); **139 (20)**	37.9
Dieldrin	13.88 ± 0.02	263	**193 (35)**; 228 (20)	66.4
Difenoconazole	18.04 ± 0.04	323	202 (35); **265 (15)**	47.3

(*continued on next page*)

Table 1 (*continued*)

Compound	RTW (min)	Precursor ion (*m/z*)	Product ions (collision energy, eV)[a]	Ion ratio (%)
Diflufenican	14.84 ± 0.03	394	238 (40); **266 (15)**	28.3
Dimethomorph	18.48 ± 0.06	301	**165 (15)**	58.9
		387	301 (15)	
Endosulfan alpha	13.54 ± 0.02	195	**125 (25)**	70.3
		241	170 (25)	
Endosulfan beta	14.31 ± 0.03	195	**125 (25)**	20.0
		241	170 (25)	
Endosulfan sulphate	14.78 ± 0.03	270	**235 (18)**	60.5
		387	289 (10)	
Endrin	14.16 ± 0.02	263	**193 (35)**; 228 (20)	70.1
EPTC	9.01 ± 0.01	189	**128 (5)**; 86 (10)	20.0
Ethion	14.18 ± 0.02	231	**175 (15)**; 203 (10)	53.8
Ethoprophos	10.62 ± 0.02	158	**97 (18)**; 114 (10)	62.9
Etridiazole	9.16 ± 0.03	211	108 (40); **140 (25)**	42.3
Etrimfos	11.39 ± 0.01	292	152 (20); **181 (10)**	78.4
Famoxadone	18.99 ± 0.05	330	196 (25); **224 (10)**	20.0
Fenamiphos	13.58 ± 0.05	303	**154 (20)**; 180 (20)	37.5
Fenamiphos sulphone	15.34 ± 0.08	292	213 (10)	20.4
		320	**292 (10)**	
Fenamiphos sulphoxide	15.49 ± 0.08	304	122 (20); **196 (10)**	59.4
Fenarimol	16.18 ± 0.04	330	111 (40); **139 (10)**	43.2
Fenitrothion	12.27 ± 0.03	260	109 (15); **125 (15)**	74.6
Fenoxicarb	15.35 ± 0.05	255	157 (25); **186 (10)**	37.3
Fenpropathrin	15.34 ± 0.02	265	181 (30); **210 (10)**	41.7
Fenthion	12.50 ± 0.02	278	125 (40); **245 (10)**	66.6
Fentoate	13.03 ± 0.02	274	121 (10); **125 (18)**	71.3
Fenvalerate + Esfenvalerate	17.73 ± 0.03	225	**119 (20)**; 147 (10)	73.9
Fipronil	13.70 ± 0.04	367	**213 (30)**; 255 (22)	50.1
Fipronil sulphone	14.50 ± 0.12	351	228 (25); **255 (20)**	68.7
		420	255 (35); 351 (15)	
Flucythrinate	17.73 ± 0.03	225	**119 (20)**; 147 (10)	77.2
Fludioxonil	14.36 ± 0.04	248	**127 (30)**; 154 (20)	56.4
Folpet	13.29 ± 0.03	260	**103 (10)**; 104 (10)	20.0
Fonofos	11.27 ± 0.02	246	**109 (18)**; 137 (10)	75.1
Formothion	12.41 ± 0.02	224	**125 (20)**; 155 (10)	20.0
Fosalone	15.78 ± 0.04	367	111 (30); **182 (10)**	40.1
Furalaxyl	13.08 ± 0.02	242	**95 (15)**	20.0
		301	225 (10)	
Furathiocarb	15.52 ± 0.02	194	**161 (10)**; 179 (10)	57.6
Heptachlor	12.11 ± 0.01	237	143 (25)	78.8
		272	**237 (15)**	
Heptachlor epoxide cis	13.11 ± 0.02	289	219 (28); **253 (10)**	61.1
Heptachlor epoxide trans	13.06 ± 0.02	353	**263 (15)**; 282 (15)	65.0
Heptenophos	10.62 ± 0.02	215	**89 (15)**; 200 (10)	58.6
Hexachlorobenzene	10.87 ± 0.02	284	214 (30); **249 (20)**	63.6
Hexaconazole	13.65 ± 0.02	214	124 (30); **159 (25)**	44.7
Hexazynone	14.68 ± 0.01	171	**71 (15)**; 85 (15)	20.0
Iprodione	15.80 ± 0.04	314	**245 (12)**; 271 (10)	69.9
Isocarbophos	12.76 ± 0.01	230	155 (25); **198 (10)**	76.4
Isodrin	12.58 ± 0.01	263	**193 (35)**; 228 (20)	66.8
Isofenphos	12.92 ± 0.01	213	**121 (15)**; 185 (5)	45.0
Isofenphos methyl	12.76 ± 0.01	241	**121 (20)**; 199 (10)	68.6
Kresoxim methyl	13.72 ± 0.02	206	116 (10); **132 (10)**	62.0
Lambda cyhalothrin	15.82 ± 0.02	181	127 (30)	31.0
		197	**161 (10)**	
Lindane	11.44 ± 0.19	219	109 (35); **183 (10)**	46.0
Malathion	12.29 ± 0.01	173	**99 (15)**; 127 (5)	46.5
Metalaxyl	12.05 ± 0.02	249	146 (20); **190 (10)**	56.0
Metamidophos	7.44 ± 0.07	141	64 (20); **95 (10)**	33.9
Methidation	13.31 ± 0.03	145	58 (15); **85 (10)**	41.2
Methoxychlor	15.37 ± 0.02	227	**169 (30)**; 184 (20)	63.7
Mevinphos	9.05 ± 0.06	192	**127 (10)**; 164 (5)	53.5
Mirex	16.14 ± 0.02	272	**237 (15)**	25.0
		332	262 (35)	
Myclobutanil	13.83 ± 0.03	179	**125 (15)**; 129 (20)	33.4
Norflurazon	15.26 ± 0.01	303	**145 (20)**; 173 (10)	39.6
Nuarimol	14.93 ± 0.03	235	111 (32); **139 (15)**	66.4
OPP	9.67 ± 0.02	170	115 (35); **141 (22)**	58.5
Oxadiazon	13.65 ± 0.04	175	**112 (15)**	20.0
		258	112 (25)	
Oxadixyl	14.34 ± 0.04	163	117 (25); **132 (10)**	58.3
Oxyfluorfen	13.71 ± 0.02	300	**223 (20)**	79.2
		361	300 (15)	
Paraoxon methyl	12.13 ± 0.02	230	**106 (20)**; 136 (10)	61.3
Parathion ethyl	12.54 ± 0.02	291	91 (22); **109 (15)**	39.0

Table 1 (*continued*)

Compound	RTW (min)	Precursor ion (*m/z*)	Product ions (collision energy, eV)[a]	Ion ratio (%)
Parathion methyl	11.98 ± 0.03	263	79 (28); **109 (15)**	45.4
Penconazole	12.99 ± 0.03	248	**157 (25)**; 192 (15)	50.1
Pendimethalin	12.85 ± 0.02	252	**161 (15)**; 191 (10)	77.1
Pentachloroaniline	11.82 ± 0.03	265	**194 (25)**; 230 (15)	54.5
Pentachloroanisol	11.17 ± 0.03	280	**237 (25)**; 265 (10)	49.0
Permethrin	16.49 ± 0.02	163	**127 (10)**	68.1
		183	128 (25)	
Phosmet	15.91 ± 0.05	160	**77 (25)**; 133 (12)	20.0
Phosmet oxon	15.40 ± 0.05	160	**77 (25)**; 133 (12)	20.0
Phtalimide	9.51 ± 0.08	147	**103 (10)**; 104 (10)	52.6
Pirimiphos ethyl	12.61 ± 0.01	333	163 (10); **168 (25)**	65.6
Pirimiphos methyl	12.14 ± 0.01	290	**125 (25)**; 151 (20)	77.3
Procymidone	13.14 ± 0.03	283	67 (30); **96 (10)**	32.6
Profluralin	11.28 ± 0.01	318	**199 (15)**; 284 (15)	37.1
Propachlor	10.17 ± 0.02	176	**57 (10)**; 92 (10)	26.6
Prochloraz	16.69 ± 0.05	180	69 (20); **138 (15)**	75.8
		308	70 (15)	
Propanil	11.78 ± 0.04	161	**99 (25)**; 126 (20)	69.0
Propargite	14.83 ± 0.02	173	**135 (15)**	39.0
		350	173 (15); 201 (10)	
Propoxur	10.15 ± 0.03	152	92 (25); **109 (8)**	31.6
Propham	9.07 ± 0.03	179	**93 (15)**; 120 (10)	73.9
Prophenophos	13.68 ± 0.02	339	251 (30); **269 (15)**	46.2
Propiconazole	14.72 ± 0.03	259	**173 (18)**; 191 (10)	69.0
Prothiofos	13.59 ± 0.02	309	221 (30); **239 (15)**	72.8
Pyrazophos	16.03 ± 0.03	265	138 (30); **210 (10)**	69.5
Pyridaben	16.59 ± 0.03	309	132 (35); **147 (15)**	40.0
Pyridafenthion	15.21 ± 0.03	340	**199 (10)**; 203 (25)	38.5
Pyrifenox	13.30 ± 0.10	262	192 (18); **200 (18)**	69.6
Pyrimethanil	11.37 ± 0.03	198	**118 (35)**; 156 (25)	41.4
Pyriproxyfen	15.80 ± 0.03	136	41 (10); **96 (12)**	60.3
Quinalphos	13.07 ± 0.02	298	**156 (12)**; 190 (10)	56.1
Quinomethione	13.52 ± 0.05	234	148 (25); **206 (10)**	59.6
Quintozene	11.17 ± 0.02	295	**237 (18)**; 265 (10)	74.0
S-421	12.18 ± 0.02	132	95 (20); **97 (20)**	86.7
Silafluorfen	17.26 ± 0.02	286	207 (10); **258 (15)**	35.5
Sulfotep	10.45 ± 0.01	322	**146 (28)**; 266 (10)	43.2
Sulprophos	14.64 ± 0.01	322	**156 (10)**; 139 (15)	35.1
Tau fluvalinate	17.65 ± 0.03	250	**55 (12)**; 200 (20)	43.0
Tebuconazole	14.96 ± 0.04	250	**125 (20)**; 153 (10)	43.5
Tecnazene	10.10 ± 0.03	261	180 (15); **203 (15)**	49.8
Tefluthrin	11.27 ± 0.01	177	87 (30); **127 (15)**	42.1
Terbutryn	12.24 ± 0.02	241	170 (15); **185 (10)**	50.3
Tetrachlorvinphos	13.35 ± 0.03	329	**109 (20)**	31.6
		331	316 (20)	
Tetraconazole	12.57 ± 0.02	336	156 (30); **218 (18)**	66.1
Tetradifon	15.73 ± 0.04	229	166 (20); **201 (15)**	43.8
Tetrahidroftalamid	9.56 ± 0.05	151	**79 (15)**; 80 (10)	62.0
Tolclophos methyl	11.97 ± 0.02	265	220 (25); **250 (15)**	62.6
Transfluthrin	12.31 ± 0.08	163	**121 (10)**; 117 (10)	20.0
Trichloronate	13.68 ± 0.02	297	223 (22); **269 (15)**	36.1
Trifluralin	10.33 ± 0.01	306	159 (25); **264 (10)**	45.7
Vinclozolin	11.90 ± 0.02	212	145 (25); **172 (15)**	72.6

[a] Quantifier ion in bold.

the number of efficiently extracted pesticides increased significantly to percentages that ranged between 78% and 92%.

Despite of great efforts performed during the cleanup step in order to obtain a selective extraction, even with the enhancing of the detection selectivity obtained by MS/MS, pesticide residues analysis is still complicated by the co-injected matrix constituents responsible for a severe matrix effect. Therefore, matrix effect was studied and the influence of the sorbents previously evaluated was investigated, reducing the level of co-extracted matrix components in the final extract. Matrix effect may be noticed as an increase or decrease in response of the detector signal compared with the response produced by solvent solutions of the analytes (a positive or negative matrix effect). This effect could negatively influence the quantitative results. Application of matrix-matched standards to compensate the matrix effects was used for the investigation of the difference between responses in matrix-matched standards and standards in solvent. The matrix factors (MF) were calculated for each studied pesticide by comparing analyte response in matrix-matched solution vs. the pesticide response obtained in pure solvent, according to the following equation:

$$\mathrm{MF} = \left(\frac{\text{response in matrix-matched solution}}{\text{response in solvent solution}} - 1\right) \times 100\% \qquad (1)$$

Exceeding the MF higher than 20% or smaller than −20% indicates a peak signal enhancing or suppressing due to the matrix effects.

Fig. 3 shows the percentage of pesticides affected by matrix effect according to MF value. The peak areas of matrix-matched standard and solvent-matched standard were compared at 20 μg/kg. Most of the pesticides exhibited signal enhancement effects because the MF values obtained were higher than 20%. The most

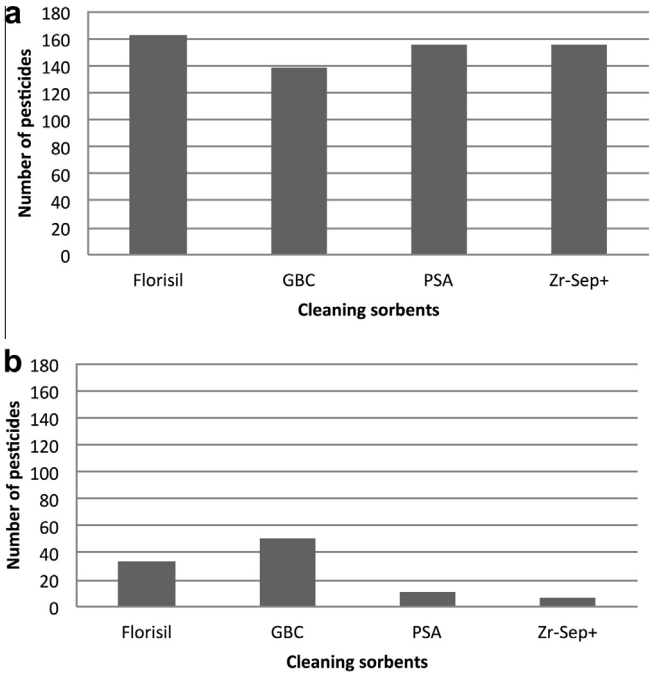

Fig. 1. Number of recovered pesticides using (a) ethyl acetate and (b) acetonitrile as extraction solvents in combination with different cleanup sorbents.

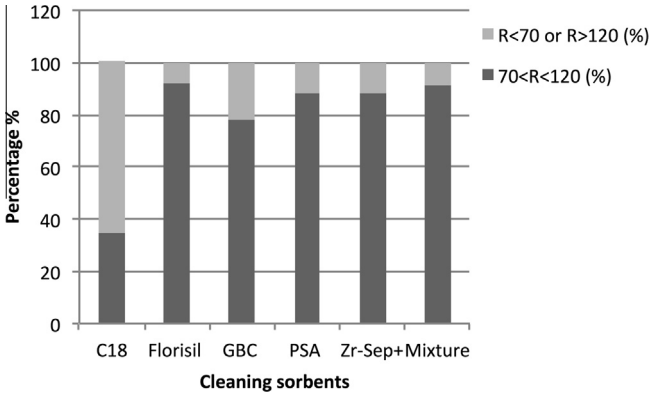

Fig. 2. Percentage of efficiently recovered pesticides from 177 pesticides using different cleanup sorbents: C18 (100 mg), GBC (100 mg), PSA (100 mg), Zr-Sep$^+$ (100 mg), mixture (C18 50 mg, GBC 50 mg, PSA 50 mg, Zr-Sep$^+$ 50 mg).

favourable MF values were noticed employing Florisil and the mixture of sorbents. Thus, 28% of the pesticide residues cleaned by these sorbents did not present matrix effect (MF values were between −20% and 20%). When GBC was used, 95% of pesticide residues evidenced matrix effect. For the rest of the sorbents, 21–28% of pesticides were extracted without matrix effect. It can be seen that most of the MFs are out of the suitable range, indicating significant differences between the matrix-matched standards and the solvent-matched standards. Therefore, matrix-matched standard solutions were used for the validation experiments to counter the matrix effect.

Moreover, the most acceptable results were obtained by Florisil and by the use of the mixture of PSA, C18, GBC and Zr-Sep$^+$. The recoveries and the matrix effects were similar for these sorbents. However, it is important to inform that the extracts obtained with the use of such a mixture of sorbents were practically colourless and the chromatographic responses were significantly more consistent (more repetitive responses), reducing the maintenance of the GC–QqQ-MS/MS equipment and improving the robustness of

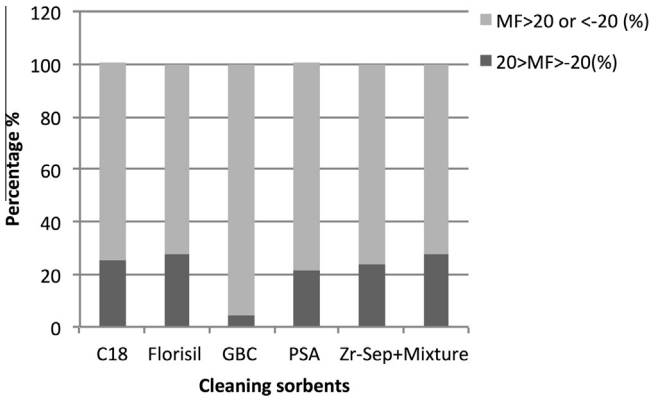

Fig. 3. Evaluation of matrix effect for different types of sorbents.

the proposed method. Therefore, the mixture of sorbents was used for further studies. However, and despite the use of the mixture of sorbents, it was observed that maintenance operations (liner and pre-column replacement) should be done in the GC system after the analysis of approximately 150 samples. Bearing in mind that the protection of the GC–QqQ-MS/MS and the analytical column is very important, the final extracts were diluted with ethyl acetate (1:1) in order to protect the system. With the combination of these two actions (clean-up and final dilution of the extract), the identification and confirmation by MS/MS has increased its reliability at very low concentrations and the quantitative results have not changed significantly in terms of recovery. Additionally, the LODs and LOQs were low enough for determining most of the studied pesticides at the concentration of 10 μg/kg.

3.2. Method validation

The validation of the method was established in terms of precision, linearity, trueness (expressed as recovery), LOD and LOQ. Precision was formulated as relative standard deviation (RSD), and it was evaluated as inter and intra-day precision. Pesticides with suitable recoveries (70–120%) were included into the validation process.

Linearity was evaluated using matrix-matched standard solutions. Blank samples were extracted and spiked with adequate volume of the pesticide working solution after the extraction. Linearity was studied at 8 concentration levels, between 0 and 100 μg/kg, and applying linear least squares regression analysis of the relative peak area (analyte/IS) vs. analyte concentration. The obtained determination coefficient (R^2) were higher than 0.98 for most of the pesticides. For two pesticides, endosulfan alpha and cypermethrin, the calibration curves were linear in the range 5–100 μg/kg, for chlorthalonil the calibration curve was linear from 10 to 100 μg/kg. For three pesticides, deltamethrin, formothion and folpet the linearity was acceptable in concentration range 20–100 μg/kg and from 25 to 100 μg/kg for heptenophos. Acephate showed low linearity in such a range (with $R^2 < 0.60$), but acceptable in the concentration range between 10 and 100 μg/kg ($R^2 = 0.98$).

Trueness was evaluated in terms of recovery, showing the results in Table 2. Recoveries for majority of pesticides were between 71% and 107% at 10 μg/kg. Some of the pesticides (2,4-DDT, 4,4-DDD, 4,4-DDE, 4,4-DDT, beta-HCH, bupirimate, chlorothalonil, dichlorvos, dichlobenil, dieldrin, endosulfan sulphate, heptachlor, hexaconazole, prophenophos, propiconazole, prothiophos) had recoveries higher than 60%, but lower than 70%. Three of the pesticides, chlorfenapyr, endosulfan alpha and pyrimiphos ethyl, had recoveries higher than 120%. At 50 μg/kg, recoveries always ranged from 77% to 120% and at 100 μg/kg between 71% and

Table 2
Validation results of the developed method.

	Recovery (%)			Interday precision (%)[a]			LOD (μg/kg)	LOQ (μg/kg)
	10 μg/kg	50 μg/kg	100 μg/kg	10 μg/kg	50 μg/kg	100 μg/kg		
2,4,6-Trichlorophenol	109 (17)[b]	97 (15)	106 (14)	21	19	17	0.1	0.5
2,4-DDD	73 (4)	93 (5)	103 (5)	10	8	9	0.5	1
2,4-DDT	66 (6)	81 (11)	97 (4)	16	14	14	0.1	0.5
4,4-DDD	65 (7)	85 (8)	96 (4)	16	14	9	0.1	0.5
4,4-DDE	68 (3)	99 (5)	95 (5)	12	8	11	0.5	1
4,4-DDT	64 (8)	91 (5)	98 (4)	17	6	12	0.1	0.5
4,4′-Dichlorobenzophenone	88 (5)	99 (5)	97 (3)	10	14	12	0.5	1
Acephate	104 (20)	120 (12)	89 (11)	5	11	12	0.1	0.5
Aclonifen	90 (20)	92 (6)	112 (7)	19	12	8	1	2
Acrinathrin	94 (3)	109 (5)	108 (5)	9	7	11	1	2
Alachlor	89 (14)	113 (6)	106 (6)	12	19	10	1	2
Aldrin	76 (9)	90 (5)	110 (2)	16	6	5	0.5	1
Alpha-HCH	83 (7)	97 (4)	96 (10)	18	7	6	0.1	0.5
Azinphos Ethyl	106 (11)	102 (9)	93 (7)	14	5	13	1	2
Azinphos Methyl	85 (8)	106 (5)	99 (2)	12	17	13	1	2
Azoxystrobin	107 (10)	96 (4)	94 (3)	20	17	18	1	2
Benalaxyl	94 (3)	99 (2)	112 (3)	7	8	7	0.5	1
Benfluralin	92 (3)	100 (3)	103 (2)	12	6	14	0.1	0.5
Beta-HCH	68 (5)	100 (5)	95 (3)	11	9	7	0.5	1
Bifenyl	82 (4)	97 (2)	94 (3)	21	19	21	0.1	0.5
Bifenox	83 (4)	96 (6)	109 (5)	14	15	15	1	2
Bifenthrin	71 (4)	91 (3)	103 (5)	20	7	6	0.1	0.5
Boscalid	85 (4)	93 (2)	93 (1)	7	5	9	0.5	1
Bromophos ethyl	78 (7)	95 (8)	107 (3)	15	8	13	0.5	1
Bromophos methyl	86 (1)	103 (4)	102 (6)	12	11	11	0.5	1
Bromopropylate	53 (10)	82 (4)	107 (4)	18	11	10	0.5	1
Buprofezin	92 (11)	98 (6)	105 (3)	16	8	11	1	2
Bupirimate	65 (8)	109 (1)	96 (4)	12	8	7	2	5
Butralin	98 (8)	105 (7)	98 (5)	14	14	12	1	2
Cadusafos	92 (10)	102 (2)	104 (3)	18	10	7	0.5	1
Carbophenothion	94 (3)	98 (2)	101 (1)	6	8	7	1	2
Chlorbenside	86 (7)	95 (2)	104 (1)	10	5	10	0.1	0.5
Chlordane	99 (14)	95 (6)	101 (4)	25	5	10	1	2
Chlorfenapyr	125 (14)	105 (5)	111 (3)	20	5	11	1	2
Chlorfenson	88 (5)	95 (2)	117 (3)	5	4	13	0.5	1
Chlorfenvinphos	72 (9)	98 (2)	102 (3)	24	4	11	0.5	1
Chlormefos	93 (4)	101 (6)	105 (2)	25	7	11	0.5	1
Chloropropylate	83 (5)	97 (1)	100 (2)	8	6	8	0.5	1
Chlorothalonil	65 (15)	86 (10)	94 (9)	19	24	5	5	10
Chlorpyrifos ethyl	84 (2)	94 (5)	112 (3)	20	7	9	0.1	0.5
Chlorpyrifos methyl	98 (3)	100 (5)	117 (3)	18	8	6	1	2
Chlorthal-dimethyl	75 (10)	99 (6)	117 (5)	13	3	9	0.5	1
Chlozolinate	86 (14)	86 (10)	99 (4)	8	8	7	1	2
Clodinafop propargyl	96 (3)	103 (5)	120 (7)	10	6	11	0.5	1
Cyanofenphos	98 (3)	100 (2)	82 (5)	7	6	13	1	2
Cycloate	99 (15)	97 (9)	108 (5)	9	6	14	0.1	0.5
Cyfluthrin	74 (2)	97 (3)	101 (1)	12	11	11	1	2
Cynidon ethyl	90 (5)	103 (4)	102 (2)	5	6	11	0.1	0.5
Cypermethrin	79 (2)	94 (5)	98 (4)	10	7	15	1	2
Cyproconazole	78 (5)	79 (4)	98 (1)	11	7	10	0.5	1
Delta-HCH	84 (8)	103 (5)	99 (3)	12	7	5	0.1	0.5
Deltamethrin	n.d.[c]	100 (2)	98 (4)	n.d.	7	12	10	20
Diazinon	83 (7)	101 (4)	104 (1)	11	8	4	0.5	1
Dichlorvos	62 (12)	82 (10)	88 (5)	20	18	15	0.1	0.5
Dichlobenil	69 (7)	91 (7)	98 (5)	21	16	6	0.1	0.5
Dichlofenthion	87 (7)	100 (2)	112 (4)	13	18	5	0.5	1
Dicloran	106 (5)	98 (1)	103 (4)	8	14	12	0.5	1
o,p′-Dicofol	73 (1)	91 (3)	112 (4)	9	16	20	0.5	1
Dieldrin	68 (5)	95 (5)	91 (2)	20	10	19	1	2
Difenoconazole	85 (8)	98 (3)	105 (2)	21	16	13	1	2
Diflufenican	78 (4)	90 (4)	107 (4)	20	11	11	0.5	1
Dimethomorph	93 (4)	83 (10)	95 (1)	20	20	20	0.1	0.5
Endosulfan alpha	122 (12)	118 (15)	97 (2)	16	11	13	1	2
Endosulfan beta	103 (19)	91 (6)	89 (4)	35	12	21	1	2
Endosulfan sulphate	68 (9)	90 (5)	114 (3)	22	11	6	1	2
Endrin	85 (16)	101 (3)	98 (3)	24	5	18	1	2
Ethion	85 (15)	90 (2)	100 (3)	7	10	8	0.1	0.5
Ethoprophos	95 (2)	99 (1)	116 (5)	7	11	10	0.5	1
Etridiazole	96 (3)	107 (6)	111 (4)	6	10	8	0.1	0.5
Etrimfos	89 (4)	101 (4)	118 (2)	15	7	12	0.5	1
Famoxadone	102 (5)	99 (10)	83 (3)	6	19	20	0.1	0.5
Fenamiphos	77 (16)	82 (9)	94 (8)	24	7	6	1	2
Fenamiphos sulphone	105 (5)	100 (4)	93 (5)	11	5	10	1	2

(*continued on next page*)

Table 2 (*continued*)

	Recovery (%)			Interday precision (%)[a]			LOD (μg/kg)	LOQ (μg/kg)
	10 μg/kg	50 μg/kg	100 μg/kg	10 μg/kg	50 μg/kg	100 μg/kg		
Fenarimol	88 (2)	94 (4)	114 (3)	5	8	13	1	2
Fenitrothion	89 (9)	104 (6)	120 (5)	19	12	7	0.5	1
Fenoxicarb	101 (2)	92 (8)	99 (8)	10	18	10	0.1	0.5
Fenpropathrin	97 (4)	97 (4)	105 (5)	12	6	8	0.1	0.5
Fenthion	79 (14)	91 (7)	114 (8)	16	20	13	1	2
Fenthoate	71 (11)	84 (12)	106 (4)	23	13	20	1	2
Fenvalerate + Esfenvalerate	75 (8)	95 (3)	103 (3)	21	4	11	0.1	0.5
Fipronil	75 (20)	117 (3)	87 (13)	25	25	23	1	2
Flucythrinate	87 (6)	94 (5)	89 (6)	8	9	9	1	2
Fludioxonil	92 (20)	82 (16)	104 (9)	18	17	14	0.5	1
Folpet	n.d.	94 (13)	84 (20)	n.d.	25	23	10	20
Fonophos	83 (8)	98 (3)	115 (4)	10	3	7	0.5	1
Formothion	n.d.	87 (6)	88 (3)	n.d.	10	5	10	20
Fosalone	91 (8)	94 (4)	104 (1)	10	9	12	1	2
Furalaxyl	75 (5)	96 (2)	99 (2)	9	4	14	0.5	1
Furathiocarb	92 (12)	97 (7)	101 (3)	29	7	10	0.1	0.5
Heptachlor	69 (12)	92 (4)	105 (4)	24	13	11	0.5	1
Heptachlor epoxide cis	79 (14)	95 (6)	106 (5)	14	10	8	1	2
Heptachlor epoxide trans	89 (7)	100 (8)	120 (8)	34	12	6	1	2
Heptenophos	n.d.	n.d.	114 (2)	n.d.	n.d.	7	10	25
Hexachlorobenzene	84 (10)	96 (2)	114 (3)	10	12	6	0.5	1
Hexaconazole	62 (11)	86 (7)	80 (5)	16	9	24	0.5	1
Isocarbophos	85 (14)	99 (6)	109 (5)	10	9	8	0.5	1
Isodrin	82 (11)	82 (2)	110 (5)	21	14	12	1	2
Isofenphos	96 (1)	95 (3)	115(3)	4	8	5	0.1	0.5
Isofenphos methyl	84 (9)	93 (5)	98 (4)	25	6	9	1	2
Kresoxim methyl	86 (9)	96 (4)	114 (5)	15	4	6	1	2
Lambda Cyhalothrin	92 (7)	101 (5)	85 (2)	25	9	12	1	2
Lindane	88 (4)	92 (2)	101 (8)	25	5	3	0.5	1
Malathion	99 (5)	99 (3)	91 (8)	6	2	17	0.5	1
Metalaxyl	91 (6)	95 (7)	105 (3)	10	6	3	1	2
Metamidophos	92 (6)	84 (4)	104 (4)	5	10	5	0.1	0.5
Methidation	112 (9)	98 (16)	118 (5)	25	5	6	0.1	0.5
Metoxychlor	87 (3)	74 (4)	90 (3)	18	13	12	1	2
Mevinphos	71 (5)	88 (5)	103 (3)	10	8	4	1	2
Mirex	74 (5)	94 (7)	109 (7)	11	5	10	0.1	0.5
Myclobutanil	84 (8)	98 (2)	103 (6)	8	6	5	0.1	0.5
Nuarimol	80 (10)	93 (2)	99 (3)	11	10	10	0.5	1
OPP	83 (11)	77 (17)	78 (8)	7	9	9	0.5	1
Oxadiazon	88 (8)	87 (4)	98 (2)	12	19	11	1	2
Oxadixyl	99 (3)	98 (1)	103 (3)	9	9	10	1	2
Oxyfluorfen	89 (20)	103 (9)	109 (4)	11	12	22	1	2
Paraoxon methyl	89 (8)	111 (17)	83 (4)	13	10	22	1	2
Parathion ethyl	94 (4)	98 (1)	113 (4)	8	6	5	0.5	1
Parathion methyl	107 (10)	101 (2)	101 (1)	7	4	9	1	2
Penconazole	87 (7)	89 (4)	100 (3)	12	7	12	0.1	0.5
Pendimethalin	85 (3)	97 (3)	116 (3)	25	10	9	0.5	1
Pentachloroaniline	87 (8)	97 (3)	110 (2)	8	1	7	1	2
Permethrin	79 (4)	96 (2)	98 (2)	10	4	10	0.5	1
Phosmet	104 (9)	103(3)	104 (8)	10	15	19	1	2
Phosmet Oxon	96 (8)	92 (1)	88 (3)	9	6	8	1	2
Phtalimide	92 (3)	97 (6)	94 (3)	5	3	15	0.1	0.5
Prochloraz	95 (5)	98 (4)	102 (4)	9	8	8	0.5	1
Procimidone	88 (6)	100 (4)	102 (4)	14	12	9	0.1	0.5
Propachlor	98 (2)	97 (3)	98 (2)	5	6	7	0.5	1
Propanil	71 (20)	94 (5)	107 (14)	23	22	9	1	2
Propargite	44 (14)	99 (7)	99 (3)	25	8	8	0.1	0.5
Prophoxur	107 (5)	101 (5)	96 (6)	4	8	5	1	2
Prophan	104 (11)	111 (9)	93 (7)	18	6	13	0.1	0.5
Prophenophos	68 (8)	81 (4)	98 (4)	21	12	5	1	2
Propiconazole	67 (6)	94 (4)	99 (5)	17	2	10	0.1	0.5
Prothiofos	69 (4)	94 (2)	108 (7)	17	6	10	0.5	1
Pyrazophos	94 (6)	105 (6)	116 (4)	6	5	9	1	2
Pyridaben	82 (7)	99 (4)	101 (6)	5	10	12	1	2
Pyridafenthion	92 (5)	101 (5)	108 (3)	6	5	16	1	2
Pyrifenox	95 (4)	89 (7)	100 (5)	16	9	12	1	2
Pyrimethanil	74 (3)	94 (4)	100 (7)	11	7	6	1	2
Pyrimiphos Ethyl	125 (18)	104 (9)	120 (3)	25	23	16	1	2
Pyrimiphos Methyl	76 (12)	98 (4)	109 (7)	16	11	15	0.5	1
Pyriproxyfen	90 (4)	99 (1)	102 (5)	18	5	15	0.5	1
Quinalphos	96 (5)	94 (7)	101 (8)	20	7	23	1	2
Quintozene	86 (8)	99 (5)	102 (7)	22	6	13	0.5	1
S-421	77 (9)	97 (2)	97 (3)	10	8	12	0.1	0.5
Silafluorfen	95 (3)	98 (3)	104 (2)	10	5	5	1	2

Table 2 (*continued*)

	Recovery (%)			Interday precision (%)[a]			LOD (μg/kg)	LOQ (μg/kg)
	10 μg/kg	50 μg/kg	100 μg/kg	10 μg/kg	50 μg/kg	100 μg/kg		
Sulfotep	89 (10)	90 (6)	101 (6)	16	8	15	0.5	1
Tau Fluvalinate	99 (3)	99 (2)	101 (2)	5	4	11	1	2
Tebuconazole	76 (1)	92 (2)	100 (2)	20	5	10	1	2
Tecnazene	90 (5)	95 (6)	104 (3)	13	6	12	0.5	1
Tefluthrin	82 (2)	98 (4)	116 (5)	9	7	6	0.1	0.5
Terbutrine	102 (5)	99 (4)	95 (3)	13	9	16	1	2
Tetrachlorvinphos	73 (6)	87 (4)	96 (1)	14	16	13	1	2
Tetraconazole	75 (15)	97 (7)	107 (5)	23	5	7	0.5	1
Tetradifon	70 (20)	94 (6)	97 (3)	22	4	10	1	2
Terahydroftalamid	98 (7)	102 (5)	74 (1)	7	4	15	1	2
Tolclophos Methyl	100 (4)	96 (5)	98 (3)	5	5	9	1	2
Transfluthrin	99 (11)	120 (9)	71 (8)	11	19	20	0.1	0.5
Trichloronate	83 (15)	82 (4)	99 (2)	19	18	10	0.1	0.5
Trifluralin	85 (6)	98 (3)	96 (4)	7	8	17	0.5	1
Vinclozolin	81 (4)	103 (4)	109 (3)	12	10	12	0.5	1

[a] Number of replicates: 5.
[b] Relative standard deviation values are given in brackets ($n = 5$).
[c] n.d.: not detected.

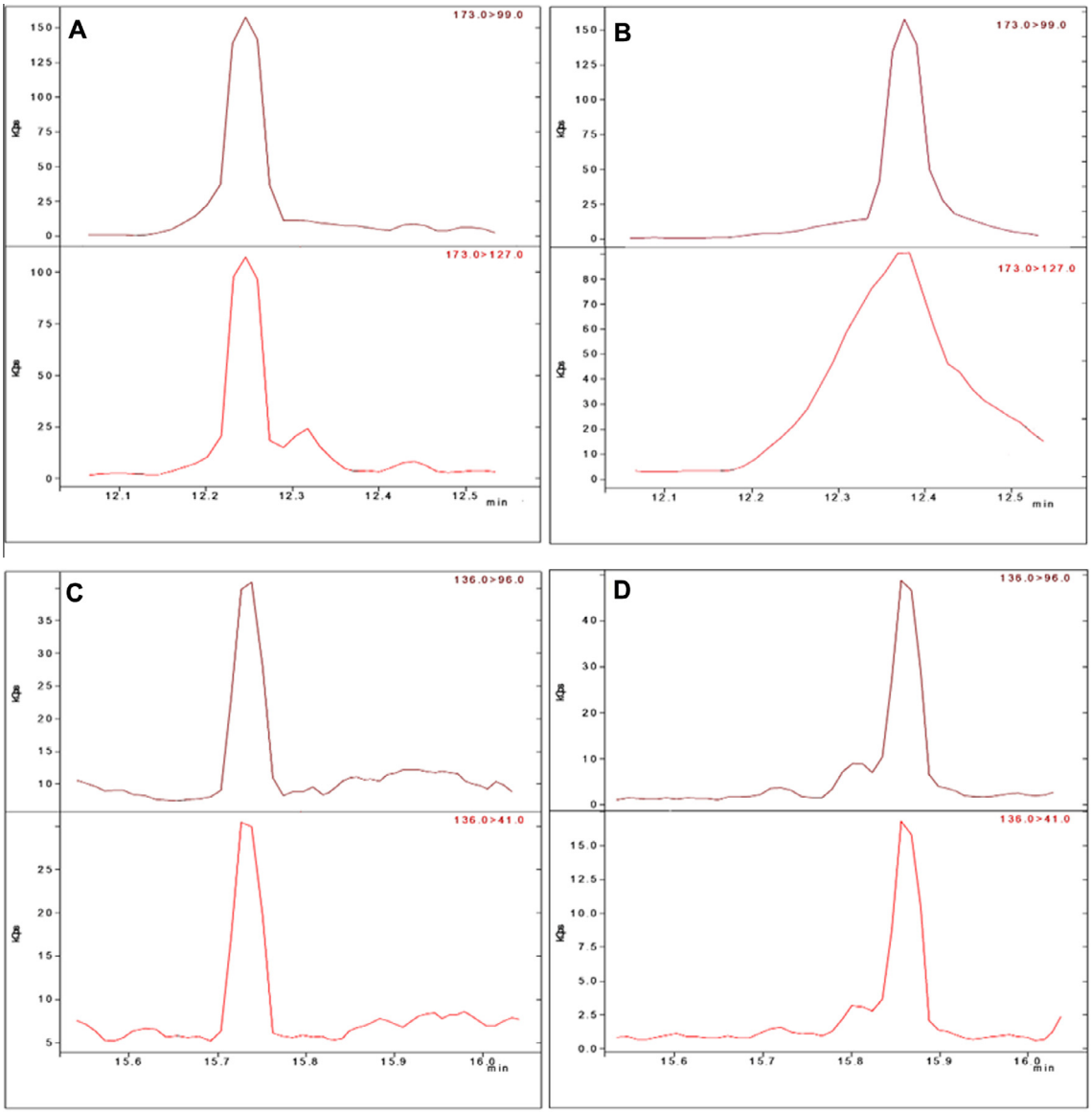

Fig. 4. GC–MS/MS chromatogram of malathion and pyriproxyfen calibration point 10 and 1 μg/kg (A and C) and in real sample (B and D).

120%. RSD values were always lower than 20%, fulfilling the established requirements for pesticide residue analysis.

Precision was studied in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). The obtained values were expressed as RSD (see Table 2). Repeatability values ranged between 1% and 20%. In the case of inter-day precision, the RSD values were lower than 25% for all pesticides.

LODs and LOQs were determined injecting blank extracts spiked with the studied pesticides at concentrations that were decreased until achieving S/N ratios at 3 (LODs) and 10 (LOQs). The obtained LODs were in the range 0.1–10 μg/kg and LOQ 0.5–20 μg/kg, as it can be seen in Table 2. The obtained LODs are lower than maximum residual limits (MRLs) in soya bean (EU Pesticides Databases).

Comparing the validated method with other published previously, it can be highlighted that the proposed method determines more than 160 pesticides, whereas other analysed 10 (Ho et al., 2013), 15 (Xu et al., 2011), 35 (Sadowska-Rociek et al., 2013) or 55 (Du et al., 2011) pesticides. Moreover the LODs were equal or lower than previous methods (Hayward et al., 2013; Mao et al., 2012), simplifying the extraction procedure in relation to more complex approaches (Mao et al., 2012). Finally, it has to be emphasised that most of the methods had been developed for dried herbs or raw materials and not for nutraceutical products, which are more complex matrices.

3.3. Sample analysis

Finally, in order to evaluate the suitability of the developed method, it was applied for the simultaneous determination of the 177 pesticides in real samples obtained in local markets. For that, 11 samples of soya-based nutraceuticals were analysed. No pesticide residues were found above the LODs for most of the samples, except one of the samples containing malathion (11.1 μg/kg), and another one with pyriproxyfen (1.5 μg/kg), showing in Fig. 4 the chromatograms corresponding to positive samples.

4. Conclusions

In this study, a new method for determination of multiclass pesticides in complex soya nutraceuticals was developed. Simple, rapid and inexpensive modified QuEChERS method was applied for the determination of 177 pesticides by GC–MS/MS. Because of the complexity of the matrix, several sorbents were evaluated, observing that a mixture of them (C18, GBC, PSA, Zr-Sep$^+$) provided the most suitable results. This fact indicates that nutraceutical matrices are more complex than raw material (i.e. soya) and specific extraction methodologies should be applied. The developed method was fully validated, providing satisfactory linearity, recovery and precision. The obtained results show good linearity for most of the pesticides, as well as good recovery and precision values. Finally, the developed methodology was applied to the analysis of real samples for testing the applicability of the method. The proposed method allows the simultaneous determination of pesticides, as well as it could be useful for routine analysis of a high number of samples because it is fast and simple.

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