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Fast gas chromatography with solid phase extraction clean-up for ultratrace analysis of pesticide residues in baby food

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Abstract

A sample preparation method based on single solvent phase extraction and solid-phase extraction (SPE-NH_2) clean-up is studied in combination with fast capillary gas chromatography (GC) to determine 18 selected pesticides belonging to various chemical classes in apples, the common raw material for baby food production and baby food, at the concentration level ≤10 μg/kg maximum residual limit (MRL). Possibilities of mass spectrometry (MS) detector and electron capture detector (ECD) in fast gas chromatography (GC) of samples with complex matrice at ultra trace levels of pesticide residues were studied and compared. MS detection in single ion monitoring (SIM) mode provided higher selectivity compared to ECD. Optimisation of extraction as well as the simplifying of the whole process of sample preparation was carried out. Recoveries obtained at concentration level of 5 μg/kg (the required value for limit of quantification (LOQ) in baby food) were >90%, except of dimethoate (77.7%) and captan (46.4%) with MS detection. The obtained LOQs were at least 1 order lower than 5 μg/kg for the majority of compounds. The repeatability of gas chromatography–mass spectrometry (GC–MS) measurements of the matrix matched standards expressed as relative standard deviation was <11% except of captan and cypermethrin.

Keywords: Fast GC; Sample preparation; Pesticide residues; Baby food; Apple

1. Introduction

In the present agricultural practice, pesticides provide an unquestionable benefit for crop protection; however, the presence of pesticide residues in food can negatively affect human health. This stimulates the establishment of legal directives to control their levels through the maximum residual limits (MRLs). Special attention is paid to the safety of children and infants, as they represent a vulnerable risk group of the population. Therefore, the European Commission (EC) specified the MRL of 10 μg/kg of pesticide residue content in baby food (on the basis of the opinions of the Scientific Committee on Food, Directive 2003/13/EC) and established the prohibition of the use of highly toxic pesticides (ADI ≤ 0.0005 mg/kg bodyweight) in the production of agricultural products intended for processed cereal-based foods and baby foods [1]).

The most common plant raw material for baby food production is apple. The appropriate multiresidue chromatographic analysis of pesticide residues in apples at the concentration level lower than 10 μg/kg expects sample preparation capable of pre-concentration, sufficient removal of matrix components in simultaneous preservation of high recoveries and good precision. The matrix of apple contains carbohydrates, chlorophylls, some lipids, sterols, glycosides, triglycerides and other components [2], therefore the sample preparation represents one of the most critical parts of analysis of pesticide residues in apples. The development of sample preparation in multiresidue gas chromatography (GC) analysis of pesticides in non-fatty food headed from simple solvent extraction followed by liquid–liquid (L–L) partitioning towards methods with cleaning-up of extracts by SPE. The

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following research was oriented to develop alternative methods such as matrix solid phase dispersion (MSPD), supercritical fluid extraction (SFE), solid-phase microextraction (SPME) to reduce solvent consumption and manual labor [3]. Despite their advantages, none of the techniques have overcome critical flaws or practical limitations to enable their widespread implementation [4]. Present multiresidue methods enabling determination of pesticide residues at concentration level 10 μg/kg and below require costly and highly sophisticated instrumentation such as GC–MS–MS. Therefore, further optimisation of existing methods or development of new methods providing reliable results at desired concentration level of 5 μg/kg (LOQ arised from a value of MRL 10 μg/kg) utilizing common routine instrumentation is required.

Connection of appropriate sample preparation technique for this purposes with fast GC on narrow-bore capillary columns provides unquestionable benefits, such as high throughput, low GC operating costs with in some cases even higher separation efficiency, higher precision and sensitivity [5,6] than conventional GC and therefore is advantageous in routine analyses. However, in the case of samples with matrix of a plant origin (complicated matrices), in the ultra trace levels of pesticide residues concentration becomes the problem of sufficiently low LOQs determination most noticeable. Mass spectrometric detection (MSD) in selected ion monitoring (SIM) mode in combination with fast GC utilizing options that reduce the analysis time at constant resolution (compared to conventional) [5,6] provides a promising solution.

The purpose of this study was to elaborate method for the determination of pesticide residues in apples and baby food at the concentration level of 5 μg/kg utilizing efficient sample preparation method combined with fast GC on narrow bore columns coupled with electron capture detector (ECD) and the most widely used quadrupole MS detector. For this purpose a column of 0.15 mm i.d. was preferred over of 0.1 mm (what represent a current limit for practical application) [5,6]. This diameter can be used in majority of GC instruments and offers more flexibility with respect to flow, loadability and operation. Optimisation of various operational parameters preceded to application of fast GC [7,8]. Studied pesticides were chosen in accordance with the consumption of pesticides applied on apple trees in south-west part of Slovakia, which are amenable to GC analysis and also represent pesticides of a wide range of polarity and other physico-chemical properties. Finally, the method was tested on real samples.

2. Experimental

2.1. Reagents and materials

Pesticides were obtained from various sources and were of purity >95% (bitertanol (Bayer, Germany), cyprodinyl, methidathion, penconazole, terbuthylazine (Ciba-Geigy, Basel, Switzerland), dimethoate (Cheminova Agro, Denmark), etofenprox (Mitsui Toatsu Chemicals, Japan), fenitrothion (Sumimoto Chemical Co., Japan), chlorpyrifos (Dow Chemical Company, USA), kresoxim-methyl (BASF, Germany), myclobutanil (Dow Agro Science, USA), pyrimethanil (Schering, Germany), tebuconazole, captan, cypermethrin, diazinon (Argovita), phosalone, chlorpyrifos-methyl (Dr. Ehrenstorfer, Germany). Stock solution of pesticides with concentration of 0.5 mg/ml was prepared by dissolving 5 mg of each compound in 10 ml of toluene (Suprasolv, Merck, Darmstadt, Germany) and was stored at −18 °C. Pesticides

Table 1
List of used pesticides; retention times, peak widths and monitored ions in SIM mode

Compound	Chemical class	Retention time (min)	Peak width at half height (s)	Monitored ions in SIM, **Target ion**
Dimethoate	Organophosphate	5.86	0.912	**87**, 125
Terbuthylazine	Triazine	6.02	0.768	**214**, 229
Diazinon	Organophosphate	6.02	0.720	**276**, 304
Pyrimethanil	Anilinopyrimidine	6.11	0.780	**198**, 199
Chlorpyrifos-methyl	Organophosphate	6.41	0.756	**286**, 288
Fenitrothion	Organophosphate	6.62	0.780	260, **277**
Chlorpyrifos	Organophosphate	6.72	0.756	286, **314**
Cyprodinyl	Anilinopyrimidine	6.96	0.852	224, **225**
Penconazole	Triazole	7.00	0.864	**248**, 250
Captan	Phtalimide	7.13	1.008	**79**, 264
Methidathion	Organophosphate	7.18	0.948	**145**, 302
Kresoxim-methyl	Oximinoacetate	7.41	0.876	**131**, 132
Myclobutanil	Triazole	7.43	1.236	**179**, 245
Tebuconazole	Triazole	8.03	1.020	**250**, 252
Phosalone	Organophosphate	8.55	0.936	182, **367**
Bitertanol 1	Triazole	9.07	1.272	168, **170**
Bitertanol 2	Triazole	9.14	1.560	168, **170**
Cypermethrin 1	Pyrethroid	9.54	1.344	163, **181**
Cypermethrin 2	Pyrethroid	9.66	1.584	163, **181**
Cypermethrin 3	Pyrethroid	9.73	2.136	163, **181**
Etofenprox	Non-ester pyrethroid	9.85	1.871	**163**, 376

and their chemical classes are listed in Table 1. Stock solution was diluted with acetone (Suprasolv, Merck, Darmstadt, Germany) to get appropriate pesticide standard solutions for preparation of spiked samples and matrix-matched standards.

Acetonitrile (MeCN) and acetone used were of gas chromatography grade (Suprasolv, Merck, Darmstadt, Germany). Magnesium sulfate (anhydrous powder) was from Lachema (Neratovice, Czech Republic). Apples were mixed with blender Braun MX 2050 (Kronberg, Germany). The sample was filtered through glass fibre paper Z4 (Papírna Perštejn, Czech Republic). The SPE columns used were 500 mg of Bond-Elut—NH_2, (1ST Ltd., Mid Glamorgan, UK), 500 mg Supelclean ENVI-Carb (Supelco, Bellefonte, USA) and 1 g Mega BE-PSA (Varian Incorporated, Harbor City, USA). Standards were weighted on Sartorius Analytic MCI balances (Sartorius, Götingen, Germany) with a precision of ±10 μg.

2.2. Sample preparation

The apples (with peel) used for this study were homogenously mixed and stored at −18 °C in a refrigerator. For optimisation purposes as well as for recovery studies and preparation of matrix matched standards chemically untreated apples were used. Untreated and real samples were apples from field experiments [1]. Apple trees were individually treated either with pesticides myclobutanil and penconazole. Two samplings were carried out, 2 days after the last crop-spraying in the season in July and in the common collection season, in September. Baby food (apple purée) was produced according to the technology of Novofruct SK, s.r.o., Nové Zámky, Slovakia).

The modified Schenck's method [9] was utilized. To disrupt the cells of apples to enhance the pesticide residue extraction, sample (25 g) was weighed into a beaker and extracted with 50 ml of acetonitrile using immersed sonication sonda (i.d. 13 mm) of the pulsed ultrasonic cell disrupter VibraCell (Sonics and Materials Inc., Danbury, CT. USA, CVX 400, frequency 20 kHz). The ultrasonic pulses at 80% amplitude with duration of 3 s paused for 3 s were applied for 1–5 min. For comparison ultrasonic bath of ultrasonic compact cleaner Teson 1 (Tesla, Slovak Republic) with power output 45 W and frequency of 38 kHz (extraction time of 5–120 min was tested) was used.

Before the injection into a GC system, solvent exchange to toluene was applied. The whole process of sample preparation is presented in Fig. 1.

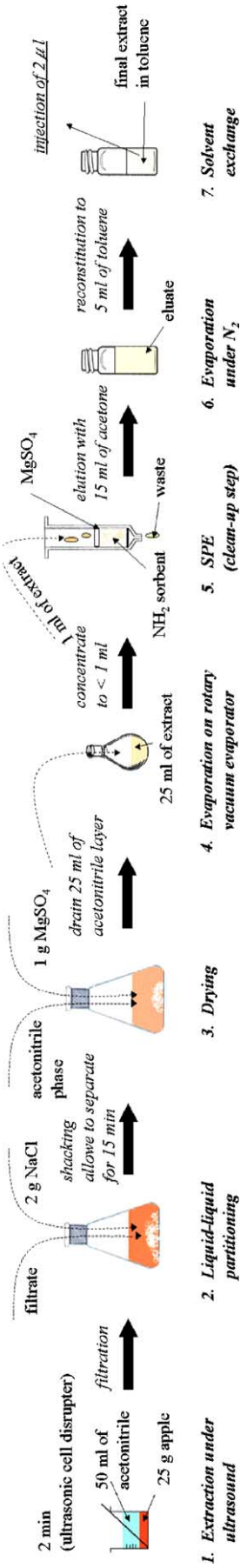

Fig. 1. Scheme of the whole process of apple sample preparation with acetonitrile extraction and SPE-NH_2 clean-up.

2.3. Chromatographic instrumentation and conditions

2.3.1. GC–ECD

The gas chromatographic analyses were carried out on a HP 6890 gas chromatograph (Hewlett Packard, Avondale, PA, USA) coupled to ECD. The system was equipped with a splitless injector with 2 mm i.d. liner. Injections were carried out by autosampler (Hewlett Packard, HP 6890 series, Avondale, PA, USA) using a 10 μl syringe (Hamilton, Reno,

Nevada, USA). GC analysis was conducted on CP-Sil 13 CB column (25 m × 0.15 mm × 0.4 μm) with 14% phenyl, 86% dimethylpolysiloxane stationary phase (Varian, Middelburg, The Netherlands) coupled with a non-polar deactivated fused silica pre-column (1 m × 0.32 mm i.d.) (Supelco, Bellefonte, PH, USA) by a glass press-fit connector (0.32/0.10, Agilent Technologies) sealed with a polyimide resin (Supelco, Bellefonte, USA). Following conditions were used: H_2 (purity >99.99%, Linde, Technoplyn, Bratislava, Slovak Republic), inlet temperature 250 °C (split vent 100 ml/min, splitless time 1 min), injection volume 2 μl, detector temperature 320 °C, programmed carrier gas flow: 2.3 ml/min (5.5 min), 2 ml/min^2, 3.4 ml/min and temperature program 100 °C (1 min), 65 °C/min, 290 °C (8 min).

2.3.2. GC–MSD

GC–MS measurements were performed on an Agilent 6890N GC connected to 5973 MSD (Agilent Technologies, Avondale, PA, USA) equipped with a programmed temperature vaporizer (PTV). Chromatographic column CP-Sil 8 Low-Bleed MS (15 m × 0.15 mm × 0.15 μm) (Varian, Middleburg, The Netherlands) was connected to the same pre-column in the same way as in GC–ECD. Helium with purity 5.0 (Linde Technoplyn, Bratislava, Slovak Republic) was used as carrier gas in constant flow mode 0.5 ml/min. PTV inlet was operated in cold splitless mode with the following conditions: temperature programme 120 °C, 400 °C/min ramp to 300 °C (1.2 min), 100 °C/min to 350 °C (3 min); split vent open time 1.5 min, injection volume 2 μl. Following oven temperature programme was used: initial temperature 120 °C hold 1 min, ramp 30 °C to 290 °C hold 5 min and a constant carrier gas flow 0.5 ml min^{-1}. MS with electron impact ionisation (EI) was used in SIM mode; two ions were selected [10] and monitored for each pesticide; dwelve time was set to 25 ms. Retention times, ions for SIM of 18 studied pesticides are listed in Table 1.

3. Results and discussion

In pesticide residue analysis the most important issues are selectivity, sensitivity of the method, accuracy of quantitation, low costs and not time consuming method with respect to the required LOQs. Due to the wide range of polarities, water solubilities and volatilies of pesticides a compromise is needed.

3.1. Method development

Fruit samples free of pesticides (from field experiments [1]) were used for the preparation of a blank matrix standard. Blank samples were first analysed by GC–MS before being spiked and none of the selected ions were found at the corresponding retention times of selected pesticides (Table 1). The ECD also shows that blanks were free of the selected pesticides.

Twenty-five grams of the homogenized sample were taken for analysis in order to achieve a representative sample. Ultrasonic bath of ultrasonic compact cleaner Teson 1 was used to test the efficiency of extraction of penconazole in real sample in the range of 5–120 min. Plateau was not reached even after 2 h of extraction. It has to be mentioned, that this ultrasonic equipment provided recovery values close to 100% for the concentration level 100 μg/kg within 5 min, but for spiked samples.

Sonication sonda of the pulsed ultrasonic cell disrupter VibraCell proved to be efficient for extraction of pesticides from well homogenized apple samples using ultrasonic pulses. Extraction time from 1 to 5 min was tested. Two minutes were enough to reach the maximum release of pesticide residues from apple cells. For further experiments pulsed ultrasonic cell disrupter VibraCell was used.

The proposed extraction is a modification of Schenck's method [9] and gives recovery data at several concentration levels of pesticide residues.

The method reduces the amount of sample to a half and so the extraction solvent. Pesticides were extracted with acetonitrile under ultrasound for a short time. Separatory funnel was substituted by an Erlenmeyer flask with a tap, what simplified manual labor and influenced reproducibility of extraction procedure. To obtain a clean extracts and reach a low concentration level of pesticide residues in apple matrices, three different SPE columns were tested. On the basis of our experiments it follows that the difference in the background of chromatograms for two different columns (–NH_2, –PSA) was not significant. Therefore –NH_2 sorbent was used for further experiments. Pigments present in apples had not significant effect on MS or ECD response according to results of further cleaning with Graphitized Carbon Black (GCB). GCB as a clean-up material has a strong affinity toward planar molecules and thus effectively removes pigments as well as sterols that are commonly present in foods [4]. From the standpoint of visual appearance, GCB clean-up had great effect. Extracts were transparent but GCB had very little effect on removing co-extractants visible in chromatograms.

Further optimization of SPE method was oriented towards recoveries and their repeatability, the eluting volume of acetone to wash-out pesticides quantitatively, type of the final solvent used for the reconstitution of pesticide residues (toluene and ethyl acetate were tested) and its volume. For elution of pesticide residues, 15 ml have to be used. Toluene was found the best solvent for dissolving the residues of pesticides from the point of view of peak shapes and repeatability of results of quantitative analysis. Final volume was adjusted to 5 ml.

Utilizing fast GC with narrow-bore capillary column with i.d. 0.15 mm total analysis time with splitless injector, CP Sil 13 CB column (25 m × 0.15 mm × 0.4 μm), hydrogen as carrier gas and ECD detection was 15 min and with PTV, helium as carrier gas, CP-Sil 8 MS (15 m × 0.15 mm × 0.15 μm) column and MS-SIM detection 16 min. With MS-SIM detection higher selectivity was obtained compared to ECD due

Table 2
LOQs from apples for fast GC–ECD and fast GC–MS

Pesticide	Configuration	
	GC–ECD LOQ[a] (μg/kg)	GC–MS LOQ[b] (μg/kg)
Dimethoate	15.80	0.38
Terbutylazine	–	0.17
Diazinon	26.60	0.50
Pyrimethanil	–	0.07
Chlorpyrifos-methyl	13.90	0.18
Fenitrothion	9.30	0.33
Chlorpyrifos	14.90	0.46
Cyprodinyl	–	0.11
Penconazole	9.10	0.17
Captan	6.20	18.84
Methidathion	31.10	0.15
Kresoxim-methyl	5.10	0.22
Myclobuthanil	4.20	0.14
Tebuconazole	–	0.29
Phosalone	2.90	0.73
Bitertanol	–	0.60
Cypermethrin	7.90	1.44
Etofenprox	–	0.08

–: not detectable by ECD.
[a] LOQ = $\frac{s_0}{\bar{s}} \times 10$, s_0—standard deviation of noise (peak height), $\bar{s}$—detector response (height).
[b] Signal to noise ratio = 10; calculated by MS software.

to careful selection of target and qualifier ions to avoid interferences from the matrix, what influences the noise level and consequently LOQ values (Table 2). Therefore for further experiments fast GC–MS was advantageous.

3.2. Reduction of matrix effects

Despite thoroughly cleaned-up of sample extracts, injected matrix significantly affects chromatographic results [10–13]. Co-eluents contribute to increasing background, peak overlapping, and their adsorption in chromatographic system causes changes in peak responses and/or decreasing separation efficiency. These effects influence LOQs (LOQs decrease due to matrix enhancement effect and increase owing to increasing background and/or adsorption in dirty liner), worsen reproducibility (by unrepeatability of adsorption processes and by more difficult integration of peaks), and the risk of false positive identification increases. In this study, several ways to reduction of matrix effects were practised:

(a) Utilization of pre-column to protect the analytical column from an excessive contamination. With splitless injection after every 30–40 injections of spiked and blank matrix samples and matrix matched standards in sequences, 2 μl of the control standard solution in toluene with concentration of 0.0125 ng/μl (equivalent to 5 μg/kg) were injected and the response and the peak width at half height were evaluated. According to our experiments it follows, that after 120 injections of matrix samples excessively contaminated pre-column had to be cut off and replaced with a new one and the liner had to be cleaned or changed.
(b) Utilisation of PTV injection in configuration with MS detection, which significantly eliminates the matrix effects by releasing high boiling co-extracted compounds to the split vent and/or by trapping in a liner [7,14].
(c) Isothermal part to the final temperature of temperature program of an oven was included to remove high boiling components from a column. In GC–MS configuration, the time of isothermal part was 5 min, in GC–ECD was longer due to higher analytical column film thickness and splitless injection utilization.
(d) Changes in peak response with a number of injections were eliminated by injections in sequences, where after three injections of a blank matrix sample (in order to occupy the active sites in a liner and stabilize conditions of an inlet) alternation of spiked samples and real samples (respectively matrix matched samples) follows.

3.3. Method validation

The linearity of response of GC–MS in SIM mode was checked with calibration matrix matched standards in blank extract in the range of concentrations from 0.0125 ng/μl to 2.5 ng/μl. One milliliter of a final solution corresponds to 2.5 g of an apple sample. For calibration the following concentration levels were used: 0.0125, 0.025, 0.125 and 2.5 ng/μl, what corresponds to 5, 10, 50 and 100 mg/kg of pesticides in apple sample; number of replicates for all concentrations levels was 5. Regression analysis was performed to generate the linear equation of the calibration curve and the coefficients of determination R^2 were in the range 0.9994–1, except for captan ($R^2 = 0.9945$) and cypermethrin ($R^2 = 0.9771$). For the illustration extracted ion chromatograms of pesticide residues in matrix matched standard solutions in toluene for concentration of pesticides 5 μg/kg is shown in Fig. 2.

Repeatability of peak areas for all pesticides expressed as relative standard deviation (R.S.D.) ($n = 5$) was in the range of 0.5–11% except of cypermethrin (20%) at the concentration of 5 μg/kg and captan. Extracted ion chromatograms of target ions of easy and difficult pesticides at two concentration levels (5 μg/kg and 100 μg/kg) illustrate the repeatability of measurements (Fig. 3).

The calculated LOQs for MS detection (for comparison also data for EC detection are included) are listed Table 2. The final apple sample extracts using acetonitrile extraction, SPE clean-up and solvent exchange to toluene was clean enough for fast GC–MS analyses in SIM mode. Fig. 2 illustrates extracted SIM chromatograms with target and qualifier ions for every pesticide for matrix standard solution at concentration level of 5 μg/kg. LOQ values and repeatability of measurements for ECD were much worse compared to GC–MS in SIM mode.

Recovery data were validated. In the following experiments, purified extracts of tested apples were spiked by repre-

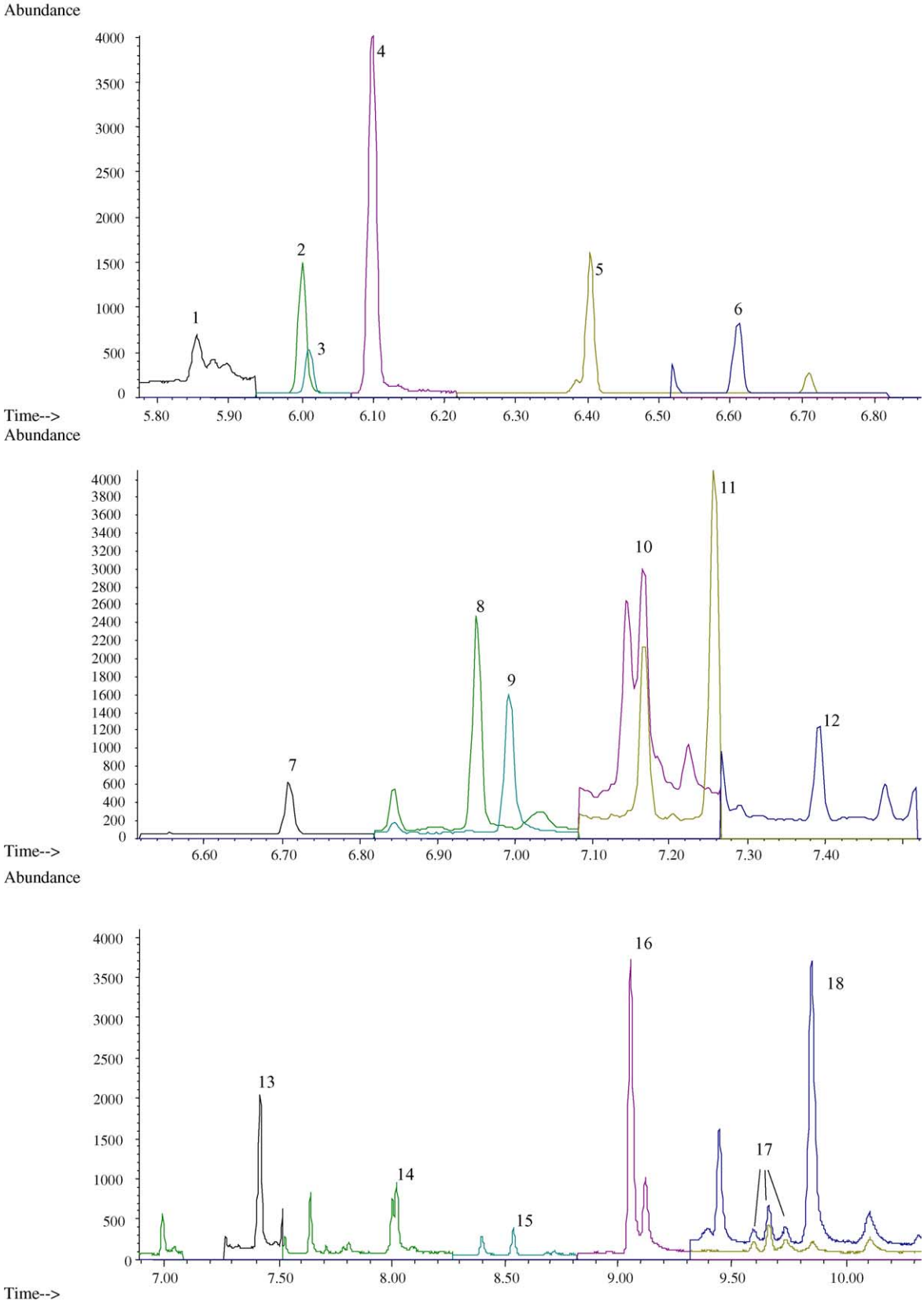

Fig. 2. Extracted ion chromatograms of pesticides in matrix matched standard solution in toluene; concentration of pesticides 5 mg/kg. 1, dimethoate; 2, terbuthylazine; 3, diazinon; 4, pyrimethanil; 5, chlorpyrifos-methyl; 6, fenitrothion; 7, chlorpyrifos; 8, cyprodinil; 9, penconazole; 10, captan; 11, methidathion; 12, kresoxim-methyl; 13, myclobutanil; 14, tebuconazole; 15, phosalone; 16, bitertanol; 17, cypermethrin; and 18, etofenprox.

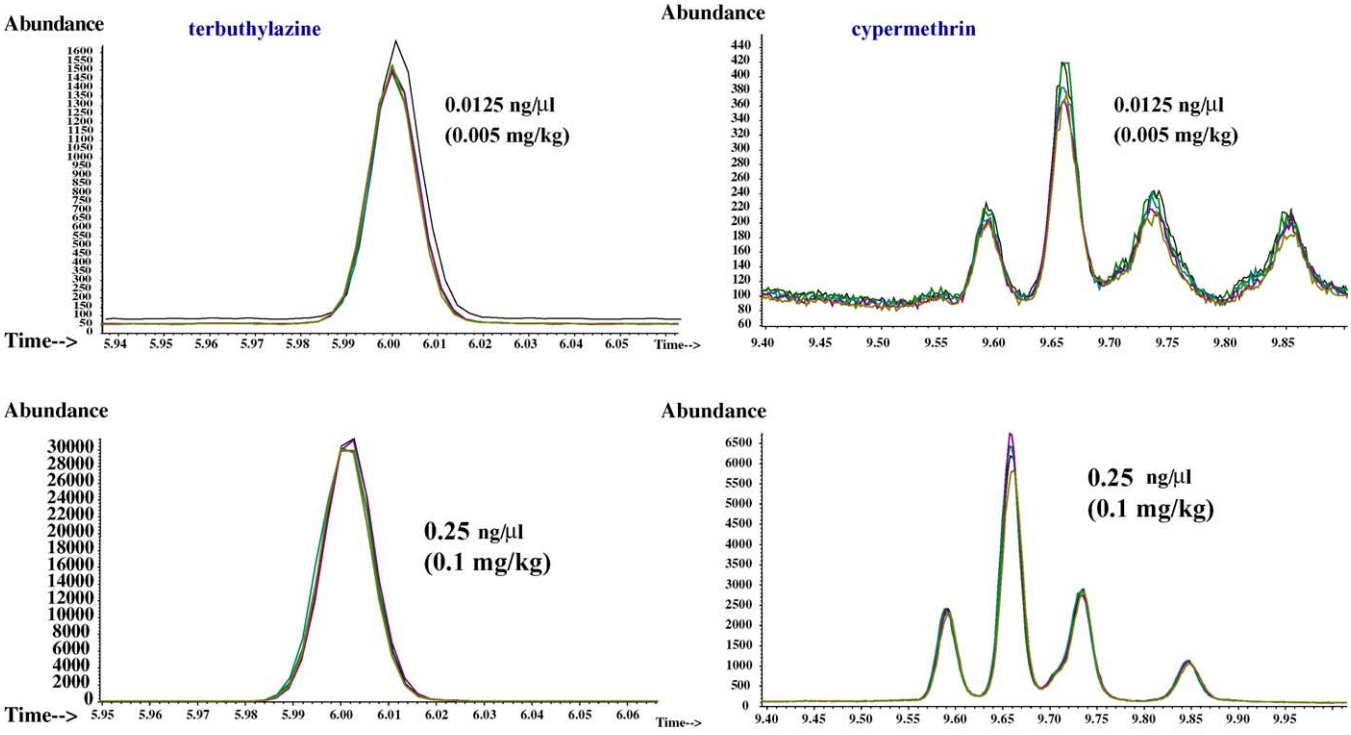

Fig. 3. Extracted ion chromatograms of terbuthylazine and cypermethrin in apple matrix extracts at concentration level 5 μg/kg (n=5).

sentative pesticides at concentration levels 5 μg/kg, 10 μg/kg, 100 μg/kg and were analysed by GC–MS to evaluate the recoveries of the method. To assess the performance of an analytical method, several criteria have to be considered before the method is employed in a practice. At the concentration five-times the limit of determination, pesticides recoveries should be 70–110% range with relative standard deviations <20% [15].

Satisfactory recoveries (>90%) using GC–MS were obtained from spiked apples at the given concentration levels, as shown by data in Table 3. Recoveries were good except captan (46.1%) and dimethoate (77.7%). Problems with the determination of captan, as one the most troublesome pesticide (decomposition or interaction with active sites in the injector liner) were published in the past times [16].

3.4. Analysis of real samples

For determination of concentration level of pesticide residues in real samples the subsequent sequence was used: 3–5× blank matrix standard, 2 parallel real sample extracts, l× matrix matched standard, 2 parallel real sample extracts, l× matrix matched standard, 2 parallel real sample extracts, l× matrix matched standard. The same sequence was used for recovery data measurements. Pesticide content found in apple samples from field experiments collected in June and September is in a good agreement with acceptable reproducibility and repeatability using fast GC–MS and GC–ECD methods (Table 4). Pesticide residues determined using fast GC–MS in apple puree samples (baby food) are shown in

Table 3
Results of the recovery (R) experiments of selected pesticide residues from apples at spiking level 5 μg/kg, 10 μg/kg and 100 μg/kg determined by fast GC–MS

Pesticide	Spiking level (μg/kg)					
	5		10		100	
	R (%)	R.S.D.[a] (%)	R (%)	R.S.D.[a] (%)	R (%)	R.S.D.[a] (%)
Dimethoate	77.7	1.7	88.5	1.9	86.1	4.4
Terbutylazine	102.9	1.0	95.4	3.1	100.3	11.1
Diazinon	103.1	9.3	95.5	1.0	95.5	3.6
Pyrimethanil	94.9	4.9	90.7	2.9	90.4	4.3
Chlorpyrifos-methyl	102.8	2.8	100.1	1.3	96.9	4.6
Fenitrothion	107.4	0.8	95.4	3.1	98.4	5.7
Chlorpyrifos	109.3	3.8	97.3	2.3	98.4	4.5
Cyprodinyl	96.8	6.0	90.0	1.6	94.6	2.9
Penconazole	99.9	3.8	94.1	1.0	98.0	3.4
Captan	46.1	56.8	108.4	22.1	99.2	25.8
Methidathion	114.3	1.3	96.6	4.5	97.5	3.3
Kresoxim-methyl	120.8	1.6	97.7	5.3	100.8	1.3
Myclobuthanil	97.6	1.3	96.4	2.0	100.2	2.9
Tebuconazole	95.3	6.5	94.3	4.5	98.7	2.9
Phosalone	123.1	11.8	95.6	9.2	101.5	3.4
Bitertanol 1	105.9	1.0	95.9	1.6	102.1	2.5
Bitertanol 2	101.2	9.8	85.6	16.4	101.5	2.7
Cypermethrin 1	112.5	9.1	84.2	40.9	99.3	1.6
Cypermethrin 2	104.8	1.4	98.7	4.9	99.8	2.8
Cypermethrin 3	96.7	5.0	96.2	8.9	110.1	2.6
Etofenprox	105.6	6.8	97.3	2.1	103.7	2.4

Two parallel sample extractions were performed with three repeated GC measurements/sample.

[a] R.S.D. of recovery experiments were calculated according to Eckschlager et al. [17].

Table 4
Pesticide residues content found in apple samples from field experiment collected in June and September using acetonitrile extraction, SPE-NH_2 clean-up and fast GC

Pesticide	Detection	Collection season					
		June			September		
		Average (μg/kg)	R.S.D.[a] (%)	$R.S.D._{GC}$ (%)	Average (μg/kg)	R.S.D.[a] (%)	$R.S.D._{GC}$ (%)
Penconazole	ECD	25.1	0.9	10.4		Under LOQ	
	MS-SIM	26.5	6.7	1.7	0.58	33.6	4.8
Myclobutanil	ECD		–		6.4	6.8	3.1
	MS-SIM		–		6.9	8.4	2.4

Two parallel sample extractions were performed with three repeated GC-measurements/one sample.
[a] R.S.D. were calculated according to Eckschlager et al. [17].

Table 5
Pesticide residues content determined in apple puree (baby food) samples, using acetonitrile extraction, SPE-NH_2 clean-up and fast GC

Pesticide	Detection	Average (μg/kg)	R.S.D.[a] (%)	$R.S.D._{GC}$ (%)
Myclobutanil	MS-SIM	12.07	3.1	3.8

Two parallel sample extractions were performed with three repeated GC-measurements/one sample.
[a] R.S.D. were calculated according to Eckschlager et al. [17].

Table 5. The presented results have shown that external calibration with matrix matched standards can be successfully utilized to obtain correct results of quantitative analysis.

4. Conclusion

Sample preparation method published by Schenk et al. [9] developed for cleaning cereal matrices was modified in such a way to be less labour and chemical consuming with a short acetonitrile extraction combined with cells disruption by the pulsed ultrasound. The clean-up achieved with the SPE columns used was evaluated by fast GC–MS or fast GC–ECD analysis. Our studies confirmed that both bonded normal phase (–NH_2 and PSA) are effective in removing the matrix co-extractants from sample extracts making possible the detection and quantitation of selected pesticide residues in apple samples at concentration level 5 μg/kg. With respect to effectivity of matrix clean-up, required concentration level for baby food (5 μg/kg), acceptable recoveries and repeatabilities the final volume of extracts was adjusted with toluene to 5 ml.

Fast GC–MS on narrow bore capillary CP-Sil 8 Low-Bleed MS (15 m × 0.15 mm × 0.15 μm) column with PTV has provided good ruggedness for fairly complicated analyses as pesticide in apples and sufficiently precise results. GC–ECD on CP Sil 13CB column compared to GC–MS in SIM mode does not afford so good selectivity on the lower concentration levels. PTV in cold splitless mode was more efficient in preventing problems connected with matrix effects and elimination of less volatile matrix constituents causing GC system performance deterioration than classical hot splitless inlet.

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