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Gas chromatographic–tandem mass spectrometric analysis of pesticides residues in produce using concurrent solvent recondensation-large volume injection

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ABSTRACT

In the present work, the feasibility of the combined use of concurrent solvent recondensation-large volume injection (CSR-LVI) and interspersed calibration for pesticide residue analysis was investigated. Splitless injections of 5–20 μL extracts containing 0.25–1 g sample per mL^{-1} were made into a Carbofrit packed liner and a 0.53 mm I.D., uncoated and deactivated retention gap. The determination was achieved by gas chromatography–tandem quadrupole mass spectrometry (GC–QqQ-MS/MS). The evaluation of the proposed approach was based on analysis of real samples representing a diverse range of commodities such as apples, barley malt, blackcurrants, carrots, clemetines, grapes, leek, plums, rapeseed (green plants) rucola, strawberries and tomatoes. The samples contained a total of 36 different incurred pesticides at different concentration levels. Also, analyses were carried out of artificial samples representing six differing matrices (apples, blackcurrants, carrots, huckleberry, strawberry and tomatoes) which were spiked with pesticides at known concentrations before proceeding with the extraction. When using 15 and 20 μL CSR-LVI injection, a decrease of about 30% in peak heights compared with injection of 5 μL was observed. In the case of 5 and 10 μL injections, no significant difference was observed when employing to the quantification of the incurred and spiked pesticide residues. In the evaluated experimental variants, overall recoveries of the pesticides were 92 ± 5% with relative standard deviations of 12 ± 4% on average. All individual recoveries were in the range between 72 and 103 with RSD between 4 and 21%. About 15% of the instrument run time was saved by the application of interspersed calibration with standards injected between sample extracts.

1. Introduction

Classical isothermal splitless injection is still the most widely used sample introduction technique in analysis of trace components such as pesticide residues by gas chromatography. In this process, the sample extract is rapidly vaporized in the injector liner which is placed in a chamber kept at a temperature of 200–300 °C, then the vapors are transferred to the column by the carrier gas. Since the vapors are stored in the liner until the transfer is complete (the split vent is switched off), the injection volume of sample extract is limited by the liner volume. The injector liners are available in various shapes and sizes (e.g. straight, tapered, dimpled, buffled), and their internal volume can differ to some extent. In addition, the resulting volume of the vaporized sample extract is dependent on the solvent type, injection temperature and inlet pressure. A typical solvent introduction volume in classical splitless injection is 1–2 μL [1].

With increased public concern over potential health hazards associated with pesticide residues in food, many research efforts are directed at the development of highly sensitive and selective analytical procedures to determine pesticides in a variety of food matrices. In gas chromatographic analysis of trace components, the need to significantly increase sensitivity requires that larger volumes of sample extracts are injected. Due to the demand for lower detection limits, and the ability to resolve target analytes from matrix coextractives potentially present in sample extracts, the performance of the chromatographic system is often limited by either the detection technique or compatibility of the injection port. Key issues when selecting an injector type include properties of the analyte, such as potential for thermal degradation and the ability of the GC systems to handle large volume injections (LVI) [2].

At the present time, the sample preparation technique known as "quick, easy, cheap, effective, rugged and safe" procedure (QuEChERS) which is based extraction and liquid–liquid partitioning with acetonitrile followed by a dispersive solid phase extraction (dispersive-SPE) clean-up step, has become a prevailing technique in multiresidue analysis of pesticides in food such as vegetables and fruits [3], cereals [4], green leafy vegetables [5], olives and olive oil

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Table 1
GC–MS/MS acquisition method conditions for 36 target pesticides.

Pesticide	Retention time (min)	MRM transitions, *m/z* (CE, eV)		Dwell time (ms)
		Quantification	Identification	
Pyrimethanil	13.45	198 > 118 (30)	198 > 158 (25)	50
Pirimicarb	13.77	238 > 166 (10)	238 > 72 (25)	50
Chlorpyrifos-methyl	14.34	288 > 93 (20)	286 > 286 (10)	22
Metalaxyl	14.59	206 > 132 (15)	206 > 105 (15)	22
Pirimiphos-methyl	14.81	290 > 151 (15)	305 > 180 (10)	22
Chlorpyrifos	15.25	314 > 258 (15)	314 > 286 (15)	18
Tetraconazole	15.38	336 > 218 (15)	336 > 183 (30)	22
Dicofol	15.65	139 > 111 (10)	139 > 75 (25)	22
Pendimethalin	15.91	252 > 162 (15)	252 > 191 (15)	22
Cyprodinil	15.95	225 > 224 (10)	225 > 208 (15)	22
Procymidone	16.32	283 > 96 (10)	283 > 67 (20)	22
Thiabendazole	16.42	201 > 174 (15)	201 > 130 (25)	22
Endosulphan-alfa	17.03	241 > 206 (10)	272 > 237 (15)	20
Fludioxonil	17.04	248 > 127 (30)	248 > 154 (20)	20
Bupirymate	17.30	273 > 193 (10)	273 > 108 (15)	22
DDE-pp′	17.31	246 > 176 (25)	318 > 248 (15)	22
Flusilazole	17.34	233 > 165 (15)	233 > 152 (15)	22
DDD-pp′	18.14	235 > 165 (20)	235 > 199 (20)	25
Endosulfan-beta	18.18	241 > 206 (10)	241 > 170 (20)	25
DDT-op′	18.21	235 > 165 (20)	235 > 199 (20)	25
Propiconazole 1	18.66	259 > 69 (10)	259 > 173 (15)	33
Propiconazole 2	18.77	259 > 69 (10)	259 > 173 (15)	33
DDT-pp′	18.87	235 > 165 (20)	235 > 199 (20)	33
Enosulfan-sulphate	18.90	272 > 237 (15)	241 > 206 (10)	21
Fenhexamid	18.92	301 > 97 (15)	301 > 266 (5)	21
Propargite	19.04	350 > 81 (15)	350 > 201 (5)	21
Tebuconazole	19.13	250 > 125 (20)	250 > 70 (10)	27
Bifenthrin	19.60	181 > 166 (10)	181 > 165 (20)	27
Fenazaquin	20.12	145 > 117 (10)	160 > 145 (10)	40
Cyhalothrin-lambda 1	20.39	197 > 141 (10)	197 > 161 (5)	25
Cyhalothrin-lambda 2	20.58	197 > 141 (10)	197 > 161 (5)	25
Cypermethrin 1	23.01	165 > 127 (5)	181 > 152 (15)	29
Cypermethrin 2	23.13	165 > 127 (5)	181 > 152 (15)	29
Boscalid	23.14	140 > 112 (10)	140 > 76 (20)	44
Pyraclostrobin	24.77	164 > 132 (20)	132 > 77 (20)	100
Difenoconazole 1	25.59	265 > 139 (25)	323 > 265 (10)	67
Indoxacarb	25.71	264 > 176 (10)	264 > 148 (15)	67
Difenoconazole 2	25.74	265 > 139 (25)	323 > 265 (10)	67
Deltamethrin 1	25.76	253 > 174 (10)	253 > 172 (5)	67
Deltamethrin 2	26.25	253 > 174 (10)	253 > 172 (5)	67
Azoxystrobin	26.61	344 > 329 (10)	344 > 156 (30)	80

[6], and soil [7]. The major drawback of this technique is the poor enrichment factor compared with other earlier developed methods for pesticide multiresidue analysis [8].

For the injection of large volumes up to hundreds of μL of sample extract, on-column and programmable temperature vaporizing (PTV) injection techniques have been used [2,9–11]. The most critical issue in LVI injection is a huge solvent vapor volume resulting from the expansion of the high amount of the injected solvent. In case of on-column injection this problem is solved by using a retention gap, which provides room for the large volume of the injected solvent to condense and expand [12] while in case of PTV injection, separation of the solvent vapor from analytes is done through venting of the vapor in the injector port. [13].

In order to be able to attain lower reporting levels when injecting diluted extracts, large volume injections can be carried out to transfer more analyte onto the GC column than with traditional splitless injection, while eliminating the interference effects of excess solvent. When large volume injections are made by using programmable temperature vaporizing (PTV) injectors, they can be equipped with cryogenic cooling to reduce equilibration time before reaching the initial temperature of the programme [14]. In this technique, the sample is injected at a slow rate while the injector temperature is set a few degrees below the injection solvent boiling point (e.g. 75 °C in case of ethyl acetate having a boiling temperature of 77 °C). The split valve is left open for a period of time to vent the solvent then it is closed when the injector is rapidly heated to vaporize the solute material onto the GC column where the separation of analytes is made. Another approach for large volume injection is on-column injection technique which allows to introduce high volumes of extracts by using a retention gap before the analytical column. The main advantage of on-column injection is reduction of breakdown of thermally labile compounds. Its principal disadvantage is the difficulty to inject dirty samples without loosing inertness and efficiency of the chromatographic system. In pesticide residue analysis this technique is rather rarely used [12].

Concurrent solvent recondensation (CRS) is another alternative technique for large volume injection. This is a splitless injection technique which is based on utilization of a high pressure increase in the injector port resulting from expansion of the solvent vapors which accelerate transfer of the sample from the injector into the uncoated precolumn by recondensation of the solvent. With liquid band formation, the sample vapors are transferred into the gas chromatography column as rapidly as they are formed in the injector. The sample transfer is fast because of concurrent recondensation of the solvent, obtained by keeping the oven temperature below the solvent boiling point [15,16].

The present paper describes the application of CSR-LVI injection as a tool to overcome the limitation of a maximum injection volume of 1–2 μL with classical splitless injection technique. Experiments were conducted based on analysis of real and artificial (spiked) samples representing a diverse range of commodities and a total of 36 representative pesticides at different concentration levels. An

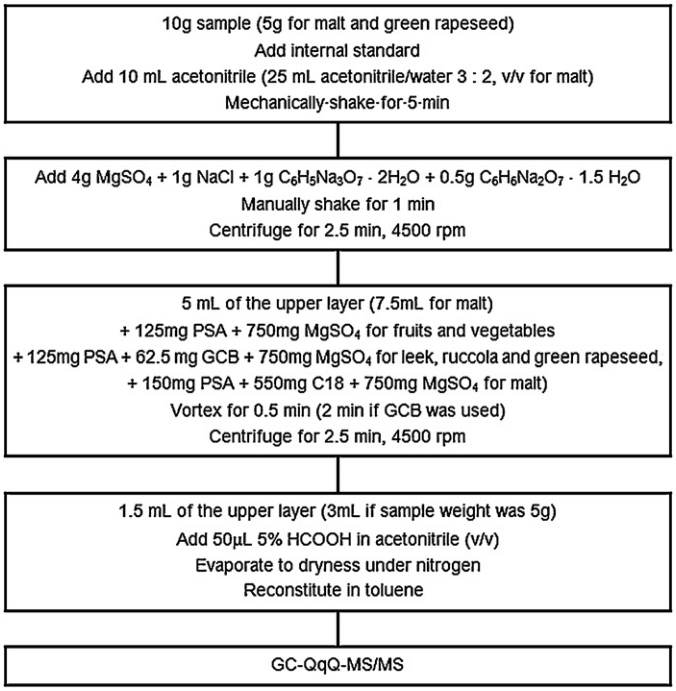

Fig. 1. Scheme of the acetonitrile extraction-based method for sample preparation before CSR-LVI GC–QqQ-MS/MS analysis.

additional aspect included in this study was to carry out a comparison of performance of two different calibration approaches, namely bracketing and interspersed calibration, in terms of results' accuracy and overall analysis time. In this paper, the comprehensive investigation of the combined use of CSR-LVI technique and interspersed calibration approach for GC–QqQ-MS/MS multiresidue analysis of pesticides in produce is described for the first time.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile and acetone (for residue analysis) was purchased from S. Witko (Łódź, Poland). Toluene (for residue analysis) and formic acid (ACS grade) were purchased from Merck (Darmstadt, Germany). Anhydrous magnesium sulphate (reagent grade), sodium citrate tribasic dihydrate (ACS reagent), and di-sodium hydrogen citrate sesquihydrate (pure) were all purchased from Sigma–Aldrich Sp. z o.o. (Poznań, Poland). Pure sodium chlorine was purchased from POCH (Gliwice, Poland). Bondesil PSA (40 μm) sorbent was purchased from Candela (Warszawa, Poland), C18 (50 μm) sorbent was purchased from Anaserwis (Poznań, Poland) and EnviCarb sorbent (120/400 sieved fraction) was purchased from Sigma–Aldrich Sp. z o.o (Poznań, Poland).

2.2. Analytical standards

Certified pesticide analytical standards of the highest purity available were purchased from Dr. Ehrenstorfer (Ausburg, Germany). Internal standard – triphenylphosphate (TPP) was purchased from Sigma–Aldrich Sp. z o.o. (Poznań, Poland). Pesticide stock solutions were prepared at approximate concentrations of 1000 μg mL^{-1}. Purity of the pesticide standard was taken into account when calculating actual concentrations of standard solutions. Of these stock solutions, a single composite mixture of all pesticides was prepared. Subsequent dilutions were made to obtain working standards. The single composite mixtures at appropriate concentrations were used to calibrate the GC–QqQ-MS/MS instrument and spike samples in recovery experiments.

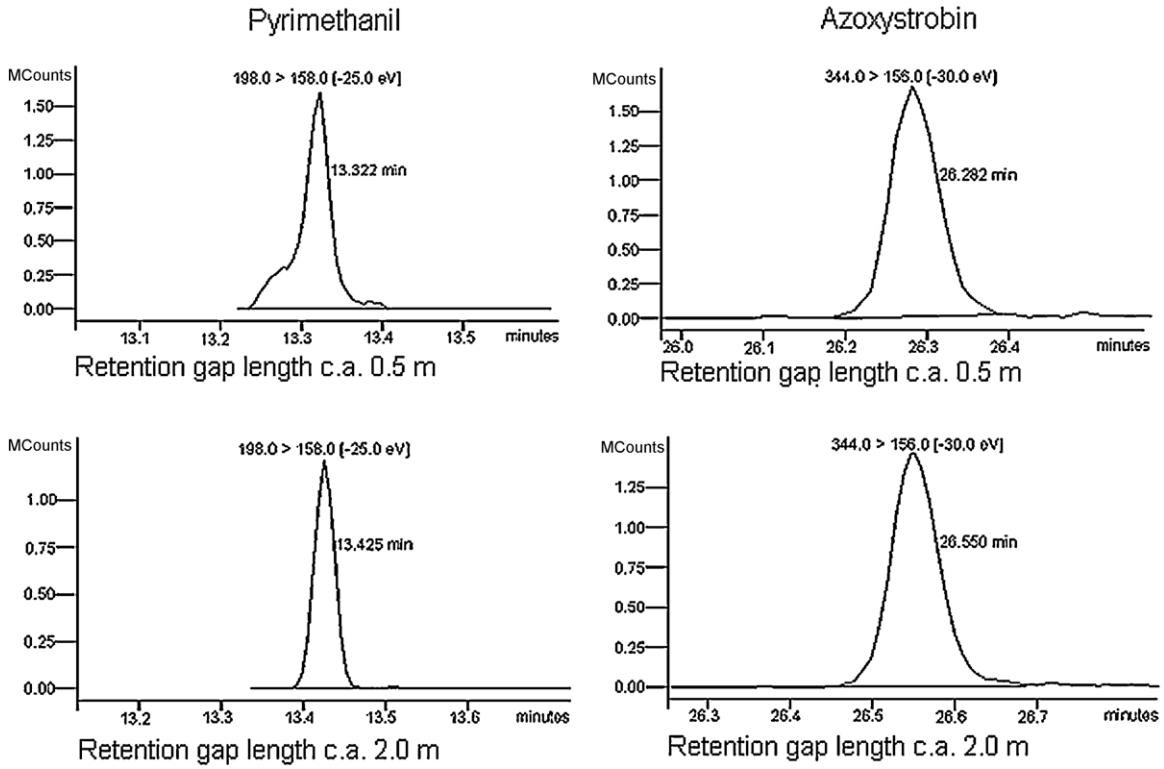

Fig. 2. Influence of retention gap length on the peak shapes of pyrimethanil and azoxystrobin.

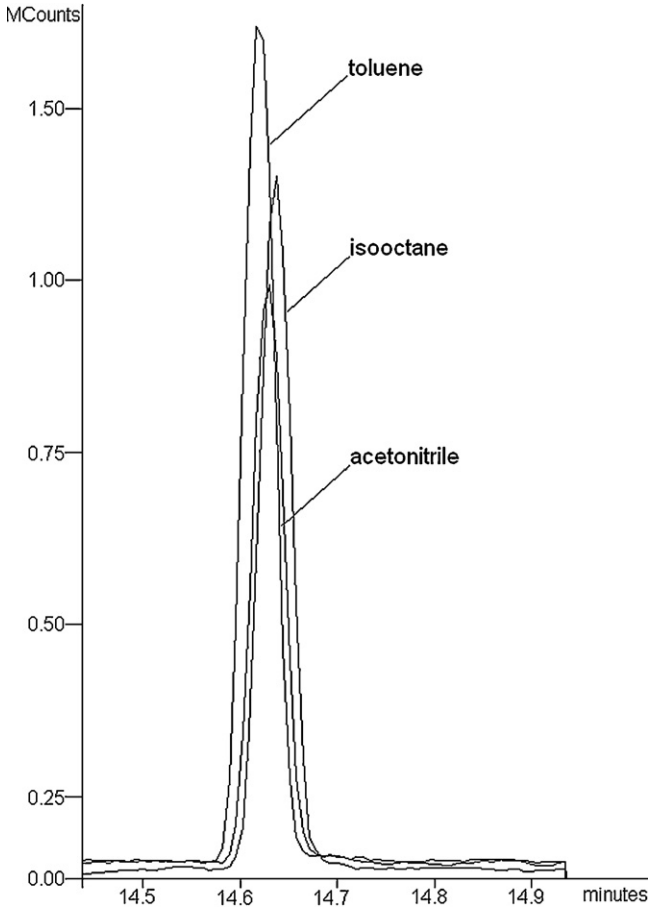

Fig. 3. Peak heights of pirimiphos-methyl (transition 305 > 180) obtained by injections of 5 μL standard at 0.05 μg mL^{-1} prepared in toluene, acetonitrile and isooctane.

2.3. GC–QqQ-MS/MS conditions

GC–QqQ-MS/MS analysis was carried out on a CP-3800 series gas chromatograph coupled with a 1200 triple quadrupole mass spectrometer (Varian Inc., Middelburg, Netherlands). The system was equipped with electronic flow control (EFC), a 1079 universal capillary injector, and a CP-8400 autosampler. Analytes were separated on a DB-5 MS 30 m × 0.25 mm × 0.5 μm column (Agilent Technologies, Folsom, USA) protected by a 2 m × 0.53 mm guard column of uncoated fused silica at the inlet end. The column head pressure was initially 11.6 psi for 3 min, then 1.87 psi min^{-1} to 16 psi and 0.6 psi min^{-1} to 24.9 psi mL min, then held for 10 min, using helium as the carrier gas. The column temperature was held at 80 °C for 3 min after injection then programmed at 30 °C min^{-1} to 150 °C, then programmed to 300 °C at 10 °C min^{-1} which was held for 10 min. For large-volume CSR injections, the injector port temperature was held at 250 °C for 1 min, then increased to 300 °C at 200 °C min^{-1}, which was held for 20 min. The initial split ratio was set at 20:1, at 0.01 min the split vent was closed until 1.5 min, then the split ratio was held at 100:1 until 20 min, and finally reduced to 20:1. For large-volume PTV injections, the injection port was held at 100 °C for 1 min, then increased to 300 °C at 200 °C min^{-1}, which was held for 20 min. The initial split ratio was set at 50:1, at 0.5 min the split vent was closed until 5 min, then the split ratio was held at 20:1. In both cases, the injector liner was single tapered, 5.4 mm × 3.4 mm I.D. A Carbofrit plug (Restek, Bellefonte, USA) was inserted into the injector liner. Sample extract (5–20 μL) were injected in toluene.

The triple quadrupole mass spectrometer was operated in the electron ionization mode (EI, 70 eV). The filament current was 150 μA. The multiplier voltage was set as determined by the autotune procedure (1700 V). The mass spectrometer was autotuned at least monthly with perfluorotributylamine (PFTBA) as the calibration compound. The temperatures of the transfer line, ion source and manifold were set at 290 °C, 270 °C, and 40 °C, respectively. For the MS/MS mode, argon was used as the collision gas using the collision cell pressure at 1.7 mTorr. Multiple reaction monitoring (MRM) conditions were experimentally developed for each individual pesticide on the instrument used in this work. The optimal MRM transitions (primary and secondary transitions of a precursor to product ion) for each pesticide and other parameters used are detailed in Table 1. For instrument control, data acquisition and processing, the Varian MS Workstation, version 6.6 was used.

2.4. Sample preparation procedures

The procedures based on acetonitrile extraction and liquid–liquid partitioning followed by dispersive-SPE cleanup were described previously [3–5], and are shown schematically in Fig. 1. Briefly, an amount of sample (10 g for fruits and vegetables or 5 g for barley malt and green rapeseed) was weighted into a 50 mL polypropylene centrifuge tube. A volume of acetonitrile (10 mL) or acetonitrile:water in the case of barley malt (25 mL, 3:2, v/v) was added and mechanical shaking was performed during 5 min. Then, 4 g anhydrous magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate were added and immediately vigorous manual shaking was performed for 1 min, and centrifugation at 4500 rpm was performed for 2.5 min. An aliquot of acetonitrile layer (5 mL for all matrices and 7.5 mL for barley malt) was transferred into a 15 mL polypropylene centrifuge tube containing preweighed dispersive-SPE agents (125 mg PSA and 750 mg $MgSO_4$ for fruits and vegetables, 125 mg PSA, 62.5 mg GCB and 750 mg $MgSO_4$ for leek, ruccola and green rapeseed or 150 mg PSA, 550 mg and 750 mg $MgSO_4$ for barley malt). The tubes were vortexed for 0.5 min (2 min if GCB was used) and centrifuged 2.5 min at 4500 rpm. An aliquot of the upper layer (1.5 mL if the sample weight was 10 g or 3 mL if the sample weight was 5 g) was acidified with 50 μL 5% formic acid in acetonitrile and evaporated under a stream of nitrogen, then reconstituted in toluene (1.5 mL) prior to GC–QqQ-MS/MS analysis.

2.5. Samples and extracts

Real samples for the study were selected of those previously tested to containing multiple pesticide residues. The samples represented such a diverse range of commodities as apples, barley malt, blackcurrants, carrots, clemetines, grapes, leek, plums, rapeseed (green plants) rucola, strawberries and tomatoes. They contained a total of 36 different incurred compounds at different concentration levels (azoxystrobin, bifenthrin, boscalid, bupirimate, chlorpyrifos, chlorpyrifos-methyl, cyhalothrin-lambda, cypermethrin, cyprodinil, DDD-pp′, DDE-pp′, DDT-pp′, DDT-op′, deltamethrin, dicofol, difenoconazole, endosulfan-alfa, endosulfan-beta, endosulfan-sulphate, fenazaquin, fenhexamid, fludioxonil, flusilazole, indoxacarb, metalaxyl, pendimethalin, pirimicarb, pirimiphos-methyl, procymidone, propargite, propiconazole, pyraclostrobin, pyrimethanil, tebuconazole, tetraconazole, thiabendazole). Artificial samples were made by spiking six different matrices (apples, blackcurrants, carrots, huckleberries, strawberries and tomatoes) at 0.05 mg kg^{-1} before proceeding with the sample preparation procedure. The spiked samples were analyzed together with real samples. When received in the laboratory, all samples were well comminuted,

Table 2
Comparison of CSR-LVI and PTV-LVI responses of the target pesticides as obtained by injecting 5 μL of matrix-matched standard mixture at 200 ng mL^{-1} (6 degrees of freedom, $p = 0.05$).

Pesticide	CSR-LVI		PTV-LVI		Student's *t*-test value	
	Average	St. dev.	Average	St. dev.	Calculated	Table
Azoxystrobin	160.2	28.6	294.7	27.6	6.763	2.447
Bifenthrin	209.0	22.2	216.5	13.1	0.576	2.447
Boscalid	191.5	10.3	281.7	20.0	8.006	2.447
Bupirymate	236.6	24.7	299.4	15.2	4.323	2.447
Chlorpyrifos	202.8	7.1	131.6	21.4	6.311	2.447
Chlorpyrifos-methyl	193.4	12.4	48.3	11.6	17.067	2.447
Cyhalothrin-lambda	205.8	26.7	146.9	26.2	3.149	2.447
Cypermethrin	226.1	26.9	182.3	23.4	2.454	2.447
Cyprodinil	195.8	19.6	191.1	7.5	0.452	2.447
DDD-pp′	210.0	23.5	146.7	14.8	4.553	2.447
DDE-pp′	188.5	16.5	203.1	5.5	1.682	2.447
DDT-op′	172.1	22.6	55.7	7.4	9.800	2.447
DDT-pp′	170.1	18.7	31.7	6.2	14.021	2.447
Deltamethrin	252.5	30.8	190.5	31.3	2.823	2.447
Dicofol	187.6	21.1	167.1	7.4	1.826	2.447
Difenoconazole	204.7	7.0	458.5	18.6	25.568	2.447
Endosulfan-alfa	202.5	24.8	208.6	9.3	0.464	2.447
Endosulphan-beta	200.1	19.7	166.7	7.9	3.141	2.447
Enosulfan-sulphate	199.2	18.3	46.4	11.5	14.130	2.447
Fenazaquin	197.4	20.3	206.4	6.9	0.847	2.447
Fenhexamid	197.6	11.6	180.8	27.6	1.122	2.447
Fludioxonil	207.9	16.2	244.4	16.1	3.198	2.447
Flusilazole	194.3	18.0	201.9	6.3	0.794	2.447
Indoxacarb	202.1	10.9	85.2	22.4	9.370	2.447
Metalaxyl	188.4	22.8	190.7	8.3	0.190	2.447
Pendimethalin	191.2	14.4	177.0	8.6	1.683	2.447
Pirimicarb	187.0	21.1	188.3	8.1	0.118	2.447
Pirimiphos-methyl	197.8	21.9	125.3	10.9	5.928	2.447
Procymidone	200.2	19.6	200.3	6.9	0.010	2.447
Propargite	201.7	20.2	58.5	7.3	13.357	2.447
Propiconazole	197.0	18.9	195.2	14.0	0.157	2.447
Pyraclostrobin	207.9	13.3	9.5	4.6	28.238	2.447
Pyrimethanil	188.4	19.9	184.9	6.6	0.335	2.447
Tebuconazole	197.2	19.8	219.6	6.9	2.127	2.447
Tetraconazole	197.4	20.5	215.6	7.0	1.682	2.447
Thiabendazole	187.3	18.3	258.6	35.4	3.576	2.447

placed in plastic storage bags, and stored at −20 °C until analysis. Also, final sample extracts in toluene were stored at −20 °C when needed for re-analysis.

2.6. GC–QqQ-MS/MS data treatment

Quantification by GC–QqQ-MS/MS was carried out with matrix-matched multi-level calibration curves. Matrix-matched calibration standards were prepared in mixed, equal volumes of carrot, huckleberry and tomato extracts. The samples were previously checked for the absence of pesticides under the conditions of the study. Three sets of calibration standards were prepared: (1) 0.01, 0.05, 0.2 and 0.5 μg mL^{-1}, (2) 0.005, 0.025, 0.1 and 0.25 μg mL^{-1}, (3) 0.0033, 0.017, 0.067 and 0.17 μg mL^{-1}. Volumes corresponding to 0.01, 0.05, 0.2 and 0.5 mg kg^{-1} pesticides content in samples were injected into the GC–QqQ-MS/MS system. Triphenylphosphate (TPP) was used as the internal standard (I.S.). Calculations were based on the peak area ratios of the primary MRM transition of the analyte to that of the transition of the I.S., and compared with concentrations of matrix-matched calibration standards by using the MS Workstation software.

3. Results and discussion

3.1. Optimization of CSR-LVI conditions – general considerations

In the present study, the investigated approach attempts to overcome a limitation of a maximum volume of 1–2 μL for injection with classical splitless technique by utilizing of the technique named concurrent solvent recondensation large volume injection (CSR-LVI). This work was inspired by Magni and Porzano [15] and Biedermann et al. [16] who used fast autosampler injection, wool plug in the liner and a retention gap (e.g. 5 m × 0.32 mm I.D.) to perform injections with liquid band formation to accept large volumes of solvent in a relatively simple and straightforward way. The authors evaluated the performance of the CSR-LVI injection for the analysis of polynuclear aromatic hydrocarbons by GC–FID and GC–MS. In this study, the CSR-LVI technique was evaluated for multi-residue, multi-matrix analysis of pesticides by GC–QqQ-MS/MS. The CSR-LVI injection is possible due to recondensation of the solvent inside of the capillary column causing a pressure drop at the beginning of the column. A pressure difference which occurs between column and injector liner significantly accelerates the sample transfer. The sample and solvent vapor is withdrawn from the liner by the lower pressure inside of the column and continuously condensed to form the liquid band at the beginning of the column. For the CSR technique, a fast autosampler injection is necessary to suppress evaporation inside the needle and cause the sample to leave the needle as a band.

For practical application of the CSR technique, several parameters such as liner type, liner packing material, retention gap length and temperatures of injection port and column oven had to be carefully selected. An important consideration was the choice of the packing material in the injector liner. The most frequently used injector liners are usually packed with glass wool but they are often prone to thermal degradation or adsorption of pesticides. In addition a reduced signal intensity and peak tailing are often observed when analyte interacts with active sites within liner materials [17].

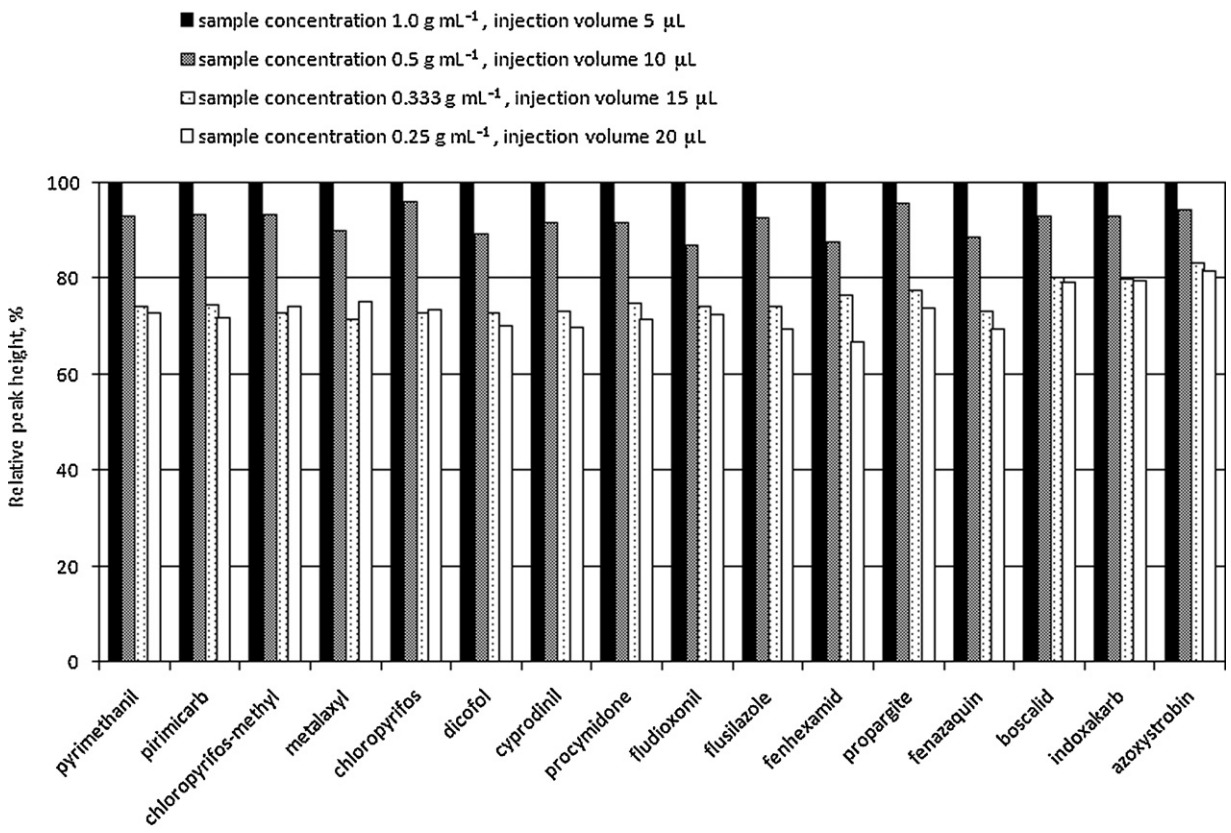

Fig. 4. Dependence of peak heights of selected pesticides representing a broad volatility range on different injected volumes of toluene extracts (5–20 µL) at different sample concentrations (1–0.25 g mL^{-1}).

An alternative injector liner packing material is Carbofrit which can be used for improved vaporization and lower pressure drop in the GC injector. Carbofrit is considered to be superior to glass wool packing because it is characterized by good inertness, high temperature stability, and capability of trapping high molecular weight contaminants which is of particular importance when injecting extracts of complex food samples [18–20]. When autosamplers make fast injections, if there is no packing material in the liner samples can be incompletely vaporized, leading to non-reproducible peak response results. This phenomenon can be compared to water droplets on a hot surface which bounce around until vaporized. A plug of packing material placed in the liner helps to prevent this phenomenon as it provides a surface for the solvent droplets to "sit" on until vaporized by the heat from the injector. The Carbofrit plug was conditioned inside the injector port by heating at 300 °C for 2 h with an increased split ratio. Subsequent priming of the GC with matrix extract prior to running a batch of samples was necessary to stabilize the system. Consistent responses were obtained after at least two injections.

Uncoated and deactivated retention gaps are generally used to enhance analyte focusing and to increase the height of chromatographic peaks [21]. The retention gap, which also serves as a guard column, can provide an additional protection and extend the lifetime of the analytical column in cases where sample extracts contain large amounts of coextractives from matrix [3–8,12,14,17,18,21–24]. The use of the retention gap is therefore an advantageous option in GC-based pesticide residue analysis in complex matrices but it is a must when CSR-LVI technique is used. The retention gap requires maintenance as the top of the retention gap must be cut off periodically and eventually replaced entirely. In this study, the retention gap of approximate length of 2 m and 0.53 mm I.D. was initially installed. After running of a batch of samples, typically encompassing 30–40 matrix containing extracts injections, a piece of 10–15 cm of the retention gap was trimmed. We found that when the retention gap became as short as approximately 0.5 m, the chromatographic peaks got distorted (discriminated). The peaks of earlier eluting compounds were more affected by this effect than those of the late eluting compounds (Fig. 2). The use of retention gap of the appropriate length was then essential for the efficient use of the CSR-LVI injection technique to allow injections of large volumes of the solvent. The retention gap precolumn must have sufficient capacity to retain most of the sample as a liquid and avoid the occurrence of discrimination of volatile compounds.

When using QuEChERS-based sample preparation methods, the extracts is obtained in acetonitrile. Acetonitrile can be accepted as a medium for the GC injection, however it is characterized by high expansion coefficient and poor focusing of chromatographic peaks caused by the high polarity of acetonitrile [17]. If sensitivity is an issue in splitless injection, then toluene can be considered to be the best exchange solvent due to its miscibility with acetonitrile, good solubility for wide range of pesticides and good responses of troublesome pesticides. As shown in Fig. 3, the use of toluene as the injection solvent can significantly increase the peak heights. In addition to that, very low volatility of toluene (boiling point 111 °C at 1 atm) make this solvent highly suitable for long-term storage of pesticide standards and sample extracts. For the CSR-LVI injection, recondensation of the solvent was starting the oven temperature programme below the solvent boiling point. At the time of injection, the injector port initial temperature was kept at 250 °C, then it was raised to 300 °C and held for 20 min to clean the Carbofrit while the split vent was open. Meanwhile, column oven temperature started

Table 3
Linearity parameters (R^2 and slope) in the range corresponding to 0.01–0.5 mg kg^{-1}, determined by using standards in toluene and matrix matched (mixed equal volumes of carrot, huckleberry and tomato extracts) as well as matrix effects measured as 100 × (1 − slope toluene/slope matrix). *A* – matrix concentration 1 g mL^{-1}, injection volume 5 μL, concentration range 0.01–0.5 μg mL^{-1}, *B* – matrix concentration 0.5 g mL^{-1}, injection volume 10 μL, concentration range 0.005–0.25 μg mL^{-1} and *C* – matrix concentration 0.333 g mL^{-1}, injection volume 15 μL, concentration range 0.0033–0.167 μg mL^{-1}.

Pesticide	Toluene		Slope			R^2			Matrix effect		
	R^2	Slope	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
Azoxystrobin	0.9961	0.000940	0.000918	0.001000	0.000923	0.9940	0.9940	0.9988	2	6	−2
Bifenthrin	0.9999	0.003800	0.003300	0.003300	0.003200	0.9991	0.9991	0.9998	13	−13	−16
Boscalid	0.9990	0.002500	0.002500	0.002700	0.002300	0.9977	0.9977	0.9999	0	8	−8
Bupirimate	0.9989	0.000661	0.000569	0.000587	0.000571	0.9997	0.9997	0.9994	14	−11	−14
Chlorpyrifos	0.9981	0.000438	0.000426	0.000402	0.000435	0.9994	0.9994	0.9993	3	−8	−1
Chlorpyrifos-methyl	0.9982	0.000968	0.000896	0.000884	0.000863	0.9988	0.9988	0.9999	7	−9	−11
Cypermethrin 1	0.9975	0.000240	0.000205	0.000244	0.000203	0.9992	0.9992	0.9997	15	2	−16
Cypermethrin 2	0.9997	0.001200	0.001000	0.001100	0.001000	0.9978	0.9978	0.9998	17	−8	−17
Cyprodinil	0.9994	0.005000	0.004600	0.004200	0.004200	0.9996	0.9996	0.9988	8	−16	−16
DDD-pp′	0.9997	0.004000	0.004300	0.003700	0.003700	0.9993	0.9993	0.9999	−8	−8	−8
DDE-pp′	0.9985	0.002100	0.001900	0.001900	0.001900	0.9999	0.9999	0.9990	10	−10	−10
DDT-op′	0.9996	0.002800	0.002000	0.002100	0.002500	0.9895	0.9895	0.9999	29	−25	−11
DDT-pp′	0.9939	0.003100	0.001900	0.000276	0.002900	0.9998	0.9998	0.9997	39	−23	−6
Deltamethrin 1	0.9957	0.000038	0.000054	0.000062	0.000051	0.9987	0.9987	0.9999	−44	65	35
Deltamethrin 2	0.9993	0.000211	0.000139	0.000147	0.000169	0.9985	0.9985	0.9995	34	−30	−20
Dicofol	0.9989	0.001700	0.001800	0.001700	0.001500	0.9967	0.9867	0.9986	−6	0	−12
Difenoconazole 2	0.9972	0.001200	0.001200	0.001300	0.001200	0.9899	0.9899	0.9985	0	8	0
Difenoconzole 1	0.9975	0.001500	0.001500	0.001500	0.001400	0.9924	0.9924	0.9981	0	0	−7
Endosulfan sulphate	0.9958	0.000386	0.000421	0.001200	0.000378	0.9955	0.9955	0.9996	−9	8	−2
Endosulfan-alfa	0.9977	0.000132	0.000120	0.000118	0.000114	0.9969	0.9969	0.9990	9	−11	−13
Endosulfan-beta	0.9979	0.000106	0.000093	0.000092	0.000086	0.9930	0.9930	0.9989	12	−13	−19
Fenazaquin	0.9997	0.004300	0.003800	0.004600	0.003300	0.9973	0.9973	0.9974	12	7	−23
Fenhexamid	0.9945	0.000313	0.000394	0.003300	0.000365	0.9996	0.9996	0.9999	−26	19	17
Fludioxonil	0.9989	0.002000	0.001900	0.001900	0.000180	0.9999	0.9999	0.9994	5	−5	−10
Flusilazole	0.9993	0.001000	0.000966	0.000906	0.000949	0.9999	0.9999	0.9998	3	−9	−5
Indoxacarb	0.9955	0.000427	0.000388	0.000405	0.000392	0.9916	0.9916	0.9991	9	−5	−8
Lambda-cyhalothrin 1	0.9998	0.000432	0.000402	0.000392	0.000379	0.9998	0.9998	0.9998	7	−9	−12
Lambda-cyhalothrin 2	0.9998	0.000387	0.000347	0.000335	0.000332	0.9999	0.9999	0.9997	10	−13	−14
Metalaxyl	0.9991	0.000439	0.000428	0.000412	0.000378	0.9922	0.9922	0.9998	3	−6	−14
Pendimethalin	0.9994	0.000330	0.000307	0.000278	0.000309	0.9999	0.9999	0.9993	7	−16	−6
Pirimicarb	0.9996	0.001500	0.001400	0.001300	0.001400	0.9999	0.9999	0.9999	7	−13	−7
Pirimifos-methyl	0.9998	0.000581	0.000536	0.000522	0.000569	0.9933	0.9933	0.9995	8	−10	−2
Pirymethanil	0.9997	0.000757	0.000768	0.000664	0.000708	0.9998	0.9998	0.9993	−1	−12	−7
Procymidone	0.9989	0.000994	0.000910	0.000895	0.000838	0.9999	0.9999	0.9999	8	−10	−16
Propargite	0.9939	0.000233	0.000227	0.004600	0.000226	0.9989	0.9989	0.9989	2	18	−3
Propiconazole 1	0.9996	0.000520	0.000479	0.000513	0.000470	0.9982	0.9982	0.9992	8	−1	−10
Propiconazole 2	0.9981	0.000379	0.000350	0.000383	0.000345	0.9930	0.9930	0.9996	8	1	−9
Pyraclostrobin	0.9946	0.000679	0.000983	0.001200	0.000946	0.9973	0.9973	0.9995	−45	77	39
Tebuconazole	0.9999	0.001300	0.001200	0.001200	0.001100	0.9992	0.9992	0.9997	0	−8	−15
Tetraconazole	0.9968	0.000349	0.000320	0.000328	0.000330	0.9993	0.9993	0.9990	8	−6	−5
Thiabendazole	0.9998	0.001900	0.001900	0.001700	0.001700	0.9975	0.9975	0.9989	0	−11	−11

at 80 °C and was held for 3 min to provide complete entrance of the analytes into the column. In this way, the effects of coextractives on chromatography were weaker because they were rather deposited in the injection port instead of the analytical column.

An experiment was carried out using PTV injection in toluene for comparison of its performance with CSR technique. Ideally, for PTV injection, the solvent evaporation should be conducted at a temperature below the boiling point of the particular solvent. As aforementioned, the boiling point of toluene is 111 °C at 1 atm, thus the injector port initial temperature was set to 100 °C with 1 min evaporation time. The initial split ratio was set at 50:1 and after 0.5 min the split vent was closed; other parameters are detailed in Section 2. The PTV injections were carried out without cryogenic cooling which in the case of the instrument used in this study took approximately 20 min to cool the injector port from 300 °C to 100 °C. Table 2 shows how the analytes responses were affected by the type of injection technique used. The compiled sets of data (i.e. for each target pesticide using CSR and PTV technique) were subjected to statistical evaluation by *F*-test for comparison of two variances. Since data sets had the same variance in all cases, they were subjected to Student's *t*-test. Statistically significant differences between means were revealed for 20 (out of 39) target compounds at 95% level of confidence. For 14 target compounds (chlorpyrifos, chlorpyrifos-methyl, cyhalothrin-lambda, cypermethrin, DDD-pp′, DDT-op′, DDT-pp′, deltamethrin, endosulfan-sulphate, endosulfan-beta, indoxacarb, pirimiphos-methyl, propargite and pyraclostrobin) the responses obtained by CSR technique were higher than those obtained by PTV technique. In contrast, for six target compounds (azoxystrobin, boscalid, bupirimate, difenoconazole, fludioxonil and thiabendazole), the responses obtained by PTV technique were higher than those obtained by PTV technique. Less pronounced degradation of susceptible DDT isomers was observed in the case of CSR technique.

3.2. Linearity and matrix effects

An experiment was conducted to investigate the influence of injection volume on the sensitivity. Fig. 4 gives the dependence of peak heights of selected pesticides representing a broad volatility range on different injected volumes of toluene extracts (5–20 μL). Sample concentrations were different in the range between 1 and 0.25 g mL^{-1} but the injected pesticides amount always was equivalent to the pesticides concentration of 0.5 mg kg^{-1}. The injected matrix-matched standards were prepared in mixed extracts of carrots, huckleberries and tomatoes (1:1:1, v/v/v). As the Fig. 4 shows, injections of 5 μL extracts containing 1 g mL^{-1} matrix gave the

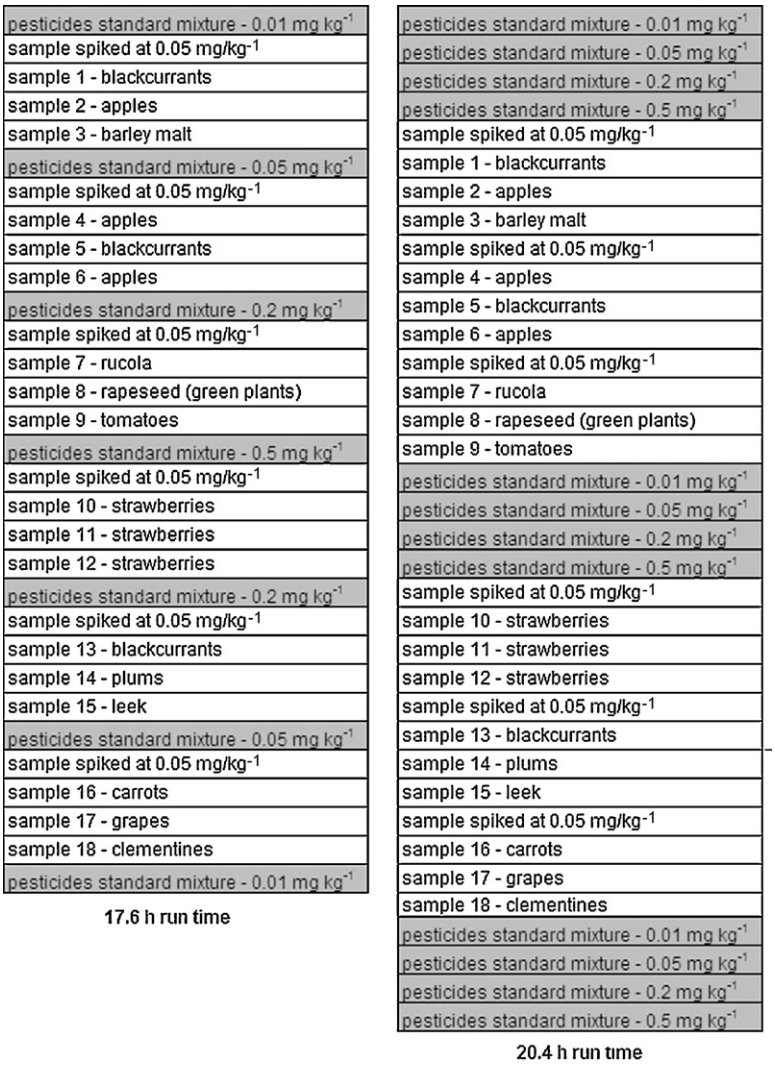

Fig. 5. Interspersed versus bracketing calibration. Sequences of injections comprising matrix-matched calibration standards in mixed equal volumes of carrot, huckleberry and tomato extracts along with 18 real samples and 6 spiked samples.

highest responses. If the injection volume was 10 μL, response diminishment by about 10% occurred for all of the pesticides whereas injections of 15 and 20 μL led to more intense diminishment by about 30% for all of the pesticides. Hence, if sensitivity is an issue, injection of larger volumes cannot be recommended, although it is feasible with a longer retention gap (>2 m). For the purpose of this work, injection volumes up to 10 μL were concluded to be optimal for CSR-LVI under the conditions of the study (i.e. using liner 5.4 mm × 3.4 mm I.D., precolumn of 1–2 m length and toluene as the injection solvent).

To assess matrix effects, calibration curves were constructed by using four sets of calibration standards, both in pure toluene and matrix matched. The injected pesticides amounts were always equivalent to the pesticides concentrations of 0.01, 0.05, 0.2 and 0.5 mg kg^{-1}. Table 3 gives the percent differences in the slopes of the linear calibration curves from the matrix-matched standards against the standards in solvent-only. For the majority of the compounds, the coefficients of determination were >0.99. It is difficult to make clear and straightforward conclusions because the type and intensity of observed matrix effects were different. Generally, the obtained values fell between −45 and 77%. If the value falls between −10 and 10%, it is deemed a negligible difference. This occurred for approximately 60% of the results. Some pesticides (e.g. bifenthrin) exhibited an enhancement effect if 5 μL were injected and a suppression effect when 10 or 15 μL were injected. In contrast, other pesticides (e.g. pyraclostrobin) exhibited a suppression effect when 5 μL were injected and an enhancement effect when 10 or 15 μL were injected. Generally, when larger volumes of diluted extracts were injected, the suppression effect became predominant. Keeping these observations in view, the 5–10 μL were decided to be the optimal injection volume under the experimental conditions of the study. This was in agreement with the results obtained in the previous experiment (Fig. 4).

3.3. Analysis of real and spiked samples: comparison of bracketing and interspersed calibration

In analytical sciences, the objective of calibration is to establish the relationship between the measurement signal and the analyte (or analytes) quantity. Preparation and utilization of analytical standards of known concentrations is of key importance to be able to quantify concentrations of analytes present in real samples [25–27]. Pesticide residues are usually quantified by using a set of calibration solutions prepared in matrix-matched extracts in order to minimize the adverse enhancement or suppression effects caused by co-extractives [22]. A variant of this method is the standard addition calibration. Positive samples, which have previously been identified by a screening method, are quantified using

Table 4
Comparison of pesticides residues results obtained in analysis of real samples by injecting the extracts using CSR-LVI technique and calculating the results from bracketing and interspersed calibration approach.

Sample no.	Matrix	Pesticide	Pesticide residue quantification result (mg kg^{-1})				RSD (%)
			CSR, 5 μL	CSR, 5 μL	CSR, 10 μL	Average result ± standard deviation	
			Bracketing calibration	Interspersed calibration	Interspersed calibration		
1	Blackcurrants	α-Endosulfan	0.051	0.054	0.044	0.050 ± 0.005	11
		β-Endosulfan	0.338	0.311	0.317	0.322 ± 0.014	4
		Endosulfan sulphate	0.239	0.235	0.259	0.244 ± 0.013	5
		Fenazaquin	0.027	0.027	0.027	0.028 ± 0.001	3
2	Apples	Pirimicarb	0.035	0.024	0.030	0.030 ± 0.005	18
		Boscalid	0.065	0.063	0.061	0.063 ± 0.004	3
		Pyraclostrobin	0.027	0.034	0.034	0.032 ± 0.004	13
		Fenazaquin	n/d	0.011	0.009	0.010 ± 0.001	11
3	Barley malt	Chlorpyrifos-methyl	0.009	0.012	0.010	0.010 ± 0.002	16
		Primiphos-methyl	0.279	0.212	0.227	0.239 ± 0.035	15
		Deltamethrin	0.040	0.051	0.050	0.047 ± 0.006	13
4	Apples	Boscalid	0.403	0,397	0.366	0.388 ± 0.020	5
		Propargite	1.35	1.18	1.34	1.29 ± 0.095	7
		Pyraclostrobin	0.145	0.141	0.127	0.138 ± 0.010	7
		Indoxacarb	0.030	0.020	0.023	0.024 ± 0.005	21
5	Blackcurrants	Pyrimethanil	0.031	0.027	0.026	0.028 ± 0.003	10
		Chlorpyrifos	0.070	0.053	0.060	0.061 ± 0.008	14
		Fenazaquin	0.095	0.080	0.071	0.082 ± 0.012	15
		Flusilazole	n/d	0.008	0.009	0.009 ± 0.001	8
		Cypermethrin	n/d	0.041	0.041	0.041 ± 0.000	1
6	Apples	Pyrimethanil	0.102	0.092	0.088	0.094 ± 0.007	8
		Pirimicarb	0.111	0.128	0.124	0.121 ± 0.009	7
		Flusilazole	0.009	0.006	0.006	0.007 ± 0.002	24
		Propargite	0.277	0.441	0.436	0.385 ± 0.093	24
		Fenazaquin	0.051	0.048	0.040	0.046 ± 0.006	12
7	Rucola	Pendimethalin	0.010	0.008	0.010	0.009 ± 0.001	15
		Bifenthrin	0.016	0.014	0.013	0.015 ± 0.001	10
		Lambda-cyhalothrin	0.035	0.063	0.062	0.053 ± 0.016	30
		Boscalid	0.006	0.006	0.007	0.006 ± 0.001	10
8	Rapeseed (green plants)	Chlorpyrifos	4.61	3.99	3.86	4.15 ± 0.40	10
		Cypermethrin	0.169	0.142	0.157	0.156 ± 0.014	9
		Deltamethrin	0.081	0.093	0.081	0.085 ± 0.007	8
9	Tomatoes	Boscalid	0.153	0.123	0.153	0.143 ± 0.017	12
		Pyraclostrobin	0.031	0.021	0.037	0.030 ± 0,008	28
		Azoxystrobin	0.085	0.068	0.079	0.077 ± 0.008	11
10	Strawberries	Bupirimate	0.016	0.020	0.019	0.018 ± 0.002	12
		β-Endosulfan	0.009	n/d	0.015	0.012 ± 0.004	33
		Endosulphate sulphate	0.018	0.019	0.014	0.017 ± 0.002	13
		Propargite	0.009	0.007	0.007	0.008 ± 0.001	15
		Fenhexamid	0.059	0.062	0.063	0.061 ± 0.002	4
		Boscalid	0.018	0.018	0.024	0.020 ± 0.003	17
11	Strawberries	Tetraconazole	0.013	0.013	0.013	0.013 ± 0.000	1
		Cyprodinil	0.189	0.193	0.193	0.192 ± 0.002	1
		Procymidone	0.317	0.330	0.324	0.323 ± 0.006	2
		Fludioxonil	0.131	0.128	0.118	0.125 ± 0.007	6
12	Strawberries	Pyrimethanil	0.058	0.057	0.056	0.057 ± 0.001	2
		Cyprodinil	0.025	0.027	0.026	0.026 ± 0.001	3
		Fludioxonil	0.014	0.016	0.017	0.016 ± 0.001	9
		Boscalid	0.151	0.256	0.201	0.203 ± 0.052	26
		Pyraclostrobin	0.036	0.045	0.038	0.040 ± 0.005	12
13	Backcurrants	Pirimicarb	0.010	0.008	0.009	0.009 ± 0.001	12
		Bupirimate	0.023	0.028	0.024	0.025 ± 0.003	10
		Propargite	0.279	0.218	0.223	0.240 ± 0.034	14
		Cypermethrin	0.019	0.015	0.014	0.016 ± 0.003	17
		Difenoconazole	0.019	0.016	0.016	0.017 ± 0.002	10
14	Plums	Propargite	1.06	0.927	0.945	0.977 ± 0.072	7
		Tebuconazole	0.089	0.091	0.083	0.088 ± 0.002	5
		Indoxacarb	0.058	0.058	0.054	0.057 ± 0.002	4
15	Leek	Chlorpyrifos	0.124	0.100	0.102	0.109 ± 0.013	12
		Cypermethrin	0.026	0.023	0.025	0.025 ± 0.002	6
		Azoxystrobin	0.110	0.065	0.077	0.088 ± 0.023	27
16	Carrots	DDT (sum)	0.044	0.033	0.040	0.039 ± 0.006	14
		Boscalid	0.085	0.081	0.079	0.081 ± 0.002	2
		Pyraclostrobin	0.017	0.016	0.017	0.017 ± 0.001	5
17	Grapes	Metalaxyl	0.543	0.517	0.460	0.506 ± 0.043	8
		Propiconazole	0.641	0.608	0.588	0.612 ± 0.027	4
		Tebuconazole	0.019	0.021	0.020	0.020 ± 0.001	4
18	Clementines	Chlorpyrifos	0.386	0.422	0.444	0.417 ± 0.029	7
		Dicofol	0.050	0.039	0.032	0.040 ± 0.009	23
		Thiabendazole	0.650	0.484	0.458	0.531 ± 0.104	20

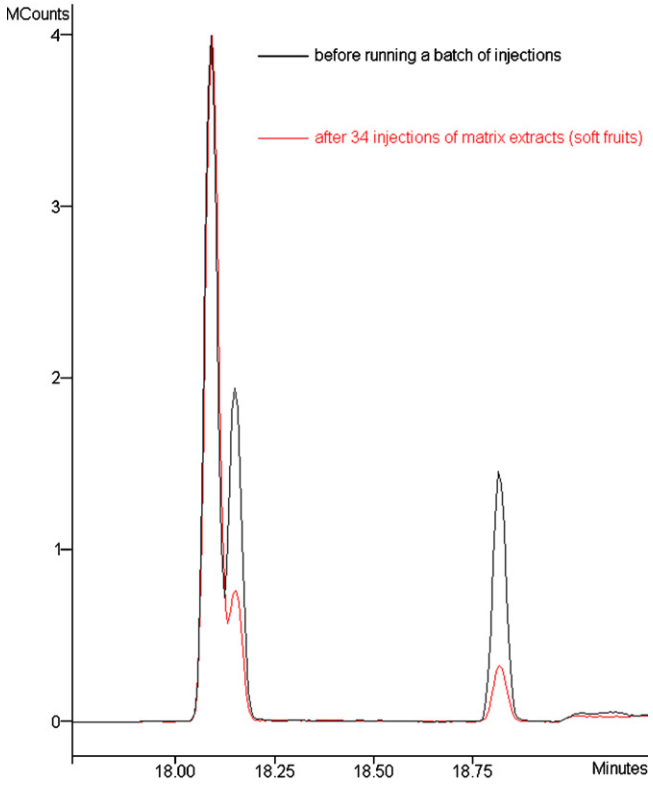

Fig. 6. Chromatographic peak profiles of DDD-pp′, DDT-op′ and DDT-pp′ (in order of appearance) before and after injections of a batch of samples.

standard addition to compensate for the matrix induced effects. With this approach, known amounts of analytes are introduced into aliquots of sample extracts containing the target compounds, so influence of coextractives is accounted in the calibration [23]. Another known approach is the addition of analyte protectants to the standards in pure solvent and samples to reduce matrix effects by blocking active sites in the injector [28] but information on its robustness in routine analysis is limited [24].

In routine analyses, calibration standards are usually injected immediately before and after each batch of real samples extracts and the calibration plots are constructed of averaged responses obtained for the target analytes. This type of calibration is known as bracketing calibration and it is often used to compensate for instrument response fluctuations over time in order to obtain a more accurate quantification when analyzing long batches of samples. Here, we evaluated a different approach in which the calibration standards were interspersed between real samples. Fig. 5 shows typical examples of sample batches which were run on a GC–MS/MS instrument by using bracketing and interspersed calibration approaches. Based on injections of 24 real and spiked samples, time savings of approximately 3 h (about 15% of the instrument run time) was achieved.

To evaluate analytical performance of CSR-LVI technique in terms of trueness and precision, analyses of real and spiked samples were carried out. Comparison of pesticide residues results was obtained in analysis of real samples by injecting the extracts with CSR-LVI technique and calculating the results by bracketing and interspersed calibration approach. In Table 4, three sets of data obtained under different experimental conditions are compiled. The relative standard deviations (RSDs) for results obtained for each pesticide were calculated to determine the overall precision. The results are in good agreement and no significant difference can be concluded. The RSD values are less than 20% for the majority of pesticides [29]. There are three instances of pesticides

Table 5
Average recoveries obtained from spiked samples by injection of 5 and 10 μL extract volumes with concurrent solvent recondensation technique (CSR). Six different matrices (apples, blackcurrants, carrots, huckleberries, strawberries and tomatoes) were spiked at 0.05 mg kg^{-1} and analyzed together with real samples. The results were quantified with reference to matrix-matched standards prepared in mixed equal volumes of carrot, huckleberry and tomato extracts.

Pesticide	Average recovery (RSD), %	
	CSR, 5 μL 1 g mL^{-1}	CSR, 10 μL 0.5 g mL^{-1}
Azoxystrobin	87.5 (7.2)	89.6 (6.4)
Bifenthrin	95.5 (12.5)	87.8 (6.2)
Boscalid	92.2 (14.4)	90.3 (9.4)
Bupirimate	91.9 (7.6)	88.1 (13.8)
Chlorpyrifos	86.1 (10.7)	90.4 (11.7)
Chlorpyrifos-methyl	89.3 (14.8)	95.1 (9.9)
Cyhalothrin-lambda 1	93.7 (12.6)	89.5 (12.1)
Cyhalothrin-lambda 2	93.9 (11.1)	97.9 (13.9)
Cypermethrin 1	95.4 (9.0)	85.3 (13.4)
Cypermethrin 2	94.5 (7.6)	90.0 (5.8)
Cyprodinil	93.6 (8.1)	91.9 (9.9)
pp′-DDD	89.5 (6.3)	89.0 (8.9)
pp′-DDE	87.2 (7.5)	85.2 (8.9)
op′-DDT	100.8 (20.8)	91.7 (16.8)
pp′-DDT	102.8 (15.0)	102.8 (20.6)
Deltamethrin 1	76.3 (20.8)	88.0 (11.3)
Deltamethrin 2	98.8 (9.8)	94.9 (15.5)
Difenoconazole 1	89.6 (6.3)	91.6 (8.3)
Difenoconazole 2	88.4 (6.5)	90.6 (7.0)
Dicofol	89.8 (12.1)	89.7 (12.4)
α-Endosulfan	90.3 (13.9)	92.1 (18.9)
β-Endosulfan	88.3 (20.3)	86.7 (15.9)
Endosulfan sulphate	97.3 (11.2	99.4 (12.2)
Fenazaquin	96.7 (15.5)	97.1 (12.2)
Fenhexamid	91.4 (15.4)	86.0 (9.9)
Fludioxonil	93.9 (8.4)	93.1 (9.50
Flusilazole	91.7 (9.5)	95.2 (9.5)
Indoxacarb	89.6 (5.2)	89.3 (8.4)
Metalaxyl	92.9 (11.9)	92.3 (11.1)
Pendimetalina	90.6 (7.7)	97.8 (11.1)
Pyrimethanil	89.0 (8.7)	96.8 (9.4)
Pirimiphos-methyl	90.9 (11.6)	97.5 (13.2)
Pirimicarb	84.8 (16.1)	86.9 (8.3)
Procymidone	94.9 (8.0)	95.7 (8.4)
Propargite	97.9 (9.5)	91.2 (17.7)
Propiconazole 1	91.2 (11.4)	91.3 (16.8)
Propiconazole 2	97.1 (8.3)	93.5 (19.2)
Pyraclostrobina	97.2 (12.5)	96.6 (14.6)
Tebuconazole	96.8 (3.6)	96.4 (4.9)
Tetraconazole	87.6 (13.6)	87.3 (17.4)
Thiabendazole	71.5 (19.2)	79.8 (18.4)

present in low concentrations which were not detected in previous, routinely performed analyses (fenazaquin in apples, flusilazole and cypermethrin in blackcurrants). It is not uncommon for chromatographic peaks suppression to occur for susceptible analytes due to matrix co-elution in complicated extracts [30]. It is difficult to make general conclusions about ruggedness of the evaluated experimental variants because this highly depends on the nature of analyzed compounds and type and complexity of matrices. In a busy routine laboratory often diverse commodities are run in one analytical batch. Observation of chromatographic behavior of susceptible compounds (e.g. DDD-pp′, DDT-op′ and DDT-pp′) advise the analyst whether false negatives can be expected (Fig. 6), and provide information about the necessity to do some maintenance of the instrument, such as trimming a piece of the guard column, replacing the injection liner and cleaning the MS ion source, to restore the instrument performance. When calibration standards were interspersed between samples, and there were shorter intervals between injections of samples and calibration standards, it was easier to investigate these problems.

Together with real samples, spiked samples of six different matrices (apples, blackcurrants, carrots, huckleberries,

strawberries and tomatoes) were analyzed by using two experimental variants. Average recoveries obtained from spiked samples by injection of 5 and 10 μL extract volumes with CSR-LVI technique are represented in Table 5. The percent recovery rates were quantified with reference to matrix-matched standards prepared in mixed equal volumes of carrot, huckleberry and tomato extracts. As shown in Table 5, in both experimental variants, the recoveries were in the range between 71.5 and 102.8 with associated RSDs in the range between 3.6 and 20.8. It is noteworthy to mention that in compliance with European Union guidance document SANCO/10684/2009, in routine multi-residue pesticides analysis, acceptable limits for a single recovery should be in the range between 60 and 140% [29].

4. Conclusions

The proposed approach employing CSR-LVI technique can be used with success for the purpose of overcoming the limitation of a maximum volume of 1–2 μL for injection with classical splitless technique in order to increase the injected sample concentration factor. The practical benefit of CSL-LVI is the feasibility for injections of a wider range of diluted sample volumes without any hardware modification. With the developed injection mode, very good sensitivity and reproducibility was achieved based on 5–10 μL injection volumes, although higher volumes could be injected with some sacrifice in sensitivity. As compared to LVI-PTV injection in toluene, ruggedness in the CSR-LVI approach was found to be improved with less pronounced degradation of susceptible DDT isomers, which was the case at least for the matrices examined in this study. Although a solvent exchange step involved in the proposed approach may be regarded as disadvantageous, evaporation of a small volume of an acetonitrile extract and reconstitution in toluene when using autosampler vials with fixed inserts (e.g. 300 μL volume) will not be a very lengthy procedure.

The application of interspersed calibration with standards placed between sample extracts instead of bracketing calibration with standards injected before and after each batch of sample extracts has led to a considerable shortening of the total duration of GC–QqQ-MS/MS analyses. Based on injections of 24 real and spiked samples, a time saving of approximately 3 h (about 15% of the instrument run time) was achieved. The obtained experimental data show high reproducibility of pesticide residue results and improved cost-effectiveness of the proposed approach. No significant difference between the evaluated experimental variants was apparent. The worked out approach can be recommended as an effective means for improving analytes enrichment factor and shortening analysis time in pesticide residue analysis by gas chromatography-based methods.

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