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Multiresidue determination of 160 pesticides in wines employing mixed-mode dispersive-solid phase extraction and gas chromatography–tandem mass spectrometry

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ABSTRACT

A new multiresidue method for the efficient screening, identification and quantification of over 160 pesticides belonging to different chemical classes in red, rose and white wines have been developed. The analysis was based on gas chromatographic-tandem quadrupole mass spectrometric determination (GC-QqQ–MS/MS). An optimization strategy involved the selection of buffering conditions and sorbents for dispersive-solid phase extraction (dispersive-SPE) in order to achieve acceptably high recoveries and reduce co-extractives in the final extracts. As a result, the optimized procedure allowed us to obtain consistent recoveries of the target pesticides including problematic ones such as captan, chlorothalonil, dichlofluanid, folpet and tolylfluanid. The attained recoveries were typically between 80 and 110% (89% on average) with RSD values typically lower than 10% (8% on average) at three spiking levels of 0.01, 0.05 and 0.2 mg kg^{-1}. Linearity was studied in the range between 0.005 and 0.2 mg kg^{-1} using pesticide standards prepared both in pure solvent and in the presence of matrix, showing coefficients of determination (R^2) higher than 0.99 for all the pesticides except for desmedipham, thiabendazole and thiamethoxam in pure solvent. The study of the ratio of the slopes obtained in solvent and in matrix provided information about the matrix effects, which was <10%, 10–20% and >20% for 33, 36 and 31% of the studied pesticides, respectively. To improve accuracy, matrix matched standards were always used for calculation of the quantification results. The expanded uncertainties were estimated by using a "top-down" approach as being 17% on average (coverage factor $k = 2$, confidence level 95%). Finally, the method was used with success to detect and quantify pesticide residues in commercial wines.

1. Introduction

Nowadays, the widespread use of pesticides in agriculture is clearly inevitable. It has brought many benefits with respect to enhanced quantity and quality of produced crops. But despite the obvious usefulness of pesticides in agriculture, there are concerns about the presence of their residues in crops which may pose a health hazard to the consumers of fresh food, processed food and drinks. Therefore, it is necessary to investigate pesticides in these products in order to identify the residues present and quantify their concentrations [1,2].

Wine is an important food commodity which is known for several thousands of years, already. It might be said that wine was given a significant place in ancient development of human culture and religious activities [3]. Grapevine cultivated in vineyards is susceptible to fungal diseases such as downy mildew, powdery mildew and gray mold (botrytis). Also, the grapevine is attacked by harmful insects such as the grapevine moth and grapevine mealybug. As wine quality strongly depends on the quality of grapes used for the fermentation process, in order to obtain high-quality wines, farmers must control grapevine pests and diseases with pesticides. During the winemaking process, yeasts cause gradual disappearance of pesticides present in must by degradation or absorption at the end of the fermentation when yeasts precipitate as lees. But there are data showing that residual amounts of pesticides can survive the winemaking process and can be present in wines as unwanted contaminants [4,5]. Apart from the compounds responsible the flavour and aroma [6–8], the presence of pesticide residues must be considered as an important factor affecting the wine quality. As a consequence, there is an increasing demand for reliable analytical methods that are adequately sensitive, selective and accurate to detect and quantify pesticide residues in wines [9].

The most useful way for determining pesticide residues in food samples is the application of multiresidue methods that allow screening for numerous compounds in a single analytical process. Depending on the properties of the target pesticides,

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either gas chromatography (GC) or liquid chromatography (HPLC) coupled to different detection systems can be used. The currently observed trend is to employ chromatography hyphenated with mass spectrometry (MS) rather than conventional detection because in terms of selectivity, mass spectrometry represents one of the most powerful detection tools for both chromatographic techniques [10]. The multiresidue chromatographic analysis of pesticides in wine requires a preconcentration step. Traditionally, this is performed either by liquid–liquid extraction (LLE) with organic solvents [5,11] or solid phase extraction with reversed phase sorbents such as C18 and Oasis HLB [12,13]. The application of solid phase microextraction (SPME) [14], ultrasound-assisted emulsification microextraction (USAEME) [14], single drop liquid–liquid microextraction (SDME) [15,16] and dispersive liquid–liquid microextraction (DLLME) [17] has also been successfully reported.

Recently, the QuEChERS (quick, easy, cheap, effective, rugged and safe) approach is being increasingly used for the pesticide multiresidue analysis in various sample matrices. This sample preparation technique involves miniaturized extraction with acetonitrile, liquid–liquid partition by salting out with sodium chloride and magnesium sulphate, and a cleanup step which is carried out by mixing the acetonitrile extract with loose sorbents rather than passing through a traditional SPE column. This cleanup technique is called dispersive-solid phase extraction (dispersive-SPE) [18]. Up to now, there has been several reports on application of the QuEChERS-based method for analysis of pesticides in wines. Zhang et al. [19] developed a method for the determination of 72 pesticides in wines. Pesticides were extracted using acetonitrile followed by dispersive-SPE cleanup with PSA and GCB sorbents. Analysis was performed by ultra-performance liquid chromatography-electrospray ionization–tandem mass spectrometry (UPLC–MS/MS). Jiang et al. [20] described a method based on QuEChERS for the determination of 77 pesticides in wine. The dispersive-SPE cleanup was carried out by applying PSA sorbent and final analysis was performed by gas chromatography–mass spectrometry (GC–MS) operated in selected ion monitoring (SIM) mode. Cuhna et al. [21] applied the QuEChERS to the determination of 27 pesticides in grapes, musts and wine. The dispersive-SPE cleanup was carried out by using PSA and C18 sorbents and the final determination was by conventional and low pressure gas chromatography–mass spectrometry (LP–GC–MS) in SIM mode. Patil et al. [22] and Dasgupta et al. [23,24] described multiresidue methods being in principle similar to the QuEChERS but ethyl acetate was used in place of acetonitrile for extraction of the analytes and the cleanup was achieved by dispersive-SPE with florisil and PSA sorbents. The determination was by gas chromatography-time-of-flight mass spectrometry (GC-TOF–MS) or two dimensional gas chromatography-time-of-flight–mass spectrometry (GC × GC-TOF–MS). These methods were applied to the simultaneous determination of a large number of pesticides and persistent organic pollutants such as dioxine-like polychlorinated biphenyls, polyaromatic hydrocarbons and bisphenol A in wines.

The main objective of the present work was to optimize and validate a new fast, sensitive and reliable analytical method, not yet described in literature, for the determination of over 160 pesticide residues in different types of wine (red, rose and white). Optimization of the sample preparation procedure entailed selection of buffering conditions during extraction and sorbents for dispersive-SPE cleanup in order to achieve acceptably high recoveries while reducing co-extractives in the final extracts. The instrumental analysis was carried out by gas chromatography coupled to tandem quadrupole mass spectrometry (GC-QqQ–MS/MS). Analytical performance of the developed method was evaluated through an extensive validation study which involved assessment of parameters such as linearity, accuracy, limits of quantification and measurement uncertainty. The special attention was devoted to the evaluation of matrix induced effects and its influence on sensitivity (calibration curve slopes) and accuracy (pesticide recoveries and RSD). Applicability of the developed and validated method was demonstrated in the analysis of 30 commercial wines for the presence of pesticide residues.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile and acetone (for residue analysis) were purchased from Witko (Łódź, Poland). Anhydrous magnesium sulphate (reagent grade), sodium citrate tribasic dihydrate (ACS reagent), and di-sodium hydrogen citrate sesquihydrate (pure), EnviCarb bulk sorbent (120/400 sieved fraction) as well as extraction tubes containing 6 g anhydrous magnesium sulphate and 1.5 g sodium acetate were all purchased from Sigma–Aldrich Sp.z o.o. (Poznań, Poland). Toluene (for residue analysis) and formic acid (ACS grade) were purchased from Merck Sp. z o.o. (Warszawa, Poland). Sodium chlorine (pure) was purchased from POCH (Gliwice, Poland). Bondesil PSA (40 μm) bulk sorbent was purchased from Candela Sp. z o.o. (Warszawa, Poland) and C18 (50 μm) bulk sorbent was purchased from Allchrom-Anaserwis (Baranowo, Poland).

2.2. Analytical standards

All high purity certified pesticide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany). Triphenylphosphate, TPP (I.S.) was purchased from Sigma–Aldrich Sp. z o.o. (Poznań, Poland). Stock solutions of approximately 1000 μg mL^{-1} were prepared in acetone. The purity of the certified standard was included in calculation of the actual concentration of each stock solution. A single composite stock standard solution at a concentration of 5 μg mL^{-1} was prepared in acetone, and working standard solutions of 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 and 1.0 μg mL^{-1} were prepared by diluting the stock solution with acetone. Matrix-matched standards were obtained by evaporating given volumes of the standards in acetone and reconstituting the residue remaining after evaporation in the wine extract in toluene at a sample concentration of 1 g mL^{-1}. The single composite mixtures at appropriate concentrations were used to calibrate the GC-QqQ–MS/MS system and to spike the wine samples in recovery experiments.

2.3. GC-QqQ–MS/MS conditions

A Varian CP-3800 series gas chromatograph coupled with a Varian 1200 triple quadrupole mass spectrometer and a CP-8400 autosampler (Varian Inc., Middelburg, The Netherlands) were employed for these analyses. Pesticides were separated on a DB-5 30 m × 0.25 mm × 0.5 μm capillary column, protected by a deactivated guard column (2 m × 0.53 mm). Helium of 99.999% purity at a flow rate of 1.2 mL min^{-1} was used as the carrier gas. The column oven temperature was programmed from 80 °C for 3 min and increased to 150 °C at 30 °C min^{-1}, increased to 300 °C at 10 °C min^{-1} which was held for 10 min. Injector temperature was programmed from 250 °C for 1 min and increased to 300 °C at 200 °C min^{-1} which was held for 20 min. The injection volume was 5 μL in splitless mode. The mass spectrometer was operated in electron impact ionization mode (EI, 70 eV). Electron multiplier voltage was set at the optimum value as determined by automatic tuning (1700 V). The autotuning procedure was performed monthly using perfluorotributylamine (PFTBA) as the compound for mass calibration. The filament current was 150 μA. The manifold ion source and transfer line temperatures were 40, 270 and 290 °C,

respectively. The collision gas for MS/MS experiments was argon of 99.9998% purity, and the pressure in the collision cell was set at 1.7 m Torr. Varian MS Workstation software, version 6.6, was used for the instrument control, data acquisition and evaluation.

2.4. Sample preparation procedures

2.4.1. Procedure A (optimized procedure)

A 10 g of wine was poured into a polypropylene centrifuge tube (50 mL), 50 μL internal standard solution (TPP at 150 μg mL^{-1}) and 10 mL acetonitrile were added. The contents were mixed vigorously with a Multi Reax vortexing device for 5 min. Hereafter, 0.5 g disodium hydrogencitrate sesquehydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulphate, and 1 g sodium chloride were added, and the mixture was immediately shaken by hand for 1 min, then centrifuged at 4000 rpm for 2.5 min. A 5 mL aliquot of the acetonitrile extract was transferred to a polypropylene centrifuge tube (15 mL) containing 0.75 g anhydrous magnesium sulphate, 0.125 g PSA and 0.250 g C18. The contents of the tube were vortexed for 0.5 min and centrifuged at 4000 rpm for 2.5 min. A 1.5 mL aliquot of the acetonitrile extract was transferred into an autosampler vial and 50 μL of 5% formic acid in acetonitrile (v/v) were added to stabilize base-sensitive pesticides. The extract was evaporated to dryness under a gentle stream of nitrogen and the residues were reconstituted in 1.5 mL toluene before GC-QqQ–MS/MS analysis.

2.4.2. Procedure B (extraction in the presence of sodium acetate buffer)

A 15 g of wine was poured into a polypropylene centrifuge tube (50 mL), 75 μL internal standard solution (TPP at 150 μg mL^{-1}) and 15 mL acetonitrile were added. The contents were mixed vigorously with a Multi Reax vortexing device for 5 min. Hereafter, 1.5 g sodium acetate, 6 g anhydrous magnesium sulphate, and 1 g sodium chloride were added, and the mixture was immediately shaken by hand for 1 min, then centrifuged at 4000 rpm for 2.5 min.

2.4.3. Procedure C (unbuffered extraction)

A 10 g of wine was poured into a polypropylene centrifuge tube (50 mL), 50 μL internal standard solution (TPP at 150 μg mL^{-1}) and 10 mL acetonitrile were added. The contents were mixed vigorously with a Multi Reax vortexing device for 5 min. Hereafter, 4 g anhydrous magnesium sulphate, and 1 g sodium chloride were added, and the mixture was immediately shaken by hand for 1 min, then centrifuged at 4000 rpm for 2.5 min.

2.4.4. Cleanup options evaluated

At the stage of optimization of sample preparation procedure, three approaches to dispersive-SPE cleanup were regarded with the above described extraction methods. For this purpose, after extraction and centrifugation, a 5 mL aliquot of the acetonitrile extract was transferred to a polypropylene centrifuge tube (15 mL) containing:

(a) 0.75 g anhydrous magnesium sulphate and 0.125 g PSA;
(b) 0.75 g anhydrous magnesium sulphate, 0.125 g PSA and 0.250 g C18;
(c) 0.75 g anhydrous magnesium sulphate, 0.125 g PSA, 0.250 g C18 and 0.050 g GCB.

In all cases the contents of the tube were vortexed for 0.5 min and centrifuged at 4000 rpm for 2.5 min. A 1.5 mL aliquot of the acetonitrile extract was transferred into an autosampler vial and 50 μL of 5% formic acid in acetonitrile (v/v) were added to stabilize base-sensitive pesticides. The extract was evaporated to dryness under a gentle stream of nitrogen and the residues were reconstituted in 1.5 mL toluene before GC-QqQ–MS/MS analysis.

2.5. Method performance

All validation studies were carried out by using wine samples previously checked to be free of the target pesticides. Linearity of calibration curves was studied over the concentration range between 0.005 and 0.2 μg mL^{-1} by GC-QqQ–MS/MS analysis of six calibration solutions at pesticides concentrations of 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 μg mL^{-1}, both in pure solvent (toluene) and matrix-matched (in red wine extract). The corresponding range of pesticides concentrations in real samples was between 0.005 and 0.2 mg kg^{-1}. Quantitation by GC-QqQ–MS/MS was based on the peak area ratios of the primary MRM transition of the analyte to that of the primary MRM transition of the internal standard (TPP). For the determination of trueness and precision, the samples were spiked with the target pesticides before proceeding with the sample preparation procedure. The recovery studies were carried out in six repetitions at three concentration levels or 0.01, 0.05 and 0.2 mg kg^{-1} for red wine. For rose and white wines, the recoveries from spiked samples were determined at 0.05 mg kg^{-1} ($n = 6$). In each experiment, average recovery values and the relative standard deviations (RSDs) were calculated. Limit of quantification (LOQ) was defined as the lowest spiking level, for which the validation criteria were met. The obtained method characteristics were assessed in compliance with the European Union guidelines SANCO/10684/2009 which stipulate the average recoveries in the range 70–120% with corresponding RSD less or equal 20% to prove the fitness for purpose of pesticide residue analytical methods [25].

3. Results and discussion

3.1. Optimization of GC-QqQ–MS/MS conditions

For optimization of the MS/MS data acquisition conditions, pesticide standards in toluene were injected and mass spectra in the range between 80 and 500 m/z were recorded in the electron ionization (EI) mode. After obtaining the full scan spectra for each compound, an abundant mass of the spectrum was selected as a precursor ion for the study of MS/MS fragmentation. In order to avoid the problem of interferences from isobaric masses resulting from common molecule fragments, ions in the high mass range were preferred as the precursor ions even in the cases where the base mass in the spectrum represented a low mass (<100 m/z). Once the precursor ions were selected, they were subjected to collision energy voltages ranged between 5 and 35 eV to study the fragmentation resulting from collision induced dissociation (CID) with argon as the collision gas. Finally, two multiple reaction monitoring (MRM) transitions of precursor ions fragmenting to product ions at specific collision energy (CE) voltage were defined for each target compound (with the exception for benalaxyl, captan, carbosulfan, dimethoate, fenhexamide, heptenophos, methidation, tolyfluanid and TPP for which only one abundant and specific MRM transition was achieved). The specific GC-QqQ–MS/MS acquisition method conditions such as precursor and product ions, collision energies and dwell times are thoroughly detailed in Table 1.

3.2. Optimization of sample preparation method

Sample preparation is a critical part of a multi-residue methods due to the widely different physicochemical properties such as polarities, water solubilities and volatilities of pesticides to be simultaneously extracted from the matrix. Wine contains significant amounts of naturally occurring matrix components such as fatty acids and their esters, phenolic compounds, alcohols, sugars,

Table 1
GC-QqQ–MS/MS conditions of the studied analytes.

Pesticide	Retention time, min	Quantification MRM	Dwell time, ms	Identification MRM	Dwell time, ms
Dichlorvos	7.56	185 > 93 (15)	87.5	185 > 109 (20)	87.5
Mevinphos	9.30	127 > 109 (15)	58.3	192 > 127 (15)	58.3
Propham	9.72	137 > 93 (10)	58.3	179 > 137 (5)	58.3
Methacriphos	10.08	208 > 110 (20)	58.3	208 > 180 (10)	58.3
Heptenophos	10.95	124 > 89 (10)	58.3		
Omethoate	11.25	156 > 110 (15)	50.0	125 > 79 (10)	50.0
Tecnazene	11.32	215 > 142 (20)	50.0	203 > 83 (15)	50.0
Propoxur	11.34	110 > 64 (20)	50.0	152 > 110 (10)	50.0
Propachlor	11.42	120 > 77 (15)	50.0	176 > 57 (5)	50.0
Diphenylomine	11.66	169 > 167 (10)	26.7	169 > 168 (5)	26.7
Ethoprophos	11.68	158 > 97 (15)	26.7	158 > 114 (5)	26.7
Phenmedipham	11.75	167 > 135 (10)	26.7	167 > 122 (15)	26.7
Trifluralin	11.79	306 > 264 (10)	26.7	264 > 160 (15)	26.7
Chlorpropham	11.86	171 > 127 (10)	26.7	213 > 171 (5)	26.7
Monocrotophos	12.04	192 > 127 (10)	26.7	192 > 164 (5)	26.7
Pencycuron	12.39	180 > 125 (20)	26.7	180 > 89 (30)	26.7
Desmedipham	12.54	181 > 109 (10)	20.0	181 > 122 (10)	20.0
HCH-alfa	12.55	181 > 145 (20)	20.0	219 > 181 (10)	20.0
HCB	12.66	284 > 214 (35)	20.0	284 > 249 (30)	20.0
Dimethoate	12.73	125 > 93 (10)	20.0		
Carbofuran	12.74	164 > 131 (10)	20.0	164 > 149 (5)	20.0
Dicloran	12.78	206 > 176 (10)	20.0	176 > 148 (10)	20.0
Simazine	12.80	201 > 173 (5)	20.0	201 > 186 (5)	20.0
Atrazine	12.87	215 > 172 (15)	20.0	215 > 173 (5)	20.0
Clomazone	13.02	204 > 107 (15)	20.0	204 > 204 (5)	20.0
HCH-beta	13.03	181 > 145 (20)	20.0	219 > 181 (10)	20.0
Quintozene	13.13	214 > 142 (25)	33.3	214 > 179 (10)	33.3
Diazinon	13.20	304 > 179 (10)	33.3	304 > 162 (10)	33.3
Lindane	13.24	181 > 145 (20)	33.3	219 > 181 (100	33.3
Propyzamide	13.24	173 > 109 (25)	33.3	173 > 145 (10)	33.3
Pyrimethanil	13.44	198 > 118 (30)	50.0	198 > 158 (25)	50.0
Chlorothalonil	13.54	266 > 133 (35)	50.0	266 > 168 (35)	50.0
Pirimicarb	13.74	238 > 166 (10)	50.0	238 > 72 (25)	50.0
Formothion	13.98	125 > 93 (10)	50.0	224 > 125 (20)	50.0
Acetochlor	14.24	223 > 132 (25)	22.2	223 > 147 (5)	22.2
Metribuzin	14.30	198 > 82 (20)	22.2	198 > 110 (5)	22.2
Chlorpyrifos-methyl	14.31	286 > 286 (10)	22.2	288 > 93 (20)	22.2
Vinclozolin	14.35	198 > 145 (15)	22.2	285 > 212 (10)	22.2
Spiroxamine 1	14.42	100 > 72 (5)	22.2	100 > 58 (10)	22.2
Parathion-methyl	14.44	263 > 109 (25)	22.2	263 > 136 (10)	22.2
Tolclofos-methyl	14.47	265 > 250 (20)	22.2	265 > 93 (25)	22.2
Heptachlor	14.51	274 > 237 (20)	22.2	274 > 239 (20)	22.2
Metalaxyl	14.55	206 > 132 (15)	22.2	206 > 105 (15)	22.2
Pirimiphos-methyl	14.55	290 > 151 (15)	22.2	305 > 180 (10)	22.2
Prometryn	14.58	241 > 58 (10)	22.2	241 > 184 (10)	22.2
Carbaryl	14.60	144 > 115 (20)	22.2	144 > 116 (10)	22.2
Ethofumesate	14.91	207 > 137 (10)	18.2	207 > 161 (5)	18.2
Fenitrothion	14.91	277 > 109 (25)	18.2	277 > 260 (10)	18.2
Spiroxamine 2	14.93	100 > 72 (5)	18.2	100 > 58 (10)	18.2
Methiocarb	14.94	168 > 153 (10)	18.2	168 > 109 (20)	18.2
Malathion	15.00	173 > 99 (15)	18.2	173 > 127 (10)	18.2
Dichlofluanid	15.11	224 > 123 (10)	18.2	167 > 124 (10)	18.2
Chlorpyrifos	15.21	314 > 258 (15)	18.2	314 > 286 (15)	18.2
Metolachlor	15.21	238 > 133 (25)	18.2	238 > 162 (10)	18.2
Flufenacet	15.28	151 > 95 (25)	18.2	151 > 136 (15)	18.2
Fenpropimorph	15.33	128 > 70 (10)	18.2	128 > 110 (5)	18.2
Tetraconazole	15.35	336 > 218 (15)	22.2	336 > 183 (30)	22.2
Parathion	15.38	291 > 81 (25)	22.2	291 > 109 (20)	22.2
Triadimefon	15.44	208 > 111 (20)	22.2	208 > 127 (15)	22.2
Aldrin	15.50	263 > 193 (30)	22.2	263 > 191 (30)	22.2
Dicofol	15.62	139 > 111 (10)	22.2	139 > 75 (10)	22.2
Thiamethoxam	15.85	212 > 139 (10)	22.2	212 > 125 (15)	22.2
Fipronil	15.85	367 > 213 (25)	22.2	367 > 215 (25)	22.2
Pendimethalin	15.88	252 > 162 (15)	22.2	252 > 191 (15)	22.2
Cyprodinil	15.92	225 > 224 (10)	22.2	225 > 208 (15)	22.2
Isofenphos	15.92	213 > 121 (10)	22.2	255 > 213 (10)	22.2
Penconazole	16.02	248 > 157 (25)	22.2	248 > 192 (15)	22.2
Chlorfenvinphos	16.05	269 > 161 (15)	22.2	323 > 267 (20)	22.2
Mecarbam	16.06	159 > 131 (10)	22.2	159 > 132 (10)	22.2
Tolylfluanid	16.11	238 > 137 (10)	22.2		
Quinalphos	16.23	146 > 118 (10)	22.2	157 > 129 (10)	22.2
Triflumizole	16.24	206 > 144 (30)	22.2	206 > 179 (15)	22.2
Heptachlor exo-epoxid	16.24	353 > 263 (15)	22.2	353 > 282 (15)	22.2
Procymidone	16.29	283 > 96 (10)	22.2	283 > 67 (20)	22.2
Triadimenol 1	16.29	168 > 70 (15)	22.2	128 > 65 (20)	22.2

Table 1 (*Continued*)

Pesticide	Retention time, min	Quantification MRM	Dwell time, ms	Identification MRM	Dwell time, ms
Heptachlor endo-epoxid	16.34	237 > 141 (25)	22.2	237 > 143 (25)	22.2
Captan	16.38	264 > 79 (10)	22.2		
Thiabendazole	16.40	201 > 174 (15)	30.8	201 > 130 (25)	30.8
Triadimenol 2	16.44	168 > 70 (15)	30.8	128 > 65 (20)	30.8
Folpet	16.50	260 > 102 (30)	30.8	260 > 130 (10)	30.8
Methidathion	16.55	145 > 85 (10)	30.8		
Picoxystrobin	16.62	335 > 115 (30)	30.8	335 > 173 (10)	30.8
Hexythiazox	16.66	227 > 149 (10)	30.8	184 > 115 (15)	30.8
Mepamipyrim	16.80	222 > 220 (20)	20.0	222 > 207 (15)	20.0
Hexaconazole	16.87	256 > 159 (20)	20.0	214 > 172 ((10)	20.0
Flutriafol	16.94	219 > 95 (25)	20.0	219 > 123 (15)	20.0
Napropamid	16.99	271 > 72 (10)	20.0	271 > 128 (5)	20.0
Endosulfan-alfa	17.00	241 > 206 (10)	20.0	272 > 237 (15)	20.0
Fludioxonil	17.01	248 > 127 (30)	20.0	248 > 154 (20)	20.0
Imazalil	17.01	173 > 145 (15)	20.0	215 > 173 (5)	20.0
Profenofos	17.15	339 > 269 (15)	20.0	339 > 188 (25)	20.0
Iprovalicarb 1	17.15	158 > 98 (10)	20.0	158 > 116 (10)	20.0
Kresoxim-methyl	17.26	206 > 131 (10)	22.2	206 > 116 (5)	22.2
Bupirimate	17.26	273 > 108 (15)	22.2	273 > 193 (10)	22.2
DDE-pp′	17.27	246 > 176 (25)	22.2	318 > 248 (15)	22.2
Myclobutanil	17.29	179 > 90 (25)	22.2	179 > 125 (10)	22.2
Flusilazole	17.30	233 > 165 (15)	22.2	233 > 152 (15)	22.2
Iprovalicarb 2	17.32	158 > 98 (10)	22.2	158 > 116 (10)	22.2
Buprofezin	17.36	175 > 132 (10)	22.2	190 > 175 (5)	22.2
Carboxin	17.46	235 > 143 (15)	25.0	235 > 87 (25)	25.0
Dieldrin	17.53	263 > 193 (30)	25.0	263 > 191 (30)	25.0
Cyproconazole	17.72	222 > 125 (25)	25.0	222 > 82 (15)	25.0
Nitrofen	17.77	202 > 139 (20)	25.0	283 > 162 (15)	25.0
Endrin	17.96	263 > 193 (30)	25.0	263 > 191 (30)	25.0
Ethion	18.00	231 > 129 (20)	25.0	231 > 175 (10)	25.0
Diniconazole	18.01	268 > 136 (30)	25.0	268 > 149 (25)	25.0
Oxadixyl	18.03	163 > 132 (5)	25.0	163 > 117 (15)	25.0
DDD-pp′	18.10	235 > 165 (20)	25.0	235 > 199 (20)	25.0
Endosulfan-beta	18.10	241 > 206 (10)	25.0	241 > 170 (20)	25.0
DDT-op′	18.17	235 > 165 (20)	25.0	235 > 199 (20)	25.0
Triazophos	18.29	161 > 106 (15)	33.3	257 > 162 (10)	33.3
Trifloxystrobin	18.37	186 > 145 (15)	33.3	190 > 130 (5)	33.3
Benalaxyl	18.52	176 > 146 (10)	33.3		
Propiconazole 1	18.61	259 > 69 (10)	33.3	259 > 173 (15)	33.3
Quinoxyfen	18.71	237 > 208 (15)	21.1	307 > 237 (15)	21.1
Propiconazole 2	18.73	259 > 69 (10)	21.1	259 > 173 (15)	21.1
Lenacil	18.75	153 > 136 (10)	21.1	153 > 153 (5)	21.1
DDT-pp′	18.83	235 > 165 (20)	21.1	235 > 199 (20)	21.1
Endosulfan-sulphate	18.86	272 > 237 (15)	21.1	241 > 206 (10)	21.1
Fenhexamid	18.88	301 > 97 (15)	21.1		
Diflufenican	18.95	266 > 246 (15)	21.1	266 > 238 (10)	21.1
Propargite	18.99	350 > 81 (15)	21.1	350 > 201 (5)	21.1
Tebuconazole	19.08	250 > 125 (20)	26.7	250 > 70 (10)	26.7
TPP (I.S.)	19.10	326 > 169 (30)	26.7		
Epoxiconazole	19.32	192 > 138 (10)	26.7	192 > 111 (25)	26.7
Bifenthrin	19.55	181 > 166 (20)	26.7	181 > 165 (20)	26.7
Bromuconazole 1	19.68	295 > 173 (10)	25.0	295 > 175 (10)	25.0
Carbosulfan	19.73	160 > 104 (10)	25.0		
Phosmet	19.73	160 > 77 (20)	25.0	160 > 133 (10)	25.0
Bromopropylate	19.75	341 > 183 (15)	25.0	341 > 185 (20)	25.0
Fenpropathrin	19.78	265 > 89 (25)	25.0	265 > 210 (10)	25.0
Methoxychlor	19.80	227 > 141 (35)	25.0	227 > 169 (30)	25.0
Tebufenpyrad	19.89	276 > 171 (15)	25.0	318 > 131 (10)	25.0
Fenazaquin	20.07	145 > 117 (10)	40.0	160 > 145 (10)	40.0
Flurtamone	20.09	199 > 157 (15)	40.0	333 > 120 (10)	40.0
Bromuconazole 2	20.10	295 > 173 (10)	40.0	295 > 175 (10)	40.0
Metconazole	20.13	250 > 125 (10)	40.0	250 > 145 (10)	40.0
Tetradifon	20.28	229 > 201 (15)	40.0	229 > 199 (15)	40.0
Cyhalothrin-lambda 1	20.34	197 > 141 (10)	25.0	197 > 161 (5)	25.0
Phosalone	20.37	182 > 111 (25)	25.0	367 > 182 (10)	25.0
Triticonazole	20.41	235 > 182 (10)	25.0	235 > 217 (10)	25.0
Pyriproxyfen	20.47	136 > 96 (5)	25.0	185 > 129 (5)	25.0
Azinphos-methyl	20.52	160 > 104 (10)	25.0	160 > 132 (10)	25.0
Cyhalothrin-lambda 2	20.52	197 > 141 (10)	25.0	197 > 161 (5)	25.0
Acrinathrin 1	20.52	181 > 152 (20)	25.0	208 > 181 (15)	25.0
Acrinathrin 2	20.60	181 > 152 (20)	25.0	208 > 181 (15)	25.0
Pyrazophos	20.80	221 > 149 (15)	25.0	221 > 193 (5)	25.0
Fenarimol	21.03	251 > 139 (15)	28.6	251 > 111 (25)	28.6
Azinphos-ethyl	21.14	160 > 104 (10)	28.6	160 > 132 (10)	28.6
Bitertanol	21.57	170 > 115 (30)	28.6	170 > 141 (15)	28.6
Permethrin 1	21.58	165 > 91 (10)	28.6	165 > 127 (5)	28.6

Table 1 (*Continued*)

Pesticide	Retention time, min	Quantification MRM	Dwell time, ms	Identification MRM	Dwell time, ms
Permethrin 2	21.74	165 > 91 (10)	28.6	165 > 127 (5)	28.6
Fluquinconazole	21.85	340 > 298 (20)	28.6	340 > 108 (30)	28.6
Pyridaben	21.90	147 > 117 (20)	28.6	309 > 147 (10)	28.6
Prochloraz	21.91	310 > 70 (15)	28.6	310 > 85 (10)	28.6
Cyfluthrin 1	22.26	206 > 151 (20)	44.4	165 > 127 (5)	44.4
Cyfluthrin 2	22.39	206 > 151 (20)	44.4	165 > 127 (5)	44.4
Fenbuconazole	22.42	198 > 129 (15)	44.4	198 > 102 (25)	44.4
Cyfluthrin-beta 1	22.49	206 > 151 (20)	44.4	165 > 127 (5)	44.4
Cypermethrin 1	22.49	181 > 152 (20)	44.4	165 > 127 (5)	44.4
Cyfluthrin-beta 2	22.55	206 > 151 (20)	44.4	165 > 127 (5)	44.4
Cypermethrin 2	22.94	181 > 152 (20)	44.4	165 > 127 (5)	44.4
Cypermethrin 3	23.05	181 > 152 (20)	44.4	165 > 127 (5)	44.4
Boscalid	23.08	140 > 112 (10)	44.4	140 > 76 (20)	44.4
Etofenprox	23.38	163 > 107 (20)	44.4	163 > 135 (15)	44.4
Fenvalerate 1	24.48	167 > 125 (10)	87.5	181 > 127 (25)	87.5
Pyraclostrobin	24.71	132 > 77 (20)	87.5	164 > 132 (20)	87.5
Fenvalerate 2	24.89	167 > 125 (10)	87.5	181 > 127 (25)	87.5
Difenoconazole 1	25.51	323 > 265 (10)	100.0	265 > 139 (25)	100.0
Indoxacarb	25.62	264 > 176 (10)	100.0	264 > 148 (15)	100.0
Difenoconazole 2	25.66	323 > 265 (10)	100.0	265 > 139 (25)	100.0
Deltamethrin 1	25.67	253 > 174 (10)	100.0	253 > 172 (5)	100.0
Deltamethrin 2	26.15	253 > 174 (10)	100.0	253 > 172 (5)	100.0
Azoxystrobin	26.53	344 > 329 (10)	75.0	344 > 156 (30)	75.0
Dimethomorph 1	26.91	301 > 165 (10)	75.0	301 > 139 (10)	75.0
Famoxadon	27.14	330 > 193 (25)	75.0	330 > 196 (25)	75.0
Dimethomorph 2	27.76	301 > 165 (10)	75.5	301 > 139 (10)	75.5
Imibenconazole	28.76	253 > 82 (10)	300.0	375 > 260 (20)	300.0

etc. which also get co-extracted with the target pesticides [26]. It is desirable to reduce the amount of co-extractives because these compounds may interfere with the analysis through co-eluted signals making identification and quantification of the analytes ambiguous. For the purpose of optimization of sample preparation procedure, the three acetonitrile extraction methods (unbuffered, citrate-buffered and acetate-buffered) were evaluated. Also, the three approaches to dispersive-SPE cleanup were evaluated using: (1) PSA sorbent, (2) the combination of PSA and C18 sorbents and (3) the combination of PSA, C18 and GCB sorbents. In this experimental design, the feasibility of nine different extraction—cleanup approaches were assessed in terms of presence of interferences after extraction and recoveries of the spiked pesticides. First, the extracts of red wine obtained through these procedures were analyzed by GC–MS in full scan mode (m/z 100–500) (Fig. 1). This revealed, a little difference between amounts of co-extractives in wine extracts obtained by unbuffered and citrate-buffered extraction but in the case of acetate buffered-extraction, the extracts contained somewhat more co-extractives. The sums of areas of chromatographic peaks in total ion chromatograms (TIC) as normalized to the highest obtained value are graphically presented in Fig. 2. A disadvantage encountered when using acetate-buffering to red wine extraction was that after acetonitrile–water partition it was extremely difficult to distinguish the acetonitrile (upper) layer from the water (lower) layer because both layers were almost of the same colour.

Keeping these observations in view, we studied the effect of different sample preparation approaches (unbuffered and citrate-buffered) on recoveries. Red wine was spiked at the concentration of 0.02 mg kg^{-1} and the GC-QqQ–MS/MS quantifications were done using calibration standards prepared red wine extract in toluene. It was found that graphitized carbon black (GCB) provided a nice cleanup with almost colourless extracts but at the same time it caused unacceptable losses with poor recoveries <70% of the pesticides susceptible to adsorption on GCB such as chlorothalonil, thiametoxam, thiabendazole, cyprodinil, diniconazole, diflufenican, pyrazofos, prochloraz, and imibenconazole. According to the results obtained, citrate-buffered extraction followed by dispersive-SPE cleanup with mixed sorbents PSA and C18 provided the most consistent recoveries with the less result variability (lower RSD) compared with other studied sample preparation methods (Fig. 3). We concluded that this sample preparation

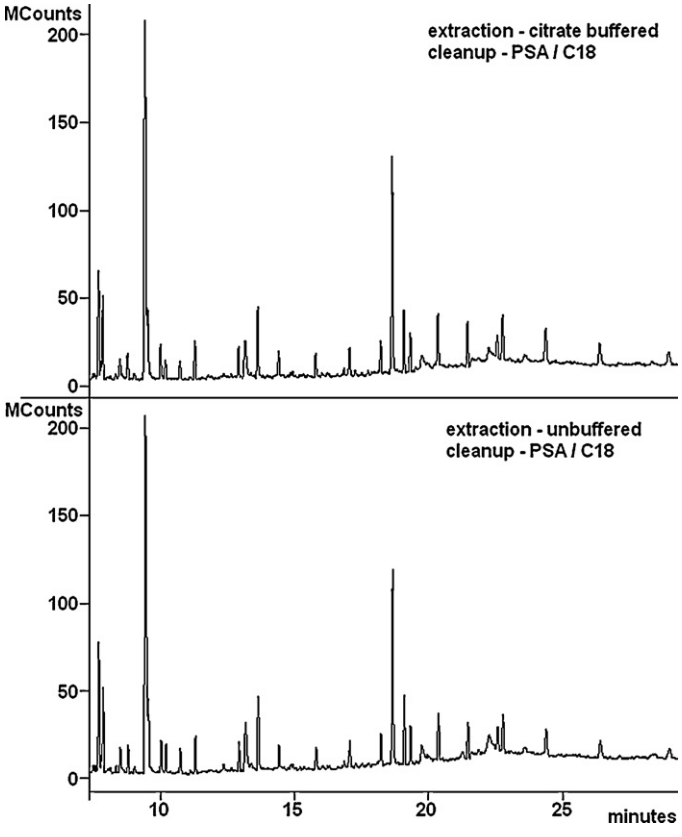

Fig. 1. Example of GC–MS total ion chromatograms (TICs) of red wine extracts.

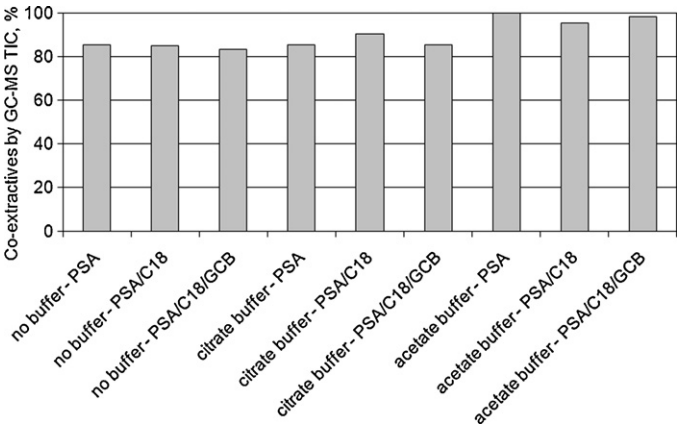

Fig. 2. Relative amounts of co-extractives from red wine as determined by GC–MS total ion chromatograms (TICs) after extraction and cleanup by different versions of sample preparation procedure. The highest total peaks area obtained when using extraction in the presence of acetate buffer and dispersive-SPE cleanup with PSA gave 100%.

approach will be the most favorable option for preparation of wine extracts before GC-QqQ–MS/MS analysis.

3.3. Method validation

The optimized method was subjected to the validation study according to single-laboratory validation approach. The analytical performance characteristics such as linearity (dynamic ranges and R^2), trueness, precision, limit of quantification (LOQ) and measurement uncertainty were evaluated. The spiking experiments were carried out on red wine produced from organically grown grapes. The wine was pretested to be free of pesticides before validation experiments were begun because it is known that organic wine can contain traces of pesticides due to cross-contamination, especially if the plot of organically grown grapes is adjacent to the plot of conventionally grown grapes [27]. The rose and white wines used for the recovery study were produced of conventionally grown grapes but were previously extracted and determined by GC-QqQ–MS/MS to be free of pesticides.

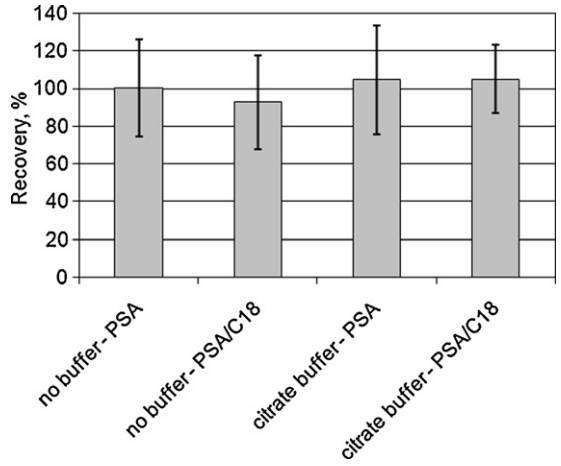

Fig. 3. Average recoveries and RSDs from red wine at 0.02 mg kg^{-1} obtained by following four versions of the sample preparation procedure (four combinations extraction—cleanup).

3.4. Linearity and matrix effects

For the linearity study, calibration curves were constructed on the basis of calibration standards prepared at six concentration levels in the range between 0.005 and 0.2 mg kg^{-1} by using pesticide standards prepared both in pure solvent (toluene) and in the presence of matrix (red wine). Excellent linearity with the coefficients of determination (R^2) > 0.99 was achieved both when using standards in pure toluene as well as matrix-matched standards for all the pesticides except for desmedipham, thiabendazole and thiametoxam in pure toluene, for which R^2 was >0.98 (Table 2). The obtained sensitivity parameters (calculated slopes of the calibration curves) were used to assess the matrix induced effects, which were evaluated by comparing solvent and matrix-matched calibration curves in terms of slope ratios 100% × (1 − toluene slope/wine slope) [28].

The matrix-induced enhancement or suppression effects are commonly encountered in gas chromatographic analysis of pesticide residues [29]. As a consequence the obtained concentration results can be erroneously high or low depending on the nature of the analytes and the nature of matrix. The study of the slopes ratios obtained in solvent against those obtained in the presence of matrix provided information about the matrix effects, which were <10%, 10–20%) and >20% for 33, 36 and 31% of the studied compounds, respectively. The response enhancement effect was predominant, it was observed for approximately 85% of the studied compounds. Matrix enhancement effect may improve peak shape by reducing tailing and improving sensitivity. This phenomenon can be considered as a positive aspect of matrix-effect. Co-extracted matrix components blocked the active sites in the gas chromatographic system and allowed a larger amount of the analyte to reach the detector. However, matrix effects should be addressed in order to avoid inaccuracy in the quantification results.

In the follow up experiment, we evaluated to what extent the matrix matched standards prepared in extracts of different types of wine (red, rose and white) might affect the recovery results. The main objective for conducting this experiment was to find out whether pesticide standards prepared in pure solvent can be used to calibrate the GC-QqQ–MS/MS system in case of unavailability of wine extract being totally free of target pesticides. A sample of 22 pesticides such as azoxystrobin, benalaxyl, boscalid, chlorothalonil, cyprodinil, difenoconazole, dimethomorph, fenarimol, fenbuconazole, fenhexamid, fludioxonil, iprovalicarb, kresoxim-methyl, metalaxyl, pyrimethanil, prochloraz, procymidone, pyraclostrobin, tebuconazole, tetradifon, triadimenol and trifloxystrobin were selected for this experiment. Although a limited number of compounds was selected for this experiment, the selected pesticides represented a broad scale of matrix effect intensity, both enhancement and suppression (Table 2). Red, rose and white wines were spiked at 0.05 mg kg^{-1} with the pesticides before proceeding with the extraction (n = 3) then the recovery results were calculated by using different types of matrix matched standards. As seen in Fig. 4, notably higher average recoveries (108.8–112.1%) with higher RSD values (12.3–15.4%) were obtained when the results were calculated with reference to calibration standards in pure toluene compared with those calculated by using matrix-matched standards. When using matrix matched standards, the recoveries were in the range 85.5–93.8 with RSD in the range 4.6–6.6%. The highest matrix enhancement effects with single recoveries in the range 120–165% were observed for chlorothalonil, fenhexamid, fludioxonil and pyraclostrobin. We concluded that in order to overcome the adverse impact of matrix effects on the quantified results, extracts of any type of wine (free of pesticide residues) can be used to prepare matrix-matched standards for construction of the calibration curves (e.g. calibration standards prepared in red wine extracts can be used to calculate pesticides residues results in white wine). As long as matrix-matched calibration standards are used

Table 2
Linearity parameters (range, slope, R^2) obtained by using standards in toluene and matrix matched (in red wine extract) as well as matrix effects measured as 100 × (1 − slope toluene/slope matrix).

Pesticide	Linearity range, mg kg^{-1}	Toluene		Matrix (red wine)		Matrix effect, %
		Slope	R^2	Slope	R^2	
Acetochlor	0.005–0.2	4.399E−04	0.9998	4.926E−04	0.9997	10.7
Acrinathrin 1	0.005–0.2	5.966E−04	0.9991	6.981E−04	0.9994	14.5
Acrinathrin 2	0.005–0.2	1.200E−03	0.9991	1.400E−03	0.9997	14.3
Aldrin	0.005–0.2	2.986E−04	0.9991	3.733E−04	0.9965	20.0
Atrazine	0.01–0.2	7.989E−05	0.9880	8.352E−05	0.9950	4.3
Azinphos-ethyl	0.01–0.2	3.620E−04	0.9976	3.903E−04	0.9989	7.3
Azinphos-methyl	0.01–0.2	2.451E−04	0.9927	3.550E−04	0.9993	30.9
Azoxystrobin	0.005–0.2	2.556E−04	0.9963	2.320E−04	0.9992	−10.2
Benalaxyl	0.02–0.2	6.246E−05	0.9986	3.550E−04	0.9998	82.4
Bifenthrin	0.005–0.2	3.300E−03	0.9985	3.300E−03	0.9999	0.0
Bitertanol	0.005–0.2	2.500E−03	0.9994	3.900E−03	0.9995	35.9
Boscalid	0.005–0.2	3.100E−03	0.9989	3.400E−03	0.9997	8.8
Bromopropylate	0.005–0.2	9.000E−04	0.9998	1.000E−03	0.9999	10.0
Bromuconazole 1	0.005–0.2	2.865E−04	0.9995	2.669E−04	0.9996	−7.3
Bromuconazole 2	0.005–0.2	2.789E−04	0.9988	2.758E−04	0.9995	−1.1
Bupirimate	0.005–0.2	4.449E−04	0.9997	4.214E−04	0.9998	−5.6
Buprofezin	0.01–0.2	2.535E−04	0.9994	2.564E−04	0.9997	1.1
Captan	0.05–0.2	2.162E−05	0.9937	2.752E−05	0.9960	21.4
Carbaryl	0.005–0.2	1.700E−03	0.9960	3.500E−03	0.9992	51.4
Carbofuran	0.005–0.2	2.670E−04	0.9987	4.711E−04	0.9995	43.3
Carbosulfan	0.01–0.2	1.780E−04	0.9938	2.320E−04	0.9987	23.3
Carboxin	0.005–0.2	7.744E−04	0.9988	8.798E−04	0.9989	12.0
Chlorfenvinphos	0.005–0.2	5.820E−04	0.9994	5.690E−04	0.9905	−2.3
Chlorothalonil	0.005–0.2	6.509E−04	0.9991	9.708E−04	0.9997	33.0
Chlorpropham	0.01–0.2	3.711E−04	0.9996	4.613E−04	0.9982	19.5
Chlorpyrifos	0.01–0.2	4.223E−04	0.9997	4.367E−04	0.9979	3.3
Chlorpyrifos-methyl	0.005–0.2	6.509E−04	0.9998	6.766E−04	0.9986	3.8
Clomazone	0.005–0.2	9.021E−04	0.9996	1.100E−03	0.9992	18.0
Cyfluthrin 1	0.005–0.2	1.077E−04	0.9995	1.268E−04	0.9992	15.0
Cyfluthrin 2	0.005–0.2	1.526E−04	0.9986	1.877E−04	0.9993	18.7
Cyfluthrin 3	0.005–0.2	2.459E−04	0.9983	2.652E−04	0.9977	7.3
Cyfluthrin 4	0.005–0.2	3.534E−04	0.9997	3.916E−04	0.9998	9.8
Cyhalothrin-lambda 1	0.005–0.2	2.367E−04	0.9981	3.172E−04	0.9989	25.4
Cyhalothrin-lambda 2	0.005–0.2	3.101E−04	0.9977	3.178E−04	0.9989	2.4
Cypermethrin 1	0.01–0.2	2.273E−04	0.9978	2.532E−04	0.9982	10.2
Cypermethrin 2	0.01–0.2	9.478E−04	0.9992	1.100E−03	0.9993	13.8
Cypermethrin 3	0.005–0.2	1.717E−04	0.9970	2.042E−04	0.9996	15.9
Cyproconazole	0.005–0.2	2.000E−03	0.9999	2.200E−03	0.9999	9.1
Cyprodinil	0.005–0.2	2.800E−03	0.9998	2.800E−03	0.9999	0.0
DDD-pp′	0.005–0.2	3.900E−03	0.9998	4.100E−03	0.9972	4.9
DDE-pp′	0.005–0.2	2.400E−03	0.9996	2.800E−03	0.9996	14.3
DDT-op′	0.005–0.2	3.200E−03	0.9999	3.600E−03	0.9965	11.1
DDT-pp′	0.005–0.2	3.500E−03	0.9997	3.900E−03	0.9997	10.3
Deltamethrin 1	0.01–0.2	1.536E−05	0.9990	1.565E−05	0.9997	1.8
Deltamethrin 2	0.01–0.2	1.517E−04	0.9994	1.537E−04	0.9997	1.3
Desmedipham	0.005–0.2	2.217E−04	0.9854	3.718E−04	0.9999	40.4
Diazinon	0.01–0.2	8.992E−05	0.9988	9.678E−05	0.9986	7.1
Dichlofluanid	0.005–0.2	6.620E−04	0.9989	7.074E−04	0.9987	2.2
Dichlorvos	0.005–0.2	3.977E−04	0.9962	6.791E−04	0.9990	41.4
Dicloran	0.005–0.2	3.039E−04	0.9971	4.165E−04	0.9987	27.0
Dicofol	0.005–0.2	1.300E−03	0.9991	1.800E−03	0.9984	27.8
Dieldrin	0.005–0.2	2.938E−04	0.9998	3.641E	0.9990	19.3
Difenoconazole 1	0.005–0.2	4.076E−04	0.9986	3.851E−04	0.9995	−5.8
Difenoconazole 2	0.005–0.2	3.774E−04	0.9996	3.568E−04	0.9994	−5.8
Diflufenican	0.005–0.2	5.929E−04	0.9989	6.564E−04	0.9996	9.7
Dimethoate	0.05–0.2	3.827E−05	0.9971	4.401E−05	0.9980	13.0
Dimethomorph 1	0.005–0.2	5.867E−04	0.9982	5.353E−04	0.9991	−9.6
Dimethomorph 2	0.005–0.2	4.118E−04	0.9968	3.867E−04	0.9993	−6.5
Dimoxystrobin	0.005–0.2	3.200E−03	0.9999	3.600E−03	0.9999	11.1
Diniconazole	0.005–0.2	4.242E−04	0.9993	5.596E−04	0.9994	24.2
Diphenylamine	0.005–0.2	1.100E−03	0.9997	1.400E−03	0.9997	21.4
Endosulfan-alfa	0.01–0.2	1.010E−04	0.9998	6.662E−05	0.9994	−9.0
Endosulfan-beta	0.01–0.2	6.347E−05	0.9998	6.662E−05	0.9962	4.7
Endosulfan-sulphate	0.005–0.2	5.142E−04	0.9997	5.961E−04	0.9998	13.8
Endrin	0.01–0.2	1.925E−04	0.9992	2.256E−04	0.9996	14.7
Epoxiconazole	0.005–0.2	1.300E−03	0.9996	1.400E−03	0.9999	7.1
Ethion	0.005–0.2	2.100E−03	0.9991	2.600E−03	0.9999	19.2
Ethofumesate	0.005–0.2	3.788E−04	0.9992	4.052E−04	0.9951	6.5
Ethoprophos	0.005–0.2	6.892E−04	0.9986	1.000E−03	0.9976	31.1
Etofenprox	0.005–0.2	8.000E−03	0.9999	8.800E−03	0.9998	9.1
Famoxadon	0.005–0.2	2.469E−04	0.9979	3.012E−04	0.9992	18.0
Fenarimol	0.005–0.2	8.516E−04	0.9998	8.618E−04	0.9992	1.2

Table 2 (*Continued*)

Pesticide	Linearity range, mg kg^{-1}	Toluene		Matrix (red wine)		Matrix effect, %
		Slope	R^2	Slope	R^2	
Fenazaquin	0.005–0.2	3.500E−03	0.9999	3.600E−03	0.9999	2.8
Fenbuconazole	0.005–0.2	2.900E−03	0.9996	2.700E−03	0.9996	−7.4
Fenhexamid	0.01–0.2	9.436E−05	0.9977	1.334E−04	0.9999	29.2
Fenitrothion	0.005–0.2	3.130E−04	0.9991	3.983E−04	0.9986	54.9
Fenpropathrin	0.005–0.2	2.316E−04	0.9993	2.471E−04	0.9999	6.2
Fenpropimorph	0.005–0.2	2.100E−03	0.9999	2.400E−03	0.9990	12.5
Fenvalerate 1	0.005–0.2	2.100E−03	0.9997	2.200E−03	0.9998	4.5
Fenvalerate 2	0.005–0.2	1.100E−03	0.9996	1.200E−03	0.9997	8.3
Fipronil	0.005–0.2	3.645E−04	0.9995	4.807E−04	0.9999	24.2
Fluchinconazole	0.005–0.2	7.650E−04	0.9995	8.044E−04	0.9991	4.9
Fludioxonil	0.005–0.2	1.900E−03	0.9924	2.700E−03	0.9997	29.6
Flufenacet	0.005–0.2	1.700E−03	0.9996	2.200E−03	0.9995	22.7
Flurtamone	0.01–0.2	3.629E−04	0.9984	4.168E−04	0.9994	12.9
Flusilazole	0.005–0.2	7.334E−04	0.9998	7.779E−04	0.9997	5.7
Flutriafol	0.005–0.2	1.900E−03	0.9999	2.100E−03	0.9999	9.5
Folpet	0.01–0.2	2.334E−04	0.9962	3.656E−04	0.9920	36.0
Formothion	0.005–0.2	1.575E−04	0.9952	2.414E−04	0.9995	34.8
HCB	0.005–0.2	6.439E−04	0.9977	8.749E−04	0.9991	26.4
HCH-alfa	0.005–0.2	6.971E−04	0.9992	8.442E−04	0.9982	17.4
HCH-beta	0.005–0.2	5.215E−04	0.9950	7.682E−04	0.9982	32.1
Heptachlor	0.005–0.2	4.150E−04	0.9995	5.022E−04	0.9991	17.4
Heptachlor endo-epoxide	0.005–0.2	4.887E−05	0.9982	5.623E−05	0.9986	13.1
Heptachlor exo-epoxide	0.005–0.2	1.311E−04	0.9971	1.580E−04	0.9996	17.0
Heptenophos	0.005–0.2	1.300E−03	0.9988	2.000E−03	0.9984	35.0
Hexaconazole	0.02–0.2	1.501E−04	0.9979	1.602E−04	0.9989	6,3
Hexythiazox	0.05–0.2	2.960E−05	0.9969	3.324E−05	0.9956	13.6
Imazalil	0.01–0.2	6.395E−04	0.9928	3.881E−04	0.9989	9.5
Imibenconazole	0.005–0.2	3.514E−04	0.9928	3.881E−04	0.9989	9.5
Indoxacarb	0.005–0.2	1.412E−04	0.9949	1.519E−04	0.9990	7.1
Iprovalicarb 1	0.01–0.2	1.173E−04	0.9994	1.252E−04	0.9990	6.3
Iprovalicarb 2	0.01–0.2	1.173E−04	0.9954	1.190E−04	0.9995	1.4
Isofenphos	0.005–0.2	1.200E−03	0.9997	1.300E−03	0.9997	7.7
Krezoxim-methyl	0.005–0.2	3.400E−04	0.9994	3.410E−04	0.9995	0.3
Lenacil	0.005–0.2	1.200E−03	0.9983	1.300E−03	0.9990	7.7
Lindane	0.005–0.2	5.285E−04	0.9995	7.153E−04	0.9986	26.1
Malathion	0.005–0.2	1.100E−03	0.9989	1.400E−03	0.9986	21.4
Mecarbam	0.01–0.2	3.939E−04	0.9974	4.427E−04	0.9998	11.0
Mepamipyrim	0.005–0.2	9.911E−04	0.9992	1.300E−03	0.9995	23.8
Metalaxyl	0.005–0.2	3.433E−04	0.9993	3.328E−04	0.9997	2.7
Metconazole	0.01–0.2	6.240E−05	0.9975	5.241E−05	0.9992	−19.1
Methacriphos	0.005–0.2	3.022E−04	0.9991	3.857E−04	0.9971	21.6
Methidathion	0.005–0.2	4.400E−03	0.9990	5.400E−03	0.9988	18.5
Methiocarb	0.005–0.2	9.139E−04	0.9980	1.400E−03	0.9999	34.7
Methoxychlor	0.005–0.2	3.400E−03	0.9998	4.000E−03	0.9998	15.0
Metolachlor	0.005–0.2	1.300E−03	0.9998	1.500E−03	0.9999	13.3
Metribuzin	0.005–0.2	6.856E−04	0.9994	5.843E−04	0.9996	−17.4
Mevinphos	0.005–0.2	8.134E−04	0.9976	1.300E−03	0.9976	37.4
Monocrotophos	0.005–0.2	2.744E−04	0.9982	4.059E−04	0.9986	32.4
Myclobutanil	0.005–0.2	7.815E−04	0.9983	9.466E−04	0.9996	17.4
Napropamid	0.005–0.2	3.389E−04	0.9988	3.660E−04	0.9978	7.4
Nitrofen	0.005–0.2	7.679E−04	0.9978	1.100E−03	0.9994	30.2
Omethoate	0.005–0.2	3.009E−04	0.9928	6.486E−04	0.9975	53.6
Oxadixyl	0.01–0.2	4.426E−04	0.9992	3.963E−04	0.9986	−11.7
Parathion	0.005–0.2	2.489E−04	0.9983	3.334E−04	0.9990	25.4
Parathion methyl	0.005–0.2	4.621E−04	0.9983	5.480E−04	0.9994	15.7
Penconazole	0.005–0.2	1.500E−03	0.9995	1.700E−03	0.9999	11.8
Pencycuron	0.005–0.2	1.200E−03	0.9997	1.400E−03	0.9999	14.3
Pendimethalin	0.005–0.2	2.938E−04	0.9984	3.468E−04	0.9993	15.3
Permethrin 1	0.02–0.2	2.129E−04	0.9998	2.268E−04	0.9997	6.1
Permethrin 2	0.02–0.2	1.857E−04	0.9998	1.945E−04	0.9986	4.5
Phenmedipham	0.005–0.2	1.868E−04	0.9887	4.145E−04	0.9982	54.9
Phosalone	0.005–0.2	1.400E−03	0.9994	1.600E−03	0.9999	12.5
Phosmet	0.005–0.2	2.900E−03	0.9954	4.500E−03	0.9999	35.6
Picoxystrobin	0.005–0.2	1.654E−04	0.9986	2.135E−04	0.9986	22.5
Pirimicarb	0.005–0.2	7.045E−04	0.9997	7.466E−04	0.9997	5.6
Pirimiphos-methyl	0.005–0.2	3.618E−04	0.9999	4.056E−04	0.9999	10.8
Prochloraz	0.01–0.2	2.551E−04	0.9988	2.011E−04	0.9992	−26.8
Procymidone	0.005–0.2	4.097E−04	0.9963	4.180E−04	0.9998	2.0
Profenofos	0.005–0.2	2.634E−04	0.9996	3.120E−04	0.9999	15.6
Prometryn	0.005–0.2	5.856E−04	0.9998	6.871E−04	0.9984	14.8
Propachlor	0.005–0.2	1.400E−03	0.9996	1.800E−03	0.9984	22.2
Propargite	0.01–0.2	5.805E−05	0.9957	6.127E−05	0.9977	5.2
Propham	0.005–0.2	6.564E−04	0.9996	1.000E−03	0.9985	34.4
Propiconazole 1	0.005–0.2	2.702E−04	0.9993	2.893E−04	0.9984	6.6

Table 2 (*Continued*)

Pesticide	Linearity range, mg kg^{-1}	Toluene		Matrix (red wine)		Matrix effect, %
		Slope	R^2	Slope	R^2	
Propiconazole 2	0.005–0.2	2.127E−04	0.9982	2.147E−04	0.9978	0.9
Propoxur	0.005–0.2	1.600E−03	0.9990	2.200E−03	0.9979	27.3
Propyzamide	0.005–0.2	1.400E−03	0.9996	1.900E−03	0.9997	26.3
Pyraclostrobin	0.005–0.2	8.192E−04	0.9944	1.900E−03	0.9978	56.9
Pyrazofos	0.005–0.2	6.212E−04	0.9995	7.083E−04	0.9997	12.3
Pyridaben	0.005–0.2	4.400E−03	0.9999	4.700E−03	0.9998	6.4
Pyrimethanil	0.005–0.2	9.623E−04	0.9995	1.300E−03	0.9995	26.0
Pyriproxyfen	0.005–0.2	4.376E−04	0.9988	4.452E−04	0.9999	1.7
Quinalphos	0.005–0.2	1.600E−03	0.9999	1.900E−03	0.9997	15.8
Quinoxyfen	0.005–0.2	7.827E−04	0.9998	9.256E−04	0.9999	15.4
Quintozene	0.005–0.2	2.236E−04	0.9993	2.907E−04	0.9985	23.1
Simazine	0.05–0.2	5.591E−05	0.9999	3.698E−05	0.9983	−51.2
Spiroxamine 1	0.01–0.2	4.697E−04	0.9998	5.223E−04	0.9993	10.1
Spiroxamine 2	0.01–0.2	5.919E−04	0.9996	6.416E−04	0.9997	7.8
Tebuconazole	0.005–0.2	1.000E−03	0.9998	1.100E−03	0.9996	9.1
Tebufenpyrad	0.005–0.2	9.584E−04	0.9999	9.646E−04	0.9995	0.6
Tecnazene	0.005–0.2	1.451E−04	0.9977	2.001E−04	0.9955	27.5
Tetraconazole	0.005–0.2	2.093E−04	0.9991	2.443E−04	0.9998	14.3
Tetradifon	0.005–0.2	2.864E−04	0.9919	2.648E−04	0.9995	−8.1
Thiabendazole	0.005–0.2	1.200E−03	0.9868	1.900E−03	0.9989	36.8
Thiamethoxam	0.01–0.2	1.958E−04	0.9853	3.040E−04	0.9990	35.6
Tolclofos-methyl	0.005–0.2	7.609E−04	0.9998	8.812E−04	0.9971	14.6
Tolylfluanid	0.005–0.2	7.649E−04	0.9998	7.692E−04	0.9988	0.6
Triadimefon	0.005–0.2	5.819E−04	0.9989	6.428E−04	0.9974	9.5
Triadimenol 1	0.005–0.2	7.167E−04	0.9995	9.829E−04	0.9997	27.1
Triadimenol 2	0.005–0.2	1.906E−04	0.9883	2.431E−04	0.9992	21.6
Triazophos	0.005–0.2	7.053E−04	0.9992	8.387E−04	0.9999	15.9
Trifloxystrobin	0.005–0.2	5.533E−04	0.9998	6.049E−04	0.9999	8.5
Triflumizol	0.005–0.2	5.511E−04	0.9995	4.781E−04	0.9984	−15.3
Trifluralin	0.005–0.2	2.677E−04	0.9989	3.040E−04	0.9991	12.0
Triticonazole	0.01–0.2	2.070E−04	0.9984	2.084E−04	0.9993	0.7
Vinclozoline	0.005–0.2	4.188E−04	0.9996	4.572E−04	0.9982	8.4

more accurate results will be obtained whereas calculations with reference to standards prepared in pure solvent, i.e. without the presence of matrix components, cannot guarantee correct quantification of pesticides concentrations in real samples.

3.5. Recovery study

For the recovery study, spiked samples were prepared from red wine at 0.01, 0.05 and 0.2 mg kg^{-1} concentration levels. Also, samples of rose and white wine were spiked at 0.05 mg kg^{-1}. The analyses were carried out six times at each spiking level. The data evaluation was performed by comparing the signal intensities of the spiked samples to those obtained by matrix-matched standard calibration. The recovery data are compiled in Table 3, as seen, the recoveries are typically between 80 and 110% (89% on average) with RSD values lower than 10% (8% on average). Inter-day recovery and precision were also evaluated to be used for estimation of measurement uncertainty since inter-day precision has a great contribution to the uncertainty budget. All the recoveries are in the acceptance range of the SANCO/10684/2009 of the European Quality Control guidelines (70–120%) in all cases, except for spiroxamine, which shows recovery lower than 60% at 0.01 mg kg^{-1} but the overall recovery is still above 70%. The problematic, base-sensitive pesticides, such as captan, folpet, tolylfluanid and dichlofluanid showed very satisfactory recoveries. Nevertheless, the recovery data for the majority of the compounds were lower than the ideal recovery of 100% by approximately 10%. We assumed that this effect could be attributed to the presence of alcohol in wine which was not fully

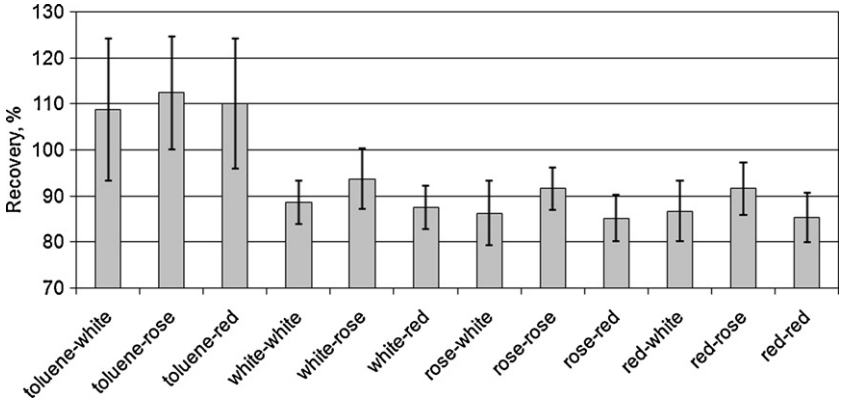

Fig. 4. Average recoveries and RSDs obtained for samples of different types of wine (red, rose and white) spiked at 0.05 mg kg^{-1} (n = 3) then quantified with reference to calibration standards prepared in pure solvent and matrix-matched (e.g. white—red means that calibration standards in white wine extract were used to determine spiking recovery of pesticides from red wine).

Table 3
Limits of quantification (LOQ), average recoveries and RSDs after application of GC-QqQ–MS/MS-based procedure to spiked red, white and rose wines as well as measurement uncertainties *U*, % (*k* = 2) calculated for overall recovery and RSD data.

Pesticide	LOQ, mg kg^{-1}	Recovery (RSD), %						*U*, % (*k* = 2)
		Red wine			White wine	Rose wine	Overall	
		0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.2 mg kg^{-1}	0.05 mg kg^{-1}	0.05 mg kg^{-1}	0.01–0.2 mg kg^{-1}	
Acetochlor	0.01	88 (6)	86 (6)	89 (4)	91 (5)	94 (5)	90 (5)	12
Acrinathrin 1	0.01	82 (11)	84 (4)	90 (2)	88 (4)	97 (5)	88 (8)	17
Acrinathrin 2	0.01	82 (5)	86 (3)	90 (3)	87 (4)	98 (5)	89 (7)	16
Aldrin	0.01	87 (10)	80 (10)	86 (3)	86 (7)	87 (6)	85 (8)	18
Atrazine	0.05	87 (31)	93 (15)	86 (3)	89 (6)	86 (13)	88 (16)	32
Azinphos-ethyl	0.01	72 (10)	89 (5)	90 (4)	91 (4)	91 (3)	87 (10)	22
Azinphos-methyl	0.01	82 (15)	81 (3)	86 (3)	89 (6)	89 (3)	86 (8)	18
Azoxystrobin	0.01	82 (10)	90 (7)	91 (3)	93 (5)	95 (5)	90 (7)	16
Benalaxyl	0.05		96 (7)	95 (4)	90 (8)	81 (5)	91 (9)	18
Bifenthrin	0.01	91 (4)	88 (7)	89 (3)	91 (4)	99 (7)	92 (7)	14
Bitertanol	0.01	89 (10)	86 (4)	85 (3)	90 (3)	92 (3)	88 (6)	13
Boscalid	0.01	81 (6)	87 (2)	86 (3)	86 (4)	93 (2)	87 (5)	13
Bromopropylate	0.01	82 (4)	89 (4)	90 (1)	90 (3)	93 (3)	89 (5)	12
Bromuconazole 1	0.01	83 (50	87 (7)	88 (2)	94 (2)	97 (4)	90 (7)	15
Bromuconazole 2	0.01	91 (8)	88 (5)	90 (3)	91 (3)	89 (4)	90 (5)	11
Bupirimate	0.01	96 (10)	91 (6)	87 (3)	88 (5)	92 (4)	91 (7)	15
Buprofezin	0.01	92 (18)	90 (5)	92 (5)	87 (8)	93 (6)	91 (9)	19
Captan	0.05		95 (20)	101 (6)	106 (14)	103 (20)	101 (16)	33
Carbaryl	0.01	85 (7)	85 (4)	87 (3)	89 (3)	92 (2)	88 (5)	12
Carbofuran	0.01	84 (9)	85 (6)	88 (6)	84 (4)	95 (8)	87 (8)	18
Carbosulfan	0.01	74 (14)	84 (6)	88 (3)	97 (4)	98 (6)	88 (12)	26
Carboxin	0.01	102 (5)	89 (4)	87 (6)	90 (3)	89 (4)	91 (7)	16
Chlorfenvinphos	0.01	88 (3)	113 (1)	110 (2)	92 (2)	92 (2)	99 (11)	22
Chlorothalonil	0.01	102 (5)	89 (6)	92 (2)	94 (3)	95 (2)	94 (6)	12
Chlorpropham	0.01	86 (8)	82 (2)	86 (4)	90 (3)	88 (6)	87 (7)	14
Chlorpyrifos	0.01	96 (10)	81 (4)	87 (2)	88 (2)	91 (7)	88 (8)	17
Chlorpyrifos-methyl	0.01	78 (8)	86 (3)	88 (2)	88 (2)	93 (5)	87 (7)	16
Clomazone	0.01	90 (6)	82 (4)	85 (1)	89 (5)	95 (6)	88 (7)	15
Cyfluthrin 1	0.01	89 (13)	90 (10)	90 (5)	91 (5)	89 (8)	90 (8)	18
Cyfluthrin 2	0.01	94 (8)	87 (9)	83 (5)	91 (4)	96 (5)	90 (8)	17
Cyfluthrin 3	0.01	81 (15)	85 (8)	90 (4)	88 (5)	96 (5)	88 (9)	20
Cyfluthrin 4	0.01	102 (9)	89 (7)	90 (3)	89 (3)	94 (5)	93 (8)	16
Cyhalothrin-lambda 1	0.01	94 (9)	94 (3)	91 (2)	90 (2)	96 (5)	93 (5)	11
Cyhalothrin-lambda 2	0.01	82 (14)	85 (8)	86 (3)	91 (5)	94 (6)	88 (9)	19
Cypermethrin 1	0.01	84 (18)	89 (8)	84 (4)	88 (6)	96 (2)	88 (10)	21
Cypermethrin 2	0.01	82 (11)	87 (4)	87 (6)	82 (8)	94 (5)	86 (9)	19
Cypermethrin 3	0.01	82 (3)	84 (5)	86 (2)	87 (4)	95 (4)	87 (6)	15
Cyproconazole	0.01	90 (7)	85 (2)	87 (2)	89 (2)	92 (2)	89 (5)	12
Cyprodinil	0.01	90 (7)	88 (3)	88 (2)	89 (4)	90 (2)	89 (4)	10
DDD-pp′	0.01	91 (3)	90 (3)	92 (2)	89 (3)	94 (4)	91 (3)	8
DDE-pp′	0.01	93 (2)	83 (4)	87 (1)	88 (4)	93 (3)	89 (5)	12
DDT-op′	0.01	99 (5)	80 (3)	87 (12)	86 (5)	92 (5)	89 (9)	20
DDT-pp′	0.01	101 (4)	85 (4)	88 (1)	87 (6)	89 (5)	90 (8)	16
Deltamethrin 1	0.01	75 (19)	95 (12)	91 (8)	84 (16)	93 (14)	88 (15)	32
Deltamethrin 2	0.01	89 (7)	91 (3)	93 (3)	87 (3)	95 (6)	91 (5)	12
Desmedipham	0.01	93 (10)	88 (12)	86 (6)	92 (5)	98 (4)	91 (9)	18
Diazinon	0.01	82 (14)	82 (6)	83 (4)	82 (8)	81 (8)	82 (8)	19
Dichlofluanid	0.01	92 (10)	88 (4)	90 (3)	87 (6)	91 (4)	90 (6)	13
Dichlorvos	0.01	90 (3)	82 (6)	84 (2)	82 (5)	93 (5)	86 (7)	16
Dicloran	0.01	90 (12)	83 (6)	88 (3)	83 (3)	91 (8)	87 (8)	18
Dicofol	0.01	83 (3)	84 (3)	84 (3)	88 (1)	91 (3)	86 (4)	12
Dieldrin	0.01	107 (7)	82 (9)	85 (2)	83 (10)	91 (5)	90 (12)	25
Difenoconazole 1	0.01	83 (8)	89 (7)	88 (2)	88 (6)	94 (4)	88 (7)	15
Difenoconazole 2	0.01	87 (12)	90 (4)	89 (3)	87 (9)	96 (5)	90 (8)	17
Diflufenican	0.01	84 (5)	89 (4)	88 (2)	90 (3)	97 (3)	89 (6)	13
Dimethoate	0.05		73 (11)	85 (13)	90 (14)	88 (17)	84 (15)	32
Dimethomorph 1	0.01	78 (7)	90 (6)	89 (3)	90 (4)	95 (3)	89 (8)	17
Dimethomorph 2	0.01	84 (8)	96 (7)	89 (4)	90 (6)	95 (5)	91 (7)	16
Dimoxystrobin	0.01	75 (9)	87 (4)	88 (2)	91 (5)	95 (1)	87 (9)	19
Diniconazole	0.01	88 (4)	86 (7)	89 (3)	90 (3)	91 (6)	89 (5)	12
Diphenylamine	0.01	95 (9)	84 (4)	89 (3)	85 (4)	90 (8)	89 (7)	16
Endosulfan-alfa	0.01	103 (18)	86 (7)	93 (6)	93 (8)	105 (12)	96 (13)	26
Endosulfan-beta	0.01	102 (8)	83 (9)	87 (5)	84 (11)	96 (12)	90 (12)	25
Endosulfan-sulphate	0.01	90 (6)	84 (5)	87 (2)	86 (3)	95 (5)	89 (6)	14
Endrin	0.01	93 (10)	87 (7)	92 (5)	91 (6)	93 (6)	91 (7)	15
Epoxiconazole	0.01	90 (4)	88 (2)	88 (2)	93 (4)	93 (3)	90 (4)	9
Ethion	0.01	94 (5)	85 (5)	90 (1)	89 (4)	92 (4)	90 (5)	11
Ethofumesate	0.01	103 (9)	83 (6)	89 (4)	93 (3)	91 (4)	92 (9)	19
Ethoprophos	0.01	81 (4)	79 (5)	84 (2)	85 (4)	90 (5)	84 (6)	15
Etofenprox	0.01	86 (4)	86 (3)	86 (2)	88 (3)	94 (2)	88 (5)	12
Famoxadon	0.01	76 (5)	92 (7)	93 (3)	88 (5)	96 (4)	89 (9)	19
Fenarimol	0.01	87 (5)	88 (2)	89 (3)	92 (3)	90 (3)	89 (3)	9

Table 3 (*Continued*)

Pesticide	LOQ, mg kg^{-1}	Recovery (RSD), %						*U*, % (*k* = 2)
		Red wine			White wine	Rose wine	Overall	
		0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.2 mg kg^{-1}	0.05 mg kg^{-1}	0.05 mg kg^{-1}	0.01–0.2 mg kg^{-1}	
Fenazaquin	0.01	83 (3)	84 (3)	86 (3)	87 (2)	87 (2)	85 (3)	11
Fenbuconazole	0.01	90 (3)	88 (4)	86 (3)	93 (4)	95 (3)	90 (5)	11
Fenhexamid	0.01	87 (18)	85 (7)	87 (3)	81 (6)	86 (9)	85 (10)	21
Fenitrothion	0.01	83 (8)	86 (4)	90 (4)	91 (4)	95 (6)	89 (7)	15
Fenpropathrin	0.01	85 (7)	83 (6)	90 (3)	89 (6)	91 (5)	87 (6)	14
Fenpropimorph	0.01	76 (10)	81 (4)	79 (12)	88 (3)	90 (3)	83 (9)	21
Fenvalerate 1	0.01	85 (4)	87 (4)	88 (2)	88 (2)	95 (3)	89 (5)	12
Fenvalerate 2	0.01	84 (6)	87 (4)	89 (2)	89 (5)	96 (2)	89 (6)	13
Fipronil	0.01	85 (7)	89 (3)	92 (2)	93 (4)	93 (3)	90 (5)	11
Fluchinconazole	0.01	84 (9)	89 (3)	88 (3)	89 (3)	91 (5)	89 (6)	13
Fludioxonil	0.01	88 (2)	87 (3)	87 (2)	89 (3)	93 (2)	89 (3)	9
Flufenacet	0.01	87 (6)	84 (4)	88 (1)	92 (1)	94 (2)	89 (5)	13
Flurtamone	0.01	83 (11)	87 (9)	91 (3)	86 (3)	90 (5)	88 (7)	16
Flusilazole	0.01	94 (7)	87 (3)	87 (1)	90 (2)	92 (3)	90 (5)	11
Flutriafol	0.01	90 (6)	88 (3)	87 (1)	89 (2)	90 (3)	89 (4)	10
Folpet	0.01	77 (19)	78 (3)	88 (5)	85 (16)	84 (14)	82 (14)	11
Formothion	0.01	73 (10)	82 (3)	87 (3)	85 (9)	93 (6)	84 (10)	30
HCB	0.01	81 (5)	77 (10)	88 (5)	79 (10)	98 (10)	85 (12)	23
HCH-alfa	0.01	79 (5)	84 (6)	87 (3)	87 (4)	93 (5)	86 (7)	26
HCH-beta	0.01	86 (5)	76 (4)	83 (3)	88 (4)	91 (5)	85 (7)	16
Heptachlor	0.01	93 (9)	79 (8)	85 (3)	83 (5)	90 (5)	86 (9)	19
Heptachlor endo-epoxide	0.01	108 (16)	90 (8)	92 (5)	83 (15)	93 (7)	93 (14)	28
Heptachlor exo-epoxide	0.01	81 (9)	83 (8)	92 (5)	88 (6)	98 (8)	86 (10)	21
Heptenophos	0.01	75 (8)	84 (5)	82 (3)	87 (6)	93 (4)	85 (9)	19
Hexaconazole	0.05		83 (6)	85 (3)	85 (8)	93 (4)	86 (8)	18
Hexythiazox	0.05		91 (20)	84 (2)	100 (16)	93 (8)	94 (17)	34
Imazalil	0.01	79 (11)	80 (6)	87 (17)	89 (6)	90 (6)	83 (9)	21
Imibenconazole	0.01	79 (11)	93 (8)	91 (6)	82 (8)	85 (4)	90 (9)	20
Indoxacarb	0.01	94 (7)	93 (8)	91 (2)	90 (4)	98 (2)	94 (5)	11
Iprovalicarb 1	0.01	93 (13)	86 (12)	87 (5)	94 (7)	97 (2)	92 (9)	19
Iprovalicarb 2	0.01	81 (13)	90 (11)	94 (8)	89 (9)	88 (8)	88 (10)	22
Isofenphos	0.01	93 (3)	84 (3)	89 (2)	90 (5)	92 (3)	90 (5)	11
Krezoxim-methyl	0.01	93 (15)	91 (4)	89 (4)	94 (6)	97 (6)	93 (8)	17
Lenacil	0.01	80 (5)	87 (2)	89 (2)	89 (5)	93 (4)	87 (6)	14
Lindane	0.01	83 (7)	87 (5)	86 (3)	85 (3)	92 (4)	87 (6)	14
Malathion	0.01	84 (6)	85 (5)	87 (2)	90 (2)	94 (3)	88 (6)	13
Mecarbam	0.01	89 (17)	89 (3)	89 (2)	94 (6)	93 (8)	91 (8)	18
Mepamipyrim	0.01	92 (6)	86 (4)	87 (2)	90 (3)	94 (2)	90 (5)	12
Metalaxyl	0.01	90 (7)	88 (7)	88 (3)	91 (3)	92 (6)	90 (5)	21
Metconazole	0.01	90 (19)	88 (9)	94 (3)	91 (7)	91 (8)	91 (10)	14
Methacriphos	0.01	91 (6)	88 (4)	89 (3)	82 (7)	91 (6)	88 (10)	14
Methidathion	0.01	78 (5)	88 (2)	88 (2)	92 (3)	94 (2)	88 (7)	16
Methiocarb	0.01	95 (6)	86 (1)	88 (2)	92 (3)	94 (2)	91 (5)	11
Methoxychlor	0.01	102 (5)	84 (3)	89 (2)	88 (5)	93 (5)	91 (8)	16
Metolachlor	0.01	91 (8)	85 (3)	88 (2)	89 (5)	93 (3)	89 (5)	12
Metribuzin	0.01	94 (7)	96 (2)	96 (3)	87 (1)	89 (6)	92 (6)	12
Mevinphos	0.01	73 (4)	84 (4)	86 (3)	87 (7)	93 (4)	85 (9)	20
Monocrotophos	0.01	88 (40	86 (5)	87 (4)	85 (2)	93 (5)	88 (6)	14
Myclobutanil	0.01	94 (4)	82 (5)	88 (1)	89 (2)	91 (2)	89 (5)	13
Napropamid	0.01	100 (5)	87 (3)	92 (3)	92 (6)	92 (5)	93 (6)	13
Nitrofen	0.01	91 (3)	86 (3)	89 (2)	90 (3)	92 (3)	90 (4)	10
Omethoate	0.01	81 (7)	80 (2)	79 (5)	85 (3)	86 (4)	82 (5)	15
Oxadixyl	0.01	84 (8)	88 (5)	91 (4)	91 (3)	92 (2)	89 (5)	13
Parathion	0.01	100 (5)	79 (3)	88 (3)	91 (3)	89 (5)	89 (8)	18
Parathion methyl	0.01	92 (6)	93 (3)	95 (3)	92 (1)	98 (16)	94 (8)	17
Penconazole	0.01	89 (7)	86 (9)	89 (2)	87 (3)	90 (2)	88 (4)	10
Pencycuron	0.01	82 (6)	89 (3)	84 (14)	88 (3)	92 (3)	87 (8)	17
Pendimethalin	0.01	95 (8)	80 (5)	82 (2)	88 (3)	96 (6)	89 (8)	18
Permethrin 1	0.05		86 (9)	88 (2)	91 (5)	89 (6)	89 (6)	14
Permethrin 2	0.05		85 (8)	88 (2)	91 (7)	94 (5)	90 (7)	15
Phenmedipham	0.01	86 (18)	92 (6)	74 (16)	88 (10)	94 (11)	87 (14)	30
Phosalone	0.01	86 (7)	88 (4)	88 (2)	91 (3)	95 (3)	89 (5)	12
Phosmet	0.01	88 (4)	86 (5)	91 (3)	89 (4)	90 (3)	89 (4)	10
Picoxystrobin	0.01	89 (11)	86 (6)	91 (2)	92 (4)	94 (5)	90 (7)	14
Pirimicarb	0.01	95 (9)	87 (3)	89 (3)	88 (5)	90 (4)	90 (6)	13
Pirimiphos-methyl	0.01	89 (6)	81 (4)	86 (3)	96 (4)	91 (3)	88 (7)	16
Prochloraz	0.01	89 (9)	81 (12)	89 (4)	87 (3)	83 (5)	86 (8)	18
Procymidone	0.01	90 (80	86 (5)	86 (2)	92 (3)	93 (8)	89 (6)	14
Profenofos	0.01	87 (13)	86 (6)	88 (3)	90 (4)	97 (6)	90 (8)	17
Prometryn	0.01	86 (13)	80 (3)	86 (4)	91 (4)	95 (4)	88 (9)	19
Propachlor	0.01	73 (5)	83 (5)	88 (2)	90 (7)	92 (4)	85 (9)	20
Propargite	0.05	94 (23)	92 (13)	94 (4)	94 (15)	94 (10)	93 (13)	27
Propham	0.01	77 (4)	85 (4)	86 (4)	85 (6)	93 (5)	85 (8)	17
Propiconazole 1	0.01	83 (8)	88 (9)	87 (2)	89 (14)	94 (10)	88 (10)	21

Table 3 (*Continued*)

Pesticide	LOQ, mg kg^{-1}	Recovery (RSD), %						*U*, % (*k* = 2)
		Red wine			White wine	Rose wine	Overall	
		0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.2 mg kg^{-1}	0.05 mg kg^{-1}	0.05 mg kg^{-1}	0.01–0.2 mg kg^{-1}	
Propiconazole 2	0.01	94 (14)	80 (5)	84 (7)	95 (7)	96 (8)	90 (11)	23
Propoxur	0.01	71 (10)	84 (4)	87 (3)	90 (9)	94 (3)	85 (11)	23
Propyzamide	0.01	85 (5)	82 (5)	87 (3)	89 (2)	92 (4)	87 (5)	13
Pyraclostrobin	0.01	80 (9)	85 (6)	89 (3)	88 (6)	93 (5)	87 (7)	16
Pyrazofos	0.01	87 (6)	86 (3)	87 (2)	90 (4)	95 (4)	89 (6)	13
Pyridaben	0.01	88 (3)	86 (3)	87 (3)	88 (4)	94 (2)	89 (4)	11
Pyrimethanil	0.01	94 (3)	83 (4)	86 (2)	90 (2)	91 (2)	89 (5)	12
Pyriproxyfen	0.01	89 (6)	93 (4)	90 (2)	91 (3)	97 (3)	92 (5)	11
Quinalphos	0.01	73 (4)	86 (4)	89 (2)	91 (1)	92 (3)	86 (8)	19
Quinoxyfen	0.01	94 (12)	88 (3)	89 (4)	89 (5)	91 (4)	90 (7)	14
Quintozene	0.01	87 (10)	78 (6)	83 (3)	84 (5)	90 (5)	84 (8)	18
Simazine	0.05		94 (12)	95 (7)	87 (11)	76 (14)	88 (13)	28
Spiroxamine 1	0.01	56 (19)	78 (8)	68 (17)	79 (10)	72 (7)	71 (16)	37
Spiroxamine 2	0.01	56 (18)	80 (9)	70 (20)	82 (8)	79 (9)	73 (18)	38
Tebuconazole	0.01	90 (8)	87 (3)	87 (2)	86 (4)	94 (2)	89 (5)	12
Tebufenpyrad	0.01	82 (8)	88 (4)	86 (2)	91 (2)	91 (3)	88 (6)	13
Tecnazene	0.01	90 (10)	79 (8)	85 (4)	81 (10)	90 (7)	85 (9)	20
Tetraconazole	0.01	87 (5)	88 (5)	90 (2)	93 (6)	99 (6)	91 (7)	14
Tetradifon	0.01	103 (19)	86 (19)	99 (3)	91 (4)	94 (3)	95 (13)	27
Thiabendazole	0.01	95 (13)	92 (6)	90 (8)	91 (17)	89 (4)	91 (10)	21
Thiamethoxam	0.01	101 (18)	86 (6)	84 (5)	90 (5)	95 (8)	91 (12)	24
Tolclofos-methyl	0.01	90 (7)	82 (4)	86 (2)	91 (3)	93 (4)	88 (6)	14
Tolylfluanid	0.01	90 (7)	89 (2)	90 (3)	90 (4)	93 (5)	92 (7)	14
Triadimefon	0.01	100 (7)	83 (4)	88 (4)	89 (5)	92 (6)	90 (8)	17
Triadimenol 1	0.01	88 (6)	85 (4)	87 (3)	89 (4)	93 (4)	88 (5)	12
Triadimenol 2	0.01	100 (9)	75 (3)	77 (7)	93 (5)	89 (8)	87 (13)	28
Triazophos	0.01	84 (7)	88 (2)	88 (2)	88 (2)	93 (4)	88 (5)	12
Trifloxystrobin	0.01	91 (6)	87 (5)	88 (2)	91 (6)	94 (1)	90 (5)	11
Triflumizol	0.01	94 (11)	90 (4)	90 (6)	87 (5)	82 (5)	88 (8)	17
Trifluralin	0.01	82 (8)	83 (8)	85 (5)	87 (6)	91 (3)	86 (7)	16
Triticonazole	0.01	89 (14)	89 (9)	88 (4)	93 (5)	94 (5)	91 (8)	17
Vinclozoline	0.01	85 (9)	89 (6)	90 (1)	89 (4)	91 (6)	89 (6)	13

Table 4
Pesticide residues detected in 30 wine samples.

Wine type	Country of origin	Pesticide residues, mg kg^{-1}			
Red	Australia				
Red	Bulgaria				
Red	Bulgaria				
Red	Bulgaria				
Red	France	Dimethomorph 0.013	Metalaxyl 0.009	Iprovalicarb 0.01	
Red	Germany	Boscalid 0.011	Dimethomorph 0.009	Iprovalicarb 0.022	Pyrimethanil 0.009
Red	Germany	Iprovalicarb 0.009			
Red	Hungary				
Red	Macedonia	Metalaxyl 0.015			
Red	Moldova	Iprovalicarb 0.008			
Red	Moldova				
Red	Poland	Azoxystrobin 0.08	Metalaxyl 0.04		
Red	Poland				
Red	Poland				
Red	Poland				
Red	Romania	Pyrimethanil 0.058			
Red	Spain				
Red	Spain				
Red	Spain	Triadimenol 0.005	Metalaxyl 0.011		
Red	Ukraine				
Red	USA	Boscalid 0.01			
Rose	France	Boscalid 0.005			
Rose	USA				
Rose	USA				
White	Chile	Tebuconazole 0.008			
White	Georgia	Metalaxyl 0.057			
White	Germany	Dimethomorph 0.01	Iprovalicarb 0.054	Pyrimethanil 0.056	
White	Hungary				
White	South Africa	Iprovalicarb 0.058			
White	South Africa	Dimethomorph 0.011	Iprovalicarb 0.011		

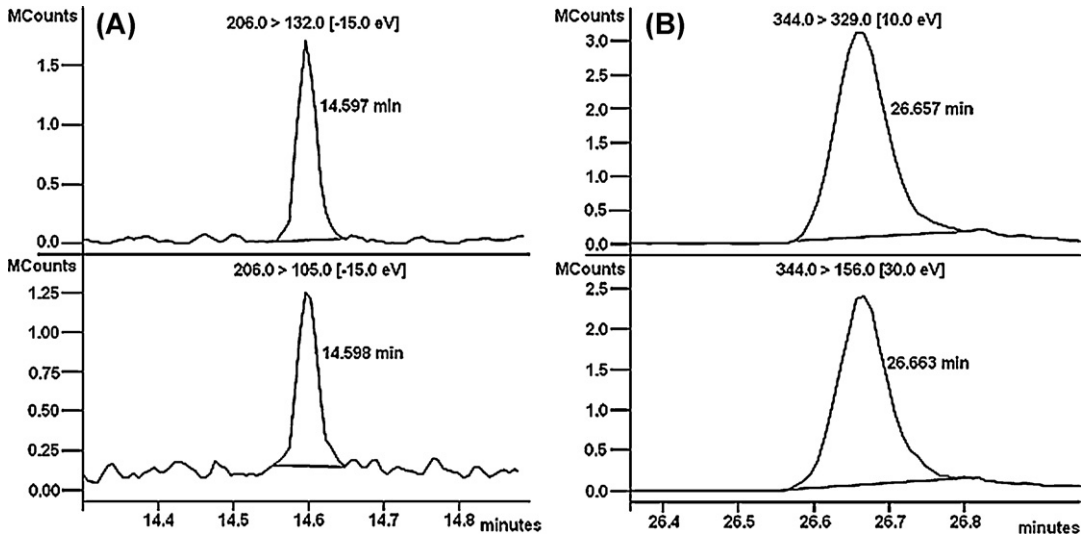

Fig. 5. GC-QqQ–MS/MS MRM chromatograms of a sample of red wine containing two pesticides: (A) metalaksyl (0.04 mg kg^{-1}) and (B) azoxystrobin (0.08 mg kg^{-1}).

transferred to the organic phase during the acetonitrile–water partition. On the other hand, all the recoveries, except for those of spiroxamine at 0.01 mg kg^{-1}, fell within the acceptance range of 70–120% with very good precision. Therefore, we decided that the slight bias of the quantification results can be considered as being negligible.

3.6. Measurement uncertainty

The measurement uncertainty was estimated according to the "top down" approach using the data obtained in the validation studies. As already mentioned, the most average recoveries showed some bias from the ideal recovery of 100%, it was then essential for us to include recovery in the calculation of the measurement uncertainty. Therefore, the uncertainty sources included in the uncertainty budget were the overall repeatability of analysis of spiked samples (measured as overall RSD) and uncertainty of the overall average recovery calculated from rectangular distribution [30]. The relative expanded uncertainty was then calculated by using the coverage factor $k = 2$ at the confidence level of 95%, and the results obtained for individual analytes are listed in Table 3. As seen, the calculated expanded uncertainties ranged between 9% (fenarimol, fludioxonil, epoxiconazole, DDD-pp′) and 38% (spiroxamine) with the average value being 17%. All the expanded uncertainty values are distinctively less than a default value of ±50% recommended by the SANCO/10684/2009 guidelines. This clearly demonstrates that the developed analytical method is suitable for its intended application.

3.7. Analysis of real samples

By the optimized and validated method, a total of 30 samples of commercial wines encompassing 22 red, 3 rose and 5 white wines were screened for pesticides residues. As seen in Table 4, the wines originating from 15 different countries were included in the survey. The years of the wines production were from 2005 to 2010. Overall, there were 10 different pesticides found in the analyzed wines at concentrations ranging from trace (<0.01 mg kg^{-1}) to 0.08 mg kg^{-1}. Of 30 analyzed samples, 15 contained pesticide residues. Six samples contained residues of more than one pesticide. The most prevalent compound was iprovalicarb (found in seven samples) followed by metalaxyl (found in five samples). Less frequent were: dimethomorph (present in four samples), and boscalid and pyrimethanil which were present in three samples. Azoxystrobin, tebuconazole and triadimenol were present each in one sample. As an example, Fig. 5 shows MRM chromatograms which reveal the presence of metalaxyl (0.04 mg kg^{-1}) and azoxystrobin (0.08 mg kg^{-1}) in a sample of red wine. All of the compounds encountered in the investigated samples were fungicides. Our results are consistent with the study conducted by Edder and Ortelli [27], where most of the analyzed wines contained pesticides, typically fungicides.

4. Conclusions

A multi-residue methodology through which a large number of pesticides in wines can be analyzed within a single chromatographic run of 30 min was successfully developed. The method was validated for a total of 161 pesticides, and the overall linearity, precision and trueness parameters were highly satisfactory. The optimization of the sample extraction procedure and application of mixed-mode dispersive-SPE for the cleanup step has resulted in significantly cleaner extracts and consistent recoveries even for problematic, base-sensitive pesticides such captan, folpet, tolylfluanid and dichlofluanid. It was revealed that in GC-QqQ–MS/MS analysis, matrix effects should be addressed in order to avoid inaccurate results and the use of matrix-matched standards rather than standards in pure solvent helped to circumvent matrix effects and improve quantification. The ruggedness of the method was demonstrated in the analysis of 30 real samples of commercial wines. Hence, we recommend the developed method as an effective approach for the surveillance of a wide range of pesticide residues in wines.

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References

[1] R.M. Gonzalez-Rodriguez, R. Rial-Otero, B. Cancho-Grande, C. Gonzalez-Barreiro, J. Simal-Gandara, Crit. Rev. Food Sci. Nutr. 51 (2011) 99–114.
[2] Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC., Off. J. Eur. Union L 70 (2005) 1–16.

[3] J. Bowers, J.M. Boursiquot, P. This, K. Chu, H. Johansson, C. Meredith, Science 285 (1999) 1562–1565.
[4] P. Caboni, P. Cabras, Adv. Food Nutr. Res. 59 (2010) 43–62.
[5] F. Čuš, H. Baša Česnik, Š. Velikonja Bolta, A. Gregorčič, Food Control 21 (2010) 1512–1518.
[6] H.H. Jeleń, A. Szczurek, Acta Sci. Pol. Technol. Aliment 9 (2010) 23–32.
[7] M. Dziadas, H.H. Jeleń, Anal. Chim. Acta 677 (2010) 43–49.
[8] A.L. Robinson, P.K. Boss, H. Heymann, P.S. Salomon, R.D. Trengove, J. Chromatogr. A 1218 (2011) 504–517.
[9] P. Edder, D. Ortelli, O. Viret, E. Cognard, A. De Montmollin, O. Zali, Food Addit. Contam. 26 (2009) 719–725.
[10] D.A. Lambropoulou, T.A. Albanis, Anal. Bioanal. Chem. 389 (2007) 1663–1683.
[11] R.M. González-Rodríguez, B. Cancho-Grande, J. Simal-Gándara, J. Chromatogr. A 1216 (2009) 6033–6042.
[12] A. Economou, H. Botitsi, S. Antoniou, D. Tsipi, J. Chromatogr. A 1216 (2009) 5856–5867.
[13] I. Carpinteiro, M. Ramil, I. Rodríquez, R. Cela, J. Chromatogr. A 1217 (2010) 7484–7892.
[14] C.G. Zambonin, M. Quinto, N. De Vietro, F. Palmisano, Food Chem. 86 (2004) 269–274.
[15] P. Viñas, N. Martínez-Castillo, N. Campillo, M. Hernández-Cordoba, J. Chromatogr. A 1217 (2010) 6569–6577.
[16] A. Garbi, V. Sakkas, Y.C. Fiamegos, C.D. Stalikas, T. Albanis, A. Economou, H. Botitsi, S. Antoniou, D. Tsipi, Talanta 82 (2010) 1286–1291.
[17] R. Montes, I. Rodríguez, M. Ramil, E. Rubí, R. Cela, J. Chromatogr. A 1216 (2009) 5459–5466.
[18] S.J. Lehotay, K. Ae Son, H. Kwon, U. Koesukwiwat, W. Fu, K. Maštovská, E. Hoh, N. Leepipatpiboon, J. Chromatogr. A 1217 (2010) 2548–2560.
[19] K. Zhang, J.W. Wong, D.G. Hayward, P. Sheladia, A.J. Krynitsky, F.J. Schenck, M.G. Webster, J.A. Ammann, S.E. Ebeler, J. Agric. Food Chem. 57 (2009) 4019–4029.
[20] Y. Jiang, X. Li, J. Xu, C. Pan, J. Zhang, W. Niu, Food Addit. Contam. 26 (2009) 859–866.
[21] S.C. Cunha, J.O. Fernandes, A. Alves, M.B.P.P. Oliveira, J. Chromatogr. A 1216 (2009) 119–126.
[22] S.H. Patil, K. Banerjee, S. Dasgupta, D.P. Oulkar, S.B. Patil, M.R. Jadhav, R.H. Savant, P.G. Adsule, M.B. Deshmukh, J. Chromatogr. A 1216 (2009) 2307–2319.
[23] S. Dasgupta, K. Banerjee, S.H. Patil, M. Ghaste, K.N. Dhumal, P.G. Adsule, J. Chromatogr. A 1217 (2010) 3881–3889.
[24] S. Dasgupta, K. Banerjee, K.N. Dhumal, P.G. Adsule, J. AOAC Int. 1 (2011) 273–285.
[25] Document No. SANCO/10684/2009, Method validation and quality control procedures for pesticide residues analysis in food and feed, European Commission, Brussels, 2009.
[26] J.F. Tomera, J. Food Sci. Technol. 10 (1999) 129–138.
[27] P. Edder, D. Ortelli, Mitt. Lebensm. Hyg. 96 (5) (2005) 311–320.
[28] B. Gilbert-López, J.F. Garcia-Reyes, P. Ortega-Barrales, A. Molina-Diaz, A.R. Fernánzez-Alba, Rapid Commun. Mass Spectrom. 21 (2007) 2059–2071.
[29] C.F. Poole, J. Chromatogr. A 1158 (2007) 241–250.
[30] R. Štěpán, J. Hajšlová, V. Kocourek, J. Tichá, Anal. Chim. Acta 520 (2004) 245–255.