Trends in Chromatography
Vol. 8, 2013

Original Communication

Experiments for miniaturization and modification of the multi-pesticide residue method EN 12393

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ABSTRACT

With the objective to miniaturize and accelerate EN 12393 sample preparation, extraction, partitioning as well as cleanup by gel permeation chromatography (GPC) were reinvestigated. Different combinations of extraction and partitioning alternatives were tested by joining two extraction and three partitioning techniques to three combinations (dispersing/dispersing; shaking/shaking; sonication/ shaking). They were evaluated in terms of applicability to routine analysis and recoveries for spiked and incurred pesticide residues. Compared to EN 12393, the combination shaking/shaking and dispersing/dispersing gave comparable results, while the combination sonication/shaking provided slightly lower recoveries, especially for incurred residues. As shaking/shaking is more convenient for routine analysis, it was selected as the preferred combination for a miniaturized method. Four high resolution GPC columns were compared with regard to separation of sunflower oil from selected pesticides with the aim to reduce the runtime of the GPC cleanup. The PSS GRAM 30 Å column provided the best performance. Methanol was used as solvent modifier to improve the elution behaviour of polar pesticides, resulting in a runtime of 25 min, which allowed a high sample throughput per column. Together with the miniaturized extraction and partitioning steps, analysis time per sample was reduced by about 30%, while hands-on time was about half as compared to EN 12393.

KEYWORDS: EN 12393, pesticides, extraction, liquid-liquid partitioning, HR-GPC, miniaturization, gel permeation chromatography, GPC cleanup

1. INTRODUCTION

In the European Union (EU), for more than 500 pesticides, maximum residue limits (MRL) are directed by the Regulation (EC) No 396/2005 [1] on maximum residue levels of pesticides in or on food and feed of plant and animal origin. For baby food designated for infants and young children as well as for infant formulae and follow-on formulae, EU legislation generally limits pesticide residues to a level of 0.01 mg kg^{-1}. Even lower MRLs (0.003-0.008 mg kg^{-1}) were set for some specific compounds, the so-called banned or restricted pesticides [2, 3]. To monitor and control the great number of pesticides at a low µg kg^{-1} level in complex and also fatty matrices, efficient and reliable sample preparation, extraction and cleanup procedures are required that are able to determine as many pesticides, metabolites and degradation products as possible. During the last decades, different powerful extraction methods were developed [4-7], whereat one of the most powerful multi-pesticide residue methods used in European laboratories is EN 12393 [8], originally developed by Specht and Tilkes and published in 1980 [5]. Since that time only few modifications were introduced, which substituted toxic dichloromethane by ethyl acetate/cyclohexane [9], or reduced the

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extraction volume and omitted the mini silica gel cleanup [10]. Compared to the more and more accepted QuEChERS method [6], the main drawbacks of EN 12393 are the huge solvent consumption during sample preparation (> 600 mL), the time and solvent consuming cleanup by gel permeation chromatography (GPC) (> 40 min; > 200 mL), and the laborious manual sample handling in big separation and filtration funnels. The original screening by GC-ECD/-FPD/-NPD is presently almost substituted by gas chromatography-mass spectrometry (GC-MS(/MS)) for both screening and confirmation purposes. However, the recent trend in residue analysis is to minimize sample amounts to improve efficiency, to reduce financial costs, applied volumes of (toxic) solvents, and time of analysis [11]. Additionally, manual operations are substituted, for example, by automated shakers, by centrifugation instead of filtration, or by automated solid phase extraction devices. Therefore, the main drawbacks of EN 12393 should be overcome by miniaturization of sample preparation including substitution of both the large and time consuming GPC system by a smaller and high performance one and the mini silica column by an SPE system, followed by GC-MS or GC-MS/MS determinations.

The aim of the present study was to miniaturize the sample preparation steps of EN 12393 by a factor of ten, but still to guarantee a limit of quantification (LOQ) of ≤ 0.01 mg kg^{-1}. For the extraction procedure, automated shaking, dispersing, and ultra sonication, which generally are reported to give satisfactory results, were compared [5, 6, 12, 13]. Both automated shaking and dispersing were also used for the partitioning step from acetone/ water to ethyl acetate/cyclohexane. Additionally, different commercially available high resolution GPC columns were evaluated in terms of cleanup efficiency concerning high molecular weight matrix compounds (e.g. triglycerides) and analysis runtime.

2. MATERIALS AND METHODS

2.1. Reagents and materials

Methanol (LiChrosolv, LC-MS grade), acetone, toluene, ethyl acetate and cyclohexane (all SupraSolv for GC analysis), anhydrous sodium sulphate (Ph. Eur) and sodium chloride (GR for analysis) were purchased from Merck KGaA (Darmstadt, Germany). Filter units (0.2 µm) and folded filters (597 ½) were purchased from Whatman GmbH (Dassel, Germany). The folded filters were soxhlet extracted for 2 h with acetone. Deionized water was used for sample preparation.

Certified pesticide standards with purity > 94%, except for cypermethrin (91.0%), flucythrinat (90.5%) and triazophos (78.5%), were obtained by Dr. Ehrensdorfer GmbH (Augsburg, Germany). Standard solutions were prepared in toluene for fortification and GC measurement as well as in ethyl acetate/cyclohexane (1:1) for GPC applications. Internal standards iodophenphos (0.5 mg L^{-1}), dichlofenthion (0.5 mg L^{-1}), and cycloat (2.5 mg L^{-1}) with a purity > 98% were purchased from Dr. Ehrensdorfer GmbH and prepared in toluene. EPA Superfund Contract Lab Program GPC Calibration mixture (CLP-GPC mixture) containing corn oil (250 g L^{-1}), bis(2-ethylhexyl) phthalate (10 g L^{-1}), methoxychlor (2.0 g L^{-1}), perylene (0.2 g L^{-1}) and sulphur (0.8 g L^{-1}) was purchased from Restek GmbH (Bad Homburg, Germany) and was diluted (1:20) with ethyl acetate/cyclohexane (1:1).

Gel permeation chromatography columns GRAM 30 Å, 7 µm (8 x 300 mm, 20 x 300 mm + precolumn 20 x 50 mm), SDV 50 Å, 3 µm (8 x 300 mm), and SDV 100 Å, 5 µm (8 x 300 mm) were purchased from Polymer Standards Service GmbH (Mainz, Germany). MZ-Gel SDplus 50 Å, 10 µm (8 x 300 mm) was obtained from MZ-Analysentechnik GmbH (Mainz, Germany), and BioBeads SX-3 column, 38–75 µm (50 g material; 300 x 25 mm i.d.) from Antec GmbH (Sindelsdorf, Germany).

Rajah extra hot chilli powder (B.E. International Foods, Middlesex, UK) and Spanish sweet pepper powder (A.L.C. Warenvertriebs-GmbH, Düsseldorf, Germany) were purchased at local supermarkets; blank rice flour was obtained from Atlantic Meals (Lisbon, Portugal) and sunflower oil from Orelrastmaslo Jsc. (Orel, Russia). Extra hot chilli powder (53 g), sweet pepper powder (79 g) and blank rice flour (368 g) were slowly mixed in a food processor (Starmix, Krups GmbH, Offenbach, Germany) for 3 h to obtain a homogeneous sample mixture for extraction experiments (chili-pepper-rice mix; CPR mix). Rice flour, which was

verifiable free of pesticide residues, was spiked at 0.1 mg kg^{-1} with a representative pesticide mixture containing 34 analytes from diverse pesticide classes (Table 1).

2.2. Instrumentation

All dispersing steps were carried out by Ultra-Turrax TP18 (IKA Werke GmbH & Co. KG, Staufen, Germany), sonication was done in a Bandelin Sonorex ultrasonic cleaning unit (Schalltec GmbH, Mörfelden-Walldorf, Germany) and shaking steps were conducted on a two-dimensional shaker SM25 at 225 rpm (Edmund Bühler GmbH, Hechingen, Germany). During sonication and dispersing, samples were cooled by an ice-water bath. For centrifugation, a Megafuge 1.0R (Thermo Fisher Scientific, Langenselbold, Germany) was used.

For quantification of pesticides during recovery experiments a combined selected ion monitoring and scan method was used on an Agilent 7890A gas chromatograph, equipped with an Agilent 7683B series injector tower (Agilent Technologies GmbH, Waldbronn, Germany) and a PTV injector (CIS4, Gerstel, Mühlheim an der Ruhr, Germany). PTV injection parameters: injection volume 5 µL; vent time 0.3 min; vent flow 200 mL min^{-1}; vent pressure 3.8 psi; temperature program: 70 °C for 0.25 min, 720 °C min^{-1} to 250 °C held to the end of GC-MS method; injection liner: Gerstel 1.5 mm i.d., baffled and deactivated glass liners. The pesticides were separated on a HP-5ms (30 m x 0.25 mm i.d., 0.25 µm) column, connected to a HP-5ms (1.5 m x 0.32 mm, 0.25 µm) pre-column at the inlet end and detected by an Agilent 5975C inert quadrupole MSD. The system was controlled by ChemStation software, which was also used for data processing and evaluation. The temperature program (70 °C for 2.0 min, 25 °C min^{-1} to 150 °C, 3 °C min^{-1} to 200 °C, 8 °C min^{-1} to 280 °C held for 10 min, 35 °C min^{-1} to 325 °C held for 3 min) was used, while transferline temperature was constantly at 280 °C. Helium was used as carrier gas at constant flow of approx. 2.5 mL min^{-1} using the retention time locking (RTL) program of ChemStation with chlorpyriphos-methyl (16.59 min) as reference substance. The quantification was done by using an adequate target ion and external calibration at the expected pesticide concentration with either matrix matched standards or solvent standards for samples of CPR mix and rice samples, respectively. For confirmation purposes, one or two qualifier ions were consulted. Matrix compounds were identified by automatic comparison of the obtained mass spectra with the spectra of NIST database.

Analytical GPC columns (8 mm i.d.) were operated on a Merck Hitachi L-6200A intelligent pump connected to a Merck Hitachi UV detector (254 nm) and a Merck Hitachi GPC integrator (Merck KGaA, Darmstadt, Germany). Samples were injected manually by a Rheodyne 7010 valve (Latek Labortechnik-Geräte GmbH & Co. Analysen-Systeme KG, Eppelheim, Germany) with a 0.2-mL sample loop. Preparative GPC columns were installed on an AccuPrep GPC system connected to an automatic evaporation system AccuVap FLX (Antec GmbH, Sindelsdorf, Germany). The system was equipped with peek tubing for high pressure applications, a switchable UV detector (254 nm) and a sample loop of 5.0 mL and 2.0 mL for recovery experiments with GPC cleanup on the BioBeads SX-3 column and acquisition of GPC elution profiles for the preparative PSS GRAM and the BioBeads SX-3 column, respectively. The solvent for elution generally was ethyl acetate/cyclohexane (1:1) at flow rates of 1.0, 5.0, and 7.5 mL min^{-1} for the analytical GPC, the BioBeads SX-3, and the preparative PSS GRAM column, respectively. For the GRAM column, methanol at different percentages was used as solvent modifier.

2.3. Sample extraction

Samples (5 g of blank rice flour, spiked rice flour, or CPR mix) were weighed into 100 mL screw capped glass centrifuge tubes. Deionized water (10 mL) was added allowing the samples to rest for 15 min. After the addition of 20 mL acetone, the extraction was conducted by dispersion, shaking or sonication. Afterwards, 3.5 g sodium chloride and 10 mL ethyl acetate/cyclohexane (1:1) were added. Liquid-liquid partitioning was performed by dispersing or shaking. After centrifugation at 4000 rpm for 5 min, 20 mL of the organic upper layer was taken by a volumetric pipette and dried by filtration through 10 g of sodium sulphate on a folded filter, which was afterwards rinsed with

Table 1. Recoveries for liquid-liquid partitioning experiments with rice flour spiked at 0.1 mg kg^{-1}, calculated with matrix-matched standards (n = 2).

	GC-MS retention time [min]	**Quantifier ion [m/z]**	**Liquid-liquid partitioning experiments**																**mod. EN 12393**	
Extraction			Dispersing 60 sec						Shaking 15 min				Ultrasonication 15 min						Dispersing 2 min	
Liquid-liquid partitioning			Dispersing 15 sec		Dispersing 30 sec		Dispersing 45 sec		Shaking 2.5 min		Shaking 10 min		Shaking 15 min		Shaking 2.5 min		Shaking 10 min		Shaking 15 min		Dispersing 1 min	
				RSD		RSD		RSD		RSD		RSD		RSD		RSD		RSD		RSD		RSD
Brompropylat	26.65	341	**85**	5	**85**	2	**67**	11.3	**73**	2.5	**78**	2	**73**	5.8	**82**	7	**78**	3.7	**76**	2.4	**95**	5.6
Carbaryl	17.1	115	**91**	6.5	**95**	6	**70**	16.2	**81**	4.7	**89**	5	**80**	9	**91**	11.6	**83**	2.8	**80**	6.7	**102**	9.2
Chlorothalonil	15.22	266	**63**	8.8	**67**	2.3	**45**	26.2	**45**	15	**54**	1	**50**	12.5	**70**	11.2	**69**	5.1	**65**	6	**24**	6.9
Chlorpropham	11.37	153	**93**	19.9	**98**	8.8	**67**	19.2	**79**	7.6	**95**	2	**91**	13.8	**84**	12.4	**84**	0.6	**76**	8.9	**100**	9.1
Chlorpyrifos	19.25	314	**85**	12.4	**97**	1.5	**67**	17.8	**75**	5.1	**83**	2.3	**80**	8.1	**81**	8.2	**81**	1.9	**78**	5.6	**98**	7.8
Chlorpyrifos-methyl	16.9	286	**86**	16.4	**98**	3.9	**67**	19.1	**74**	6.2	**85**	0.6	**82**	10.3	**80**	8.5	**82**	0.3	**76**	7.2	**100**	7.8
Chlorthion	19.7	109	**87**	9.7	**100**	5	**69**	18.5	**75**	5.6	**88**	0.2	**82**	11.4	**85**	11.8	**83**	4.1	**78**	8	**104**	7.2
Cyprodinil	20.38	224	**86**	6.7	**84**	1.2	**66**	13.3	**73**	4	**79**	1.8	**73**	5.1	**82**	6.5	**79**	2.8	**76**	3.3	**95**	6.1
DDT, p-,p'	25.28	235	**85**	6	**86**	1.5	**66**	13.1	**73**	3.3	**79**	2	**73**	6.7	**82**	6.9	**77**	3.7	**74**	3.8	**94**	6
Dichlofluanid	18.56	167	**64**	5.9	**63**	0.5	**43**	14.6	**35**	14.6	**42**	1.7	**40**	6.6	**59**	12	**60**	6.3	**56**	7.6	**15**	3.9
Dieldrin	22.9	79	**84**	9.4	**92**	0.4	**65**	13.7	**75**	2.5	**81**	4	**76**	7.7	**81**	7.8	**80**	0.9	**76**	4.3	**92**	7.4
Dimethoat	13.04	87	**91**	9	**89**	8.5	**66**	16.7	**80**	5.8	**91**	0.7	**83**	9.9	**88**	10.2	**84**	2.7	**79**	7.7	**92**	8.4
Diphenylamin	11.25	108	**114**	12.3	**102**	9.5	**97**	0.3	**90**	9.8	**96**	0	**105**	18.7	**93**	6.7	**104**	13.4	**87**	4	**59**	13.5
DMSA	10.65	108	**100**	14.4	**99**	9.2	**74**	14	**99**	4.2	**113**	0.8	**103**	11.6	**94**	11.1	**89**	0.1	**83**	10	**156**	9.8
α-Endosulfan	22.01	237	**87**	10	**95**	1.2	**67**	15.7	**77**	4.5	**84**	2.7	**80**	7.7	**83**	8.2	**81**	1.9	**79**	4.5	**87**	8.5
β-Endosulfan	23.88	237	**87**	6.9	**87**	0.8	**67**	14.9	**75**	4.1	**80**	2.9	**74**	5.3	**83**	6.5	**80**	2.6	**77**	3.2	**91**	5.8
Endosulfan-sulfat	25.12	237	**86**	5.4	**85**	0.8	**67**	12.5	**72**	4.5	**90**	22.1	**72**	4	**81**	2.2	**81**	4.3	**78**	12.5	**83**	4.9
Endrin	23.44	263	**85**	8.1	**92**	2.8	**67**	14.9	**75**	4.7	**81**	3	**76**	7.4	**82**	7.1	**80**	2.4	**78**	2.7	**94**	7.3
Esfenvalerat	39.05	167	**77**	4.5	**86**	1.9	**62**	15	**66**	2.6	**72**	2	**67**	6.4	**74**	9.7	**71**	3.8	**68**	4.1	**91**	4.8
Fluquinconazol	31.41	340	**85**	5.6	**86**	2.1	**66**	12.7	**74**	3.1	**78**	4.3	**74**	6.2	**83**	7	**79**	2.6	**76**	2.5	**95**	4.9
Folpet	21.23	260	**72**	5.3	**79**	1.3	**53**	22.7	**55**	10.4	**63**	2.2	**56**	8.8	**77**	10.8	**72**	6	**68**	5.6	**28**	5.2
Kresoxim-methyl	23.59	116	**85**	7.1	**85**	0.7	**66**	13.6	**74**	4.4	**80**	1.2	**74**	5.6	**83**	6.7	**80**	2.5	**76**	3.2	**90**	5.3
Lindane	13.89	181	**83**	21.4	**88**	6.8	**63**	20.3	**73**	6.7	**83**	1.5	**80**	10.4	**74**	5.4	**79**	0.5	**69**	7.1	**90**	11.1
Malathion	18.8	125	**87**	10.4	**91**	3.4	**65**	17.3	**77**	5.2	**87**	0.6	**80**	9	**85**	9	**83**	2.9	**77**	6.5	**93**	8.4
Metalaxyl	17.57	206	**88**	9.4	**87**	4.2	**65**	15.1	**77**	6.9	**84**	2.5	**77**	6.1	**87**	6.9	**82**	1.1	**78**	4.4	**81**	7.3

Table 1 continued..

Monolinuron	13.49	126	**92**	13.9	**98**	13.7	**69**	17.7	**83**	7	**97**	2.8	**91**	10.1	**90**	10.1	**90**	0.7	**80**	8.7	**115**	10.4
Omethoat	10.04	110	**41**	0.3	**44**	11.6	**31**	12.4	**45**	5.4	**50**	1.8	**45**	11	**46**	11.2	**45**	5.5	**43**	9.3	**53**	11.5
o-Phenylphenol	8.54	141	**76**	17.9	**73**	6.8	**64**	9.1	**68**	7.6	**79**	4.3	**76**	10.8	**72**	2.5	**74**	1.7	**67**	10.8	**57**	9.8
Permethrin	31.18	163	**85**	5.9	**90**	2.5	**69**	13.9	**77**	3.7	**82**	1.5	**74**	5.6	**79**	8.1	**77**	3.1	**75**	2.4	**88**	5
Pirimicarb	16.01	166	**90**	14.6	**87**	0.2	**65**	19.6	**77**	6.4	**85**	0.2	**79**	6.9	**84**	10.9	**82**	1.5	**78**	5.9	**98**	8.1
Pirimiphos-methyl	18.39	290	**87**	12.9	**96**	1.5	**67**	17.8	**76**	5.9	**86**	1.7	**81**	8.6	**83**	9.1	**83**	2.2	**79**	5.5	**100**	7.4
Tebuconazol	25.63	250	**83**	5.2	**82**	3.5	**65**	12.4	**73**	2.2	**79**	0.5	**73**	4.9	**79**	6.6	**77**	2.4	**72**	2.4	**87**	5.1
Tetramethrin	26.9	123	**86**	5.2	**88**	1.4	**67**	12.9	**74**	3	**79**	3.9	**74**	6.2	**82**	7.8	**78**	3.2	**76**	3.3	**83**	6.5
Average			**84**	9.5	**87**	3.8	**65**	15.3	**73**	5.7	**81**	2.6	**76**	8.4	**81**	8.4	**79**	3	**74**	5.8	**86**	7.5

3 mL ethyl acetate/cyclohexane (1:1). The combined eluates were collected in graduated 500 mL TurboVap500 tubes with 1.0 mL stem, evaporated to < 1 mL by TurboVap500 (Caliper Life Sciences GmbH, Mainz, Germany) and filled up to 1.0 mL with ethyl acetate/cyclohexane (1:1). Exactly 10 mL ethyl acetate/cyclohexane (1:1) was added, the solution dried by adding a salt mixture ($NaCl/Na_2SO_4$, 1:1), and filtrated through a 0.2 µm filter unit. GPC cleanup subsequently was conducted on BioBeads SX-3 columns (injection volume: 5.0 mL; dump time: 19 min; collect time: 31 min). To provide a precise final volume and to omit volume losses while automatic sample transfer of small solvent volumes (< 1 mL) by AccuVap FLX system, the GPC eluates were online pre-evaporated to approx. 2 mL, automatically transferred to graduated 25 mL tubes, and the evaporation chamber was automatically rinsed twice with ethyl acetate/cyclohexane (1:1). The combined eluates were manually rotary evaporated (35 °C, 0.2 bar) to < 1 mL and finally filled up to 1.0 mL. The final measuring solutions were analyzed by GC-MS.

2.4. Reference Method

The extraction procedure followed a CLF-modified version of EN 12393-2, procedure N. Samples of 12.5 g (+ 10 mL deionized water) were used for extraction. The modification concerned the following steps: the extraction time was shortened to 2 min; the evaporation residue of the crude extract was 1 mL, and 10 mL of ethyl acetate/ cyclohexane (1:1) was consecutively added; the solvent of the collected GPC (BioBeads SX-3) fraction was evaporated and filled up to 1.0 mL; cleanup on mini silica gel columns was not carried out.

2.5. Elution profile on analytical and preparative GPC columns

Elution profiles of pesticides (1 g L^{-1}), sunflower oil (250 g L^{-1}; 150 g L^{-1}) and CLP-GPC mixture (1:20) were acquired for all analytical GPC columns (8 mm i.d.) on the Merck Hitachi system. Obtained chromatograms on thermal paper were put on top of each other, compared to assess co-elution with sunflower oil and finally digitalized. Elution times for analytical PSS GRAM column in combination with different percentages of methanol in the eluent were obtained by fractionation (concentration: 0.1 mg L^{-1}; first fraction at 9.5 min; followed by 16 fractions of 1 min). Identification and evaluation were done by GC-MS analysis. Elution profiles of pesticides (1 g L^{-1}), sunflower oil (200 g L^{-1}) and CLP-GPC mixture (1:20) were acquired for preparative PSS GRAM and BioBeads SX-3 columns on AccuPrep GPC system. Chromatograms were obtained and processed with AccuPrep GPC software.

3. RESULTS AND DISCUSSION

The current EN 12393 method uses sample weights of 10–100 g and high solvent volumes for sample preparation. The samples are extracted with 200 mL acetone, followed by a partitioning step with 100 mL ethyl acetate/cyclohexane (1:1). After drying and evaporation, the residues are taken up in 15 mL ethyl acetate/cyclohexane (1:1), and 5 mL of this sample solution is injected onto a GPC column for cleanup. Finally, after cleanup with mini silica gel columns, the volume of the measuring solution is 10 mL, which represents a sample concentration of 0.12–1.17 g mL^{-1}. For the CLF-modified EN 12393 for baby food applications, a sample concentration of 0.96–31.9 g mL^{-1} with a final pesticide concentration of 0.010 to 0.319 mg L^{-1} can be obtained (3–100 g sample weight; 0.01 mg kg^{-1} pesticide residue). These concentrations, which showed excellent results during routine analysis by GC-NPD/ECD/MS, were the benchmark for the development of a new, miniaturized method based on the EN 12393 methodology. Further on, to allow the simultaneous analyses of the final solution on different measuring systems, it is common in routine laboratories to divide the volume into several vials. Therefore, the volume of the final measuring solution was set to 1.0 mL. A simple reduction of sample weights and extraction solvent volumes by a factor of ten was not feasible, as the volume of the final measuring solution would also be reduced to 0.1 mL. Without changing the volume of the final measuring solution (1.0 mL), sample weights of ≤ 3.14 g for a miniaturized EN 12393, comparable to ≤ 31.4 g sample weight of the CLF-modified EN 12393, would result in final concentrations of < 0.010 mg L^{-1} (at a pesticide residue level of 0.01 mg kg^{-1}). Hence, the ratio of extraction solvent

to sample amount had to be adjusted to obtain the right concentration and volume of the final measuring solution, while the solvent volumes for extraction/partitioning were reduced by a factor of ten to conduct the sample preparation in a 100 mL glass centrifuge tube. Due to these changes (higher sample amounts need to be extracted by less solvent volumes), the extraction properties of these adapted systems were investigated. Additionally, modifications of the extraction techniques (dispersing, shaking and sonication) were also examined.

3.1. Extraction and liquid-liquid partitioning

Three combinations of extraction and liquid-liquid partitioning were investigated, while only the time for partitioning was changed. The first approach was a combination of extraction (60 sec) and partitioning (15, 30, 45 sec) by dispersing, the second one a combination of extraction (15 min) and partitioning (2.5, 5, 15 min) by shaking on an automatic shaker and the third one a combination of extraction by sonication (15 min) and partitioning by shaking (2.5, 5, 15 min). To assess the partitioning efficiency and optimal partitioning time, blank rice flour was fortified at 0.1 mg kg^{-1} directly after the addition of acetone. Each combination was compared with the results obtained by the reference method and was evaluated within the approach individually. In comparison to the reference method (mean recovery 86%), all combinations showed good results with minor differences for the different partitioning times (Table 1). For the first approach (dispersing), mean recoveries for the 33 spiked analytes were in the range of 65%–87%. The best results were obtained for a partitioning time of 30 sec. For a slightly longer partitioning time (45 sec), a drop of efficiency was observed, also accompanied by a higher RSD, which can be referred to low recoveries (losses) for one of the samples analyzed in duplicate. The second approach (shaking) showed recoveries in the range of 73%–81%. The best results were also obtained with a medium partitioning time (10 min), which are expressed by the highest average recovery and a low RSD. The last approach (sonication) also resulted in good mean recoveries of 74%–81%. Both 2.5 and 10 min for partitioning gave the highest average recoveries of 81% and 79%, respectively, while the lowest RSD was achieved with the latter one (10 min) for the sonication experiment. The results indicated that both longer and shorter partitioning times tend to result in lower recoveries of the analytes. In comparison to the reference method, recoveries were slightly lower, but for further experiments the partitioning time was held at 30 sec (dispersing) and 10 min (shaking), which provided the highest mean recoveries.

For the evaluation of extraction efficiency, two test matrices were investigated, i.e., fortified rice flour (0.1 mg kg^{-1}) and CPR mixture containing 11 incurred residues in a range of 0.012 to 0.442 mg kg^{-1}. In this experimental setup, only the extraction times of the three different approaches were changed, while the combinations remained the same. Extraction times for dispersing were 30, 60 and 90 sec, and both for shaking and sonication 5, 10 and 15 min. All three combinations showed good results for the spiked rice flour as compared to the reference method with an average recovery of 83%. Mean recoveries of 71–79% (dispersing/dispersing), 79–82% (shaking/shaking) and 81–85% (sonication/shaking) were obtained and they were close to the reference method (Table 2). Pesticides that are pH sensitive (e.g. chlorothalonil, dichlofluanid, folpet), generally gave low recoveries as the pH was not adjusted during sample preparation, neither in the different experiments nor for the reference method. As compared to the reference method, recoveries of metribuzin were surprisingly low for nearly all miniaturized extraction procedures, which is difficult to explain.

Extraction of a spiked sample can give good evidence on the efficiency of an extraction procedure, but may not be conclusive. Therefore, a sample with incurred residues (CPR mixture) was also examined. Good recoveries were obtained for extraction by shaking (91–107%), while dispersing (85–99%) and sonication (83–89%) gave lower recoveries in relation to the reference method, for which recoveries were set at 100% (Table 3). In contrast to the spiked rice flour affording the highest recoveries by sonication, the extraction efficiency of sonication was not sufficient for the incurred residues of the CPR mixture.

Table 2. Recoveries for extraction experiments with rice flour fortified at 0.1 mg kg^{-1}, calculated with matrix matched standard (n = 2).

	GC-MS retention time [min]	**Quantifier ion [m/z]**	**Extraction experiment**																**mod. EN 12393**	
Extraction			Dispersing 30 sec		Dispersing 60 sec		Dispersing 90 sec		Shaking 5 min		Shaking 10 min		Shaking 15 min		Ultra-sonication 5 min		Ultra-sonication 10 min		Ultra-sonication 15 min		Dispersing 2 min	
Liquid-liquid partitioning			Dispersing 30 sec						Shaking 10 min						Shaking 10 min						Dispersing 1 min	
				RSD		RSD		RSD		RSD		RSD		RSD		RSD		RSD		RSD		RSD
Brompropylat	26.65	341	**80**	3.5	**81**	5	**82**	2.5	**88**	3.1	**87**	0.7	**87**	6	**94**	8.1	**93**	4.5	**89**	0.2	**95**	5.6
Carbaryl	17.1	115	**86**	1.5	**93**	2.1	**99**	5.6	**92**	0.1	**94**	5.7	**95**	9.3	**99**	12.2	**102**	2.1	**94**	1	**102**	9.2
Chlorothalonil	15.22	266	**26**	3	**28**	10.5	**27**	8.8	**21**	0.3	**18**	7.9	**18**	3.1	**33**	15.9	**35**	1.3	**34**	3.7	**24**	6.9
Chlorpropham	11.37	153	**89**	0.3	**100**	3	**110**	6.9	**105**	0.5	**100**	8.6	**102**	7.8	**105**	11	**106**	4.1	**97**	2.3	**100**	9.1
Chlorpyrifos	19.25	314	**88**	1.6	**95**	4.3	**102**	5.8	**100**	3.8	**97**	4.5	**99**	9.1	**101**	12.3	**104**	0.3	**102**	1.8	**98**	7.8
Chlorpyrifos-methyl	16.9	286	**86**	1	**96**	0.9	**105**	5.9	**102**	1.7	**97**	6.5	**100**	7.8	**99**	12.6	**103**	1.9	**99**	2.2	**100**	7.8
Chlorthion	19.7	109	**96**	1	**115**	(n = 1)	**119**	6.6	**108**	1.9	**108**	6.9	**109**	8	**110**	16.2	**117**	0.4	**110**	2.1	**104**	7.2
Cyprodinil	20.38	224	**81**	4.4	**82**	5.2	**83**	2.8	**91**	3.4	**89**	1.6	**89**	7.1	**96**	8.9	**95**	3.2	**91**	0	**95**	6.1
DDT, p-,p'	25.28	235	**83**	3.3	**86**	4	**87**	3.4	**91**	4.1	**90**	1.1	**90**	7.2	**96**	8.9	**96**	2.9	**91**	0.7	**94**	6
Dichlofluanid	18.56	167	**14**	7.4	**15**	1.1	**17**	14.3	**16**	7	**14**	2.6	**12**	10.3	**16**	1	**15**	3.6	**15**	8.1	**15**	3.9
Dieldrin	22.9	79	**82**	2.5	**83**	4.4	**87**	6.4	**94**	5.1	**89**	3.5	**91**	5.6	**96**	8.5	**94**	2.3	**90**	1.4	**92**	7.4
Dimethoat	13.04	87	**88**	(n = 1)	**94**	1.6	**100**	4.9	**95**	1.3	**94**	6.2	**95**	8.1	**97**	12.2	**99**	1.3	**92**	1.4	**92**	8.4
Diphenylamin	11.25	108	**55**	1.4	**52**	28.8	**63**	4.5	**62**	4.1	**55**	0.9	**70**	13.9	**57**	7.1	**61**	7.3	**58**	2.9	**59**	13.5
α-Endosulfan	22.01	237	**81**	3.2	**82**	2.3	**84**	5.9	**91**	4.4	**87**	4.4	**88**	7.7	**92**	8.4	**90**	1.5	**86**	1.9	**87**	8.5
β-Endosulfan	23.88	237	**83**	3.9	**83**	4.3	**83**	2.8	**91**	3.6	**89**	1.9	**88**	6.5	**95**	7.6	**94**	2.9	**92**	0.5	**91**	5.8
Endosulfan-sulfat	25.12	237	**74**	3.4	**75**	4	**74**	2.7	**82**	4.1	**79**	1.8	**79**	6.7	**88**	8.2	**87**	3.6	**82**	0.3	**83**	4.9
Endrin	23.44	263	**85**	3	**89**	3.3	**94**	4.6	**95**	3.5	**93**	3.6	**95**	6.5	**96**	10.1	**97**	2	**94**	1.4	**94**	7.3
Esfenvalerat	39.05	167	**77**	3.8	**78**	4	**80**	5.7	**87**	2.4	**85**	0.2	**84**	6.1	**91**	9.5	**91**	3.4	**86**	0.7	**91**	4.8
Fluquinconazol	31.41	340	**79**	3.9	**79**	4.3	**80**	2.4	**86**	1.6	**86**	0.8	**86**	5.7	**91**	7.7	**91**	3.5	**86**	0.5	**95**	4.9
Folpet	21.23	260	**30**	4.6	**24**	23.4	**30**	14.4	**25**	1.2	**24**	8.9	**26**	3.8	**43**	19.7	**49**	1.1	**47**	2.5	**28**	5.2
Hexachlorobenzene	12.76	282	**60**	1.9	**61**	13.5	**67**	9.9	**73**	1.8	**66**	1.8	**65**	12.6	**64**	11.2	**60**	10	**55**	2.5	**66**	9.8
Kresoxim-methyl	23.59	116	**81**	3.8	**80**	5.6	**81**	3.1	**89**	3.2	**87**	2	**86**	7.1	**94**	8.8	**93**	3.3	**88**	1.2	**90**	5.3
Lindane	13.89	181	**77**	1	**81**	5.9	**87**	11.7	**98**	0.2	**88**	7.8	**90**	7	**91**	7	**84**	5.5	**77**	1.3	**90**	11.1
Malathion	18.8	125	**87**	1.1	**92**	3.7	**95**	4.2	**100**	2.3	**96**	5.2	**97**	8.3	**101**	11.9	**104**	0.7	**99**	1.9	**93**	8.4

Table 2 continued..

Metalaxyl	17.57	206	**74**	4.2	**75**	5	**75**	4.3	**82**	2.5	**79**	2	**79**	7.5	**88**	9.3	**86**	2.7	**81**	1.6	**81**	7.3
Metribuzin	16.56	198	**35**	1.9	**58**	18	**38**	31.4	**59**	24.8	**74**	20.9	**31**	19.5	**35**	27.3	**37**	4.9	**45**	6.7	**89**	7
Monolinuron	13.49	126	**95**	2.2	**117**	6.9	**123**	6.9	**122**	2.7	**114**	10.6	**116**	10.6	**126**	11.2	**126**	4.3	**115**	5.9	**115**	10.4
Omethoat	10.04	110	**44**	0.4	**44**	17.1	**49**	8.7	**54**	0.1	**56**	7.8	**57**	9.1	**56**	10.6	**58**	3.4	**50**	2.7	**53**	11.5
o-Phenylphenol	8.54	141	**46**	1.1	**50**	2.1	**53**	7.7	**58**	2.9	**55**	6.4	**54**	9.9	**58**	10.5	**58**	6	**53**	4	**57**	9.8
Permethrin	31.18	163	**77**	5.8	**75**	4.1	**76**	1.7	**78**	3.2	**75**	0.9	**76**	6	**88**	7.7	**87**	3.4	**83**	0.3	**88**	5
Pirimicarb	16.01	166	**83**	3.3	**85**	5	**87**	3.9	**95**	2.3	**92**	4	**92**	7.8	**98**	9.6	**97**	2.7	**91**	0.5	**98**	8.1
Pirimiphos-methyl	18.39	290	**86**	2.2	**95**	8	**97**	5.3	**101**	2.7	**97**	4.8	**98**	8.6	**99**	11.9	**103**	0.4	**98**	2.3	**100**	7.4
Tebuconazol	25.63	250	**75**	2.2	**79**	6.6	**78**	1.9	**82**	3	**81**	0.8	**81**	5.6	**86**	8.6	**86**	3.5	**82**	0.7	**87**	5.1
Tetramethrin	26.9	123	**74**	3.6	**74**	4.5	**74**	2.3	**77**	2.1	**77**	1.9	**76**	6.8	**89**	9.1	**88**	3.8	**83**	0.9	**83**	6.5
Average			**72**	2.8	**76**	6.7	**79**	6.5	**82**	3.3	**80**	4.6	**79**	8	**84**	10.6	**85**	3.2	**80**	2	**83**	7.4

Table 3. Recoveries for extraction experiments with CPR mix, calculated with solvent standards (n = 2). Recoveries of the reference method (mod. EN 12393) were set to 100%.

	GC-MS retention time [min]	**Quantifier ion [m/z]**	**Extraction experiments**																**mod. EN 12393**	
Extraction			Dispersing 30 sec		Dispersing 60 sec		Dispersing 90 sec		Shaking 5 min		Shaking 10 min		Shaking 15 min		Ultra-sonication 5 min		Ultra-sonication 10 min		Ultra-sonication 15 min		Dispersing 2 min	
Liquid-liquid partitioning			Dispersing 30 sec						Shaking 10 min						Shaking 10 min						Dispersing 1 min	
				RSD		RSD		RSD		RSD		RSD		RSD		RSD		RSD		RSD	mg kg^{-1}	RSD
Azoxystrobin	36.57	344	**111**	16	**95**	1	**88**	10	**112**	4	**113**	3	**95**	8	**86**	12	**90**	3	**93**	1	**0.026**	2
Chlorfenpyr	25.26	247	**117**	22	**116**	13	**106**	9	**124**	2	**108**	16	**92**	9	**81**	10	**74**	1	**101**	9	**0.011**	12
Chlorpyriphos	19.22	314	**94**	18	**82**	0	**79**	8	**99**	2	**96**	10	**81**	17	**73**	1	**79**	2	**78**	6	**0.029**	1
Cypermethrin	32.7	163	**75**	13	**76**	14	**69**	6	**99**	7	**104**	1	**85**	16	**76**	5	**84**	9	**84**	5	**0.435**	2
Dicofol	28.79	139	**124**	17	**101**	12	**109**	7	**120**	6	**132**	1	**103**	14	**97**	4	**96**	5	**111**	4	**0.034**	4
Ethion	26	231	**103**	17	**89**	1	**84**	7	**108**	4	**102**	6	**90**	12	**83**	1	**85**	1	**85**	2	**0.225**	3
Mycobutanil	24.42	179	**66**	(n = 1)	**89**	28	**66**	25	**73**	18	**70**	11	**87**	14	**75**	1	**64**	10	**63**	4	**0.012**	1
Pendimethalin	20.97	252	**100**	23	**84**	1	**81**	7	**98**	5	**96**	4	**84**	12	**76**	6	**79**	2	**82**	1	**0.005**	3
Permethrin	31.38/ 31.56	163	**86**	10	**72**	2	**70**	0	**80**	5	**74**	2	**68**	14	**59**	1	**59**	4	**60**	0	**0.051**	2
Phosalone	29.67	182	**137**	11	**123**	1	**112**	13	**134**	2	**130**	3	**117**	9	**110**	2	**110**	1	**111**	1	**0.035**	1
Profenofos	23.9	208	**125**	23	**102**	2	**96**	4	**132**	5	**122**	6	**104**	14	**95**	2	**89**	5	**117**	3	**0.028**	6
Tebuconazole	27.42	250	**81**	18	**71**	6	**72**	9	**115**	8	**104**	32	**92**	28	**100**	1	**99**	0	**102**	3	**0.013**	1
Triazophos	26.45	313	**104**	15	**92**	0	**104**	18	**111**	0	**105**	4	**92**	12	**88**	2	**90**	3	**90**	2	**0.075**	2

For the final assessment, dispersing and shaking were taken into account as an extraction technique for a miniaturized sample preparation following the EN 12393 methodology. Besides slightly higher recoveries for the sample with incurred residues, shaking has major advantages over dispersing in terms of sample handling. In one sequence, more than 20 samples can be extracted simultaneously on an automated shaker and it does not require manual work during extraction or partitioning. On the contrary, depending on the number of dispersing tools available, only a few samples can be handled at the same time in the case of the dispersing technique. Additionally, solvent and time consuming cleaning of the dispersing instruments has to be done manually, and the risk of a cross contamination is still present. Therefore and due to the good recovery results, 'shaking for 10 min' was selected as extraction and partitioning step for a miniaturized method.

In comparison with the original and the CLF modified EN 12393, the new extraction and partitioning procedure significantly reduced the solvent consumption by a factor of ten (Table 4), which is clearly reflected in the consumable costs (reduction by 90%). Additionally, time for extraction and partitioning steps decreased by nearly 50%, calculated for a set of 14 and 20 samples for the reference and miniaturized method, respectively. Due to automatic shaking, also the hands-on time was decreased by 60% (< 5 min per sample) for the extraction and partitioning steps, which offers higher efficiency in the laboratory.

3.2. Gel permeation chromatography

The traditional BioBeads SX-3 column for extract cleanup has some major drawbacks. First of all, elution of all analytes from the column lasts up to 45 min. Due to this extended cleanup time, huge amounts of solvent are consumed and not more than 18 samples can be run on one instrument in a shift. With the primary aim to speed up the GPC cleanup to a time slot of below 25 min, different analytical GPC columns were tested additionally regarding a clear separation of high molecular matrix compounds like triglycerides from the pesticide fraction. Therefore, elution profiles for sunflower oil (30 mg/50 mg on column) with analytes that are known to elute early from BioBeads SX-3 (hexaflumuron, fluazinam, flucythrinat, λ-cyhalothrin, flumethrin, cyfluthrin, deltamethrin, permethrin) were acquired. This spectrum of analytes was extended by more polar analytes (dichlorvos, acephate, methomyl, and methamidophos) and a commercially available calibration mixture for BioBeads SX-3 columns (methoxychlor, perylene, sulphur) as well as dimethomorph and chinomethionat. The results showed that all columns based on styrene-divinylbenzene (PSS SDV, MZ SDgel) had difficulties to separate pyrethroids from sunflower oil (Figure 1). The elution order was identical for

Table 4. Solvent consumption, analysis time, and hands-on time for the miniaturized EN 12393 as compared to the reference method.

	Modified EN 12393 (reference method)	**Miniaturized EN 12393**
Solvent consumption [mL]		
- Extraction & partitioning	375	38
- GPC cleanup	210	197
Analysis time [min]		
- Extraction & partitioning	22	12
- GPC cleanup[1)]	66.5	50.5
Hands-on time [min]		
- Extraction & partitioning	11.7	4.8
- GPC cleanup	2.5	2.5

[1)]Including evaporation and rinsing.

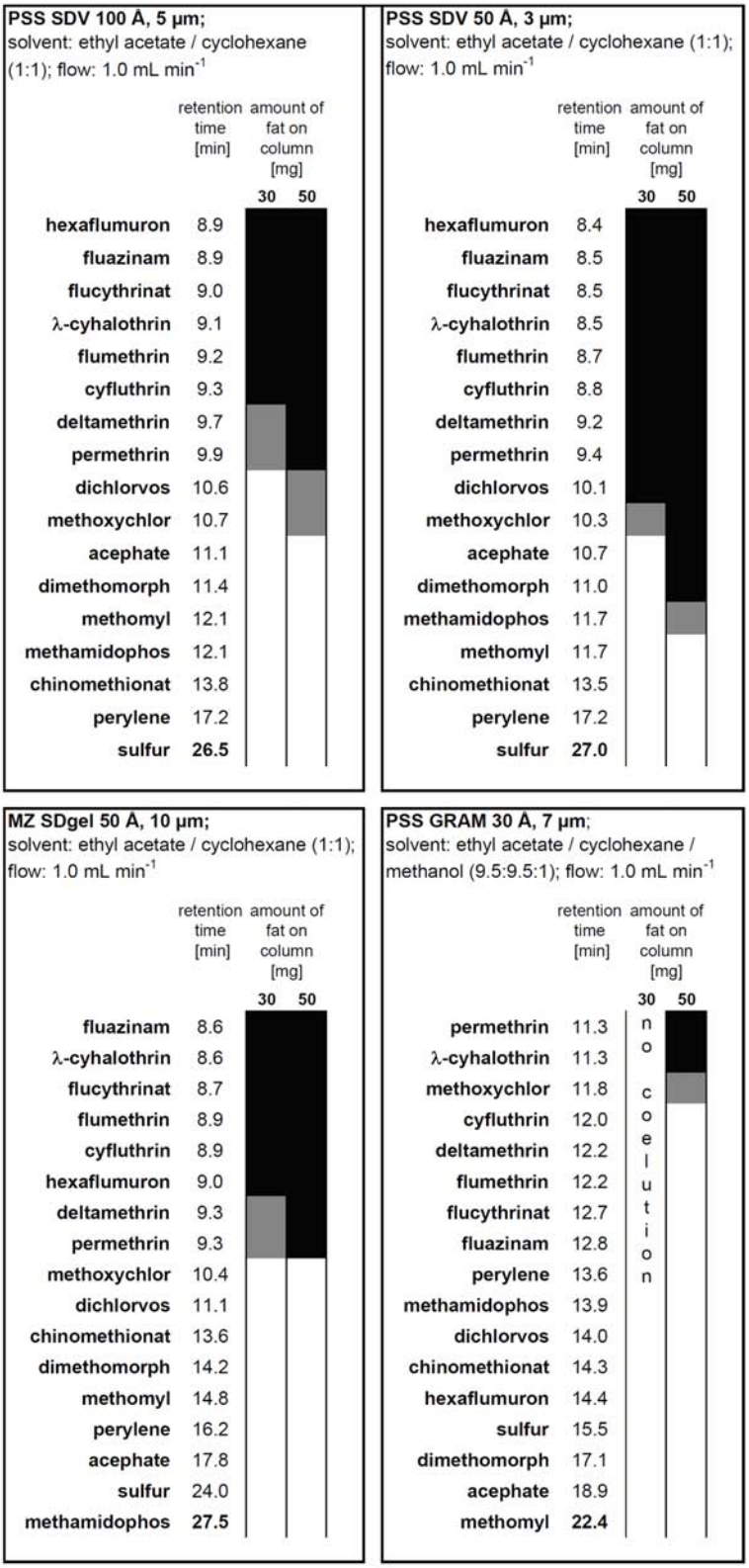

Figure 1. Elution profiles of four different analytical HR-GPC columns (8 mm x 300 mm) for selected pesticides and sunflower oil (black: full co-elution with sunflower oil; grey: partial co-elution with sunflower oil; white: no co-elution).

both PSS SDV materials, and there were no remarkable differences in retention times. However, the sunflower oil peak was much broader on the 50 Å column and affected more analytes than on the 100 Å column. The third styrene-divinylbenzene material (MZ SDgel) seems to have more polar properties resulting in stronger retention of methomyl, acephate or methamidophos than the PSS SDV material did. For all three styrene-divinylbenzene materials, a total runtime of less than 30 min was achieved, but the resolution of sunflower oil and pesticides was not sufficient. On the contrary, PSS GRAM, a highly cross-linked polyester, resulted in a good separation of the tested analytes. For the load of 30 mg sunflower oil, no co-elution of pesticides was observed, while only permethrin, λ-cyhalothrin and partly methoxychlor were affected, when a load of 50 mg sunflower oil was injected onto the column. Unfortunately, polar analytes (acephate 32.0 min, methomyl 32.4 min, methamidophos 45.23 min) were strongly retained due to the polar character of the polyester-copolymer, when the traditional GPC eluent ethyl acetate/cyclohexane (1:1) was used. Increasing the percentage of ethyl acetate from 50% to 75% accelerated the elution of acephate (19.4 min), methomyl (19.3 min) and methamidophos (26.0 min), but separation capacity was lost, and many pesticides co-eluted with sunflower oil (elution profiles not shown). However, adding 5% methanol to ethyl acetate/ cyclohexane (1:1) resulted in excellent separation of sunflower oil from pesticides within a total runtime of less than 23 min for the tested analytes (Figure 2).

The influence of different percentages of methanol added to the elution solvent was also studied. The results showed that compounds with log $K_{OW} < 1$ were highly affected by the methanol dosage. Acephate (log K_{OW} -0.9) was shifted by 17.5 min to an elution time of 14.5 min with 15% methanol, methamidophos (log K_{OW} -0.8) even by 29.5 min to 15.5 min, both in comparison to a run without the addition of methanol. Further tests showed that compounds with log K_{OW} from 1 to approximately 3.2-3.7 were only slightly affected,

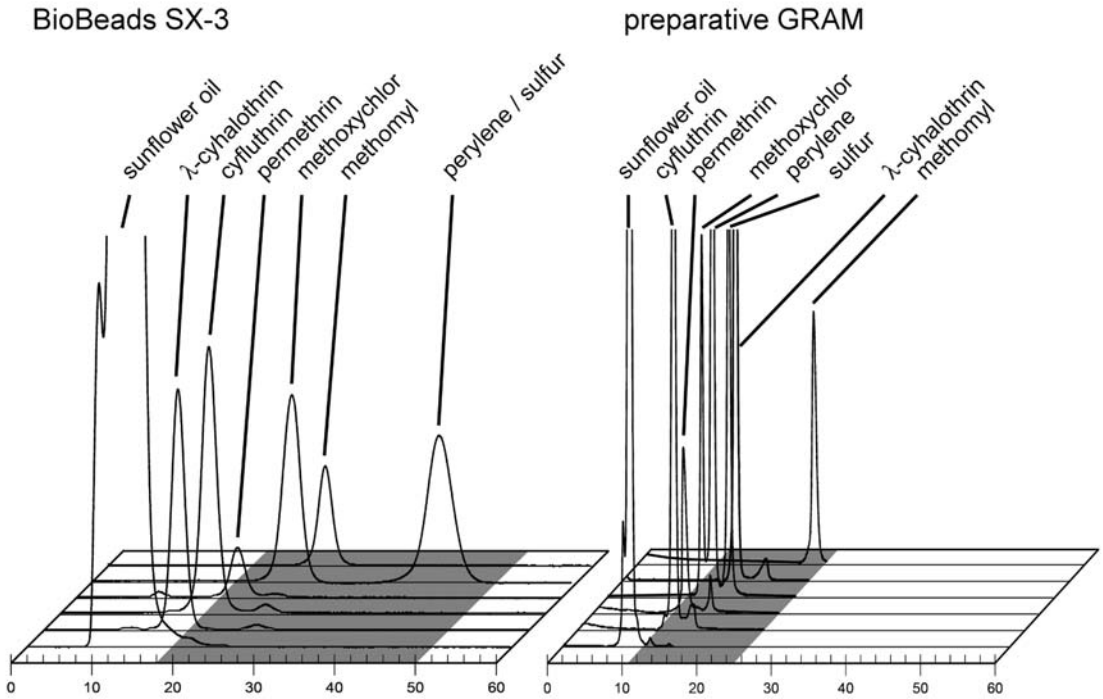

Figure 2. Separation of selected analytes from sunflower oil (400 mg on column) on BioBeads SX-3 column (flow 5 mL min^{-1}; ethyl acetate/cyclohexane 1:1) and PSS GRAM column (flow 7.5 mL, ethyl acetate/ cyclohexane/methanol, 9.5:9.5:1); grey areas: pesticide fraction (collecting period).

e.g. dichlorvos (log K_{OW} 1.9; Δ t = 1.5 min). Compounds with log K_{OW} > 3.7 like λ-cyhalothrin (log K_{OW} 6.9; Δ t = 0 min) were generally not affected. To move polar analytes into an elution window of < 25 min, 5% methanol dosage is sufficient. PSS GRAM 30 Å had the best properties to separate high molecular matrix compounds from the pesticide fraction under the described conditions. Further experiments on the analytical PSS GRAM 30 Å showed that beta-carotene (0.1 mg on column), α-tocopherol, stigmasterol and cholesterol (all 1.0 mg on column) also had retention times < 10.5 min and can be separated from the pesticide fraction. However, complete separation of linoleic acid was not possible. Therefore, fatty acids need a further selective cleanup as by an amino phase SPE.

These preliminary tests with only few pesticides but especially pyrethroids showed that an improvement of the cleanup was clearly achieved with the PSS GRAM 30 Å column in comparison with the traditional BioBeads SX-3 column. To ensure suitability of the new column for a cleanup of food samples and satisfactory recoveries of relevant pesticides, 310 analytes (Table 5) were applied on a preparative PSS GRAM 30 Å column (20 x 300 mm, flow 7.5 mL min^{-1}). The start time (10.8 min) for collecting the pesticide fraction was defined by the elution profile of sunflower oil (400 mg on column) and permethrin. Only four of the 310 tested pesticides showed a complete co-elution with sunflower oil and could not be recovered (carbosulfane, butylate, tefluthrine, fenpropimorph), while of those four pesticides only tefluthrine could not satisfactorily be recovered on BioBeads SX-3. Another compound, dimethipin, remained on the column and eluted later than 25 min. Comparing the BioBeads SX-3 and the preparative PSS GRAM 30 Å it could be shown that λ-cyhalothrin and cyfluthrin was not fully separated from sunflower oil by BioBeads SX-3, while PSS GRAM gave good results for both compounds (Figure 2). PSS GRAM is also able to remove sterols from extracts, which often can be detected in GC-MS total ion chromatograms and may deposit in the injection liner or on the column. Thus, the exchange of BioBeads SX-3 by PSS GRAM 30 Å results in an improvement of the EN 12393 GPC cleanup, concerning separation

Table 5. All pesticides (305) that elute between 10.8 min and 25.0 min (pesticide fraction) on preparative PSS GRAM 30 Å column (20 x 50 mm pre-column/20 x 300 mm column).

2.4-D methyl ester	Carfentrazon-ethyl
2.4-D-butoyl ester	Chlorbensid
2.4-D-butyl ester	Chlorbromuron
4.4'-dichlorobenzo-phenone	Chlordan
Acephate	Chlordan-oxy
Acetochlor	Chlorfenapyr
Aclonifen	Chlorfenprop-methyl
alpha-Endosulfan	Chlorfenson
HCH-alpha	Chlorfenvinphos
HCH-beta	Chlormephos
d-HCH	Chlorobenzilate/ Chloropropylate
Alachlor	Chloroneb
Aldrin	Chlorothalonil
Allethrin	Chlorpropham
Ametryn	Chlorpyrifos-ethyl
Atrazin	Chlorpyrifos-methyl
Atrazin-desethyl	Chlorthal-dimethyl
Azinphos-ethyl	Chlorthiophos
Azoxystrobin	Chlozolinat
Benalaxyl	Cinidon-ethyl
Bendiocarb	Clodinafop-propargyl
beta-Endosulfan	Clomazon
Benfluralin	Cloquintocet-1-methylhexyl
Benoxacor	Coumaphos
Bifenox	Crotoxyphos
Bifenthrin	Cyanacine
Biphenyl	Cyanofenphos
Bitertanol	Cyanophos
Boscalid	Cycloate
Bromacil	Cycluron
Bromophos-ethyl	Cyflufenamid
Bromophos-methyl	Cyfluthrin
Bromopropylate	Cyhalofop-butyl

Table 5 continued..

Bromoxynil-octanoat	Cyhalothrin-lambda
Bupirimate	Cypermethrin
Buprofezin	Cyphenothrin
Butralin	Cyproconazol
Cadusafos	Cyprodinil
Carbaryl	DDD, o-, p'-
Carbaryl-phenol	DDD, p-, p'-
Carbofuran	DDE, o-, p'-
Carbofuran-phenol	DDE, p-, p'-
Carbophenthion	DDT, o-, p'-
DDT, p-, p'-	Ethoxyquin
Deltamethrin	Etoxazol
Demeton-S-metyl	Etrimfos
Demeton-S-metyl	Famoxadon
Desmetryn	Famphur
Dialifos	Fenamiphos
Di-allate	Fenarimol
Diazinon	Fenbuconazol
Dichlobenil	Fenchlorphos
Dichlofluanid	Fenhexamid
Dichlorimid	Fenitrothion
Dichlorvos	Fenoxaprop-ethyl
Diclofop-methyl	Fenpropathrin
Dicloran	Fenpropidin
Dicofol	Fenson
Dieldrin	Fensulfothion
Diethofencarb	Fenthion
Difenconazol	Flamprop-isopropyl
Diflufenican	Flamprop-methyl
Dimepiperat	Fluazifop-butyl
Dimethachlor	Flucythrinat
Dimethenamid	Fludioxonil
Dimethoat	Flufenacet
Dimethomorph	Fluopicolid
Dimoxystrobin	Fluotrimazol
Dioxathion	Fluquinconazol
Diphenylamin	Flurochloridone

Table 5 continued..

Disulfoton	Fluroxypyr-1-methylheptylester
Disulfoton-sulfon	Flurtamon
Ditalimfos	Flusilazole
DMSA	Flutriafol
DMST	Fluvalinate
Endosulfan-sulfate	Folpet
Endrin	Fonofos
Endrin ketone	Formothion
EPN	Fosthiazate
Epoxiconazol	Fuberidazole
EPTC	Furalaxyl
Esfenvalerat	Halfenprox
Ethion	Haloxyfop-2-ethoxyethyl
Ethofenprox	Haloxyfop-methyl
Ethofumesate	Heptachlor
Ethoprophos	Heptachlor-epoxid
Heptenophos	PCB 180
Hexachlorobenzene	PCB 28
Hexaconazole	PCB 52
Iodofenphos	Penconazole
Iprodione	Pencycuron
Isodrin	Pendimethalin
Isofenphos	Pentachloranisol
Isofenphos-methyl	Pentachlorobenzol
Isoprothiolan	Pentanochlor
Isoxadifen-ethyl	Permethrin
Kresoxim-methyl	Perthane
Leptophos	Phenkapton
Lindane	Phenothrin
Linuron	Phenthoat
Malaoxon	Phorat
Malathion	Phosalon
Mecarbam	Phosphamidon
Mefenpyr-diethyl	Picoxystrobin
Mepanipyrim	Pirimicarb
Metalaxyl	Pirimiphos methyl

Table 5 continued..

Metazachlor	Pirimiphos-ethyl
Metconazol	Pretilachlor
Methacrifos	Prochloraz
Methamidophos	Procymidon
Methidathion	Profenofos
Methoxychlor	Prometryn
Metolachlor	Propachlor
Metribuzin	Propanil
Mevinphos	Propaquizafop
Molinat	Propargite
Monalid	Propazine
Monocrotophos	Propetamphos
Monolinuron	Propham
Myclobutanil	Propiconazol
Naled	Propyzamide
Nitrofen	Prothiofos
Nuarimol	Prothoat
Omethoate	Pyraclostrobine
o-phenylphenol	Pyrazophos
Oxadiazon	Pyridaben
Oxyfluorfen	Pyridaphenthion
Paraoxon	Pyrifenox
Paraoxon-methyl	Pyrimethanil
Parathion-ethyl	Quinalphos
Parathion-methyl	Quinoxyfen
PBO	Quintozen
PCB 101	Quizalofop-ethyl
PCB 118	Resmethrin
PCB 138	Rotenon
PCB 153	Simazin
Spirodiclofen	Thionazin
Sulfotep	Tolclofos-methyl
Tebuconazole	Tolylfluanid
Tebufenpyrad	Triadimefon
Tecnazene	Triadimenol
Terbacil	Tri-allate
Terbufos	Triamiphos

Table 5 continued..

Terbufos-sulfone	Triazophos
Terbuthylazine	Trichloronat
Terbutryn	Tricyclazole
Tetrachlorvinphos	Trifloxystrobin
Tetraconazol	Triflumizol
Tetradifon	Trifluralin
Tetrahydrophthalimide	Triticonazole
Tetramethrin	Vinclozolin
Tetrasul	Zoxamide
Thiobencarb	

of matrix compounds, and particularly in a reduction of analysis time by 24% (Table 4). A cleanup run including online evaporation and rinsing of the instruments with BioBeads SX-3 lasted 66.5 min (reduced runtime of 40 min + 26.5 min for evaporation and rinsing), while the PSS GRAM 30 Å column only took 50.5 min (25 min runtime + 25.5 min for evaporation & rinsing). However, regarding the different flow rates, the solvent consumption of both columns was nearly identical. In terms of costs, the GRAM column is slightly above BioBeads SX-3, but there are presently no data available to compare the life time and stability of both columns.

4. CONCLUSIONS

The presented results showed that a miniaturization of a multi-pesticide residue method based on the EN 12393 methodology gave excellent recoveries for extraction and partitioning experiments. Due to the advantages of shaking in terms of sample handling (closed system, no risk of cross contamination, simultaneous processing of more than 20 samples, and reduced hands-on time), this procedure is to be preferred for high throughput sample preparation. The developed modifications reduced solvent amounts and analysis time, thus leading to a strong reduction of costs. The newly introduced PSS GRAM column singularly resulted in excellent separation performance for more than 305 tested pesticides from sunflower oil, while GPC runs simultaneously were reduced to 25 min.

Combining the miniaturized extraction and partitioning steps and the new HR-GPC cleanup, solvent consumption, and hands-on time were generally reduced by about 90% and 50%, respectively, while analysis time was reduced by about 30%. In conclusion, a package of modifications can be presented, well suited for the introduction of a rapid and miniaturized EN 12393.

CONFLICT OF INTEREST STATEMENT

The authors of this publication declare that there is no conflict of interest.

REFERENCES

1. Regulation (EC) No. 396/2005, Official Journal of the European Union L 70 (2005), 1.
2. Commission Directive 2006/125/EC, Official Journal of the European Union L 339 (2006), 16.
3. Commission Directive 2006/141/EC, Official Journal of the European Union L 141 (2006), 1.
4. Luke, M. A., Froberg, J. E. and Masumoto, H. T. 1975, J. AOAC Int., 58, 1020.
5. Specht, W. and Tillkes, M. 1980, Fres. J. Anal. Chem., 301, 300.
6. Anastassiades, M., Štajnbaher, D., Schenck, F. J. and Lehotay, S. J. 2003, J. AOAC Int., 86, 412.
7. Klein, J. and Alder, L. 2003, J. AOAC Int., 86, 1015.
8. EN 12393-2, Foods of plant origin: Multiresidue methods for the gas chromatographic determination of pesticide residues - Part 2: Methods for extraction and clean-up, European Committee for Standardisation, Brussels, Belgium (2009).
9. Specht, W., Pelz, S. and Gilsbach, W. 1995, Fres. J. Anal. Chem., 353, 183.
10. Stan, H. J. and Linkerhägner, M. 1996, J. Chromatogr. A, 750, 369.
11. Hercegova, A., Dömötörova, M. and Matisova, E. 2007, J. Chromatogr. A, 1153, 54.
12. Tadeo, J. L., Sanchez-Brunete, C., Albero, B. and Garcia-Valcarcel, A. I. 2010, J. Chromatogr. A, 1217, 2415.
13. Hercegova, A., Dömötörova, M., Matisova, E., Kirchner, M., Otrekal, R. and Štefuca, V. 2005, J. Chromatogr. A, 1084, 46.