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Rapid analysis of multiple pesticide residues in fruit-based baby food using programmed temperature vaporiser injection–low-pressure gas chromatography–high-resolution time-of-flight mass spectrometry ☆

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Abstract

A rapid method using programmed temperature vaporiser injection–low-pressure gas chromatography–high-resolution time-of-flight mass spectrometry (PTV–LP-GC–HR-TOF-MS) for the analysis of multiple pesticide residues in fruit-based baby food was developed. The fast and inexpensive buffered QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction method and "conventional" approach that employs ethyl acetate extraction followed by gel permeation chromatography (GPC) cleanup were employed for sample preparation. A PTV injector in solvent venting mode was used to reduce volume of acetonitrile and acetic acid (from the buffered QuEChERS extracts) that caused higher column bleed without their elimination. Otherwise, the time-to-digital converter would become saturated in HR-TOF-MS. For fast GC separation allowing analysis of 100 analytes within a 7 min runtime, both a high temperature programming rate and vacuum conditions in a megabore GC column were employed. The use of HR-TOF-MS allowed the unbiased identification and reliable quantification of target analytes through the application of a narrow mass window (0.02 Da) for extracting analyte ions and the availability of full spectral information even at very low levels. With only a few exceptions, the lowest calibration levels for the pesticides tested were ≤0.01 mg/kg for both sample preparation methods, which meets the EU maximum residue limit set for pesticide residues in cereal-based foods and baby foods (2003/13/EC).

Keywords: Sample preparation; Low-pressure gas chromatography; Time-of-flight mass spectrometry; Pesticide residues; Baby food analysis

1. Introduction

Currently, more than 800 pesticide active ingredients in a wide range of commercial products are registered for use in agriculture to meet food supply demands [1]. Under certain circumstances, however, residues of active ingredients occur in treated crops at the time of harvest. Because of potential health risk for consumers, resulting from acute and/or chronic dietary exposure, maximum residue limits (MRLs) for many pesticides have been established in the EU [2]. Those MRLs typically ranging between 0.01 and 10 mg/kg [3]. In 1999, the EU introduced legislation (1999/39/EC) limiting all pesticide residues to a maximum of 0.01 mg/kg potentially occurring in processed cereal-based foods and baby foods for infants and young children [4]. The recent amendment (2003/13/EC) specifies compounds for which even lower MRLs (0.003–0.008 mg/kg) are required [5]. The rapid and cost-effective multiple residue analysis at very low levels within a single run represents a challenging task for both regulatory agencies and the food producers.

In practice, any multiresidue method (MRM) consists of the following basic steps: (i) isolation of residues from a representative sample (extraction); (ii) separation of co-extracted matrix components (cleanup); (iii) identification and quantification of target analytes (determinative step), and if the need is important enough, this is followed by (iv) confirmation of results by an additional analysis [6].

For extraction, although different organic solvents, and mixtures of organic solvents, have been used to extract a wide range of pesticides with different physico-chemical properties from food, the use of acetone, ethyl acetate (EtOAc), and acetonitrile (MeCN) has predominated in MRMs [7]. These solvents

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provide high pesticide recoveries over a wide polarity range; however, at the same time a lot of matrix components are co-extracted. To achieve required performance characteristics, cleanup techniques, such as gel permeation chromatography (GPC), solid-phase extraction (SPE), and/or liquid–liquid partitioning (LLP) are commonly employed for their removing. These procedures lead to increasing overall cost of the method, extending analysis time and requiring additional labour [8]. A recently published MRM that takes advantage of the enhanced possibilities offered by modern analytical instrumentation is the QuEChERS approach (quick, easy, cheap, effective, rugged, and safe), which is designed to deliver extracts that are directly applicable to both GC and LC analysis. During the development of this method, great emphasis was placed on streamlining this sample preparation procedure whenever possible by simplifying or omitting impractical, laborious, and time-consuming steps [9]. The buffered QuEChERS method involves initial extraction with MeCN containing 1% acetic acid (HOAc), LLP after addition of a mixture of anhydrous $MgSO_4$ and sodium acetate (NaOAc), which removes some polar matrix components, followed by a simple cleanup step in which the extract is mixed with primary secondary amine (PSA) sorbent and anhydrous $MgSO_4$ (dispersive-SPE). The dispersive-SPE step reduces the amount of matrix co-extractives common in foods, particularly fatty acids. After these steps the extract is ready for GC–MS and LC–MS (directly or after dilution with water containing formic acid) [10].

Fast GC separation is generally desirable since the decreased time of analysis can act to increase sample throughput, and consequently, the laboratory operating costs per sample can be reduced significantly. Either changing column geometry (shorter column length, smaller column inner diameter, thinner film of stationary phase) or its operational parameters (faster temperature program rate, isothermal analysis, optimal carrier gas, higher carrier gas flow-rate, vacuum outlet operation) are the strategies enabling fast runs. In practice, combination of both tactics is commonly employed [11,12]. One of the rather underestimated strategy in this context is low-pressure GC, where a megabore analytical column (typically 10 m × 0.53 mm, 0.25–1 μm) is connected through a connector to a short, narrow restriction column at the inlet [13–16]. Under these experimental settings in GC–MS, the entire analytical column is kept under vacuum conditions while the inlet remains at usual column head pressures in GC. Either "classical" (*e.g.* hot splitless) or more advanced sample introduction techniques (*e.g.* programmed temperature vaporiser, PTV; direct sample introduction/difficult matrix introduction, DSI/DMI) can be used in LP-GC. Reduction in the column outlet pressure leads to higher diffusivity of the solute in the gas phase, which shifts the optimum carrier gas linear velocity to a higher value. Consequently, faster GC separation can be achieved at the same column dimensions as normal operation with a disproportionately smaller loss of separation power [17].

In the separation step for pesticide residues, either GC and/or LC are used in MRMs. In the case of GC, element/group selective detectors such as electron capture (ECD), nitrogen–phosphorus (NPD), and/or flame photometric (FPD) are traditionally used, but mass spectrometry (MS) employing quadrupole, ion trap, and/or time-of-flight analysers is the most common detector in use now for pesticide residue analysis. Typically, MS or MS/MS are employed for detection of target compounds [18–20]. The use of GC–MS allows the identification and quantification of a wide range of even trace amounts of GC-amenable pesticide residues in complex matrices. Currently, low-resolution (unit mass) MS detectors employing either single quadrupole or ion trap analysers are most routinely used in applications [21–25]. As powerful as MS is, the low-resolution, scanning MS systems have limits in data collection rate, avoidance of interferences, and spectral information provided for identification purposes.

The commercialisation of time-of-flight mass spectrometers (TOF-MS), provides two complementary approaches [26]: (i) instruments that feature unit mass resolution at high acquisition speed (up to 500 spectra/s), which predetermines their use as detectors coupled to fast and ultra fast GC or comprehensive two-dimensional GC (GC × GC); and (ii) instruments with a moderate acquisition speed (max. 20 spectra/s), but having high mass resolution (>7000 FWHM), which allows a greater ability to resolve the analytes from the matrix components. Additionally, mass measurement accuracy (<5 ppm) permits estimation of the elemental composition of the detected ions.

The unique feature of both TOF-MS techniques is simultaneous sampling and analysis of all ions across the whole mass range (unlike scanning instruments). This permits full spectrum sensitivity comparable to selected ion monitoring (SIM) mode of a quadrupole instrument. TOF mass analyser efficiency is up to 25% in full spectra storage, while scanning instruments yield <0.1% efficiency [27], thus mass spectral library searching at lower concentrations is possible at these conditions. In recent years, application of GC–TOF-MS (both high-resolution and high-speed instruments) has been demonstrated as a powerful and highly effective analytical tool in analysis of food and environmental contaminants (*e.g.* pesticide residues [26,28–30], polychlorinated biphenyls [31,32], brominated flame retardants [33], dioxins [34,35], polycyclic aromatic hydrocarbons [36], toxaphene [37], acrylamide [38]), flavour compounds [39–42]; drug screening [43], petrochemical analysis [44], and metabolomic studies [45], demonstrating great potential of this technique not only for quantification of target analytes, but also for identification of non-target compounds in diverse (often complex) matrices. Recently, Cajka and Hajslova reviewed the advantages and limitations of GC–TOF-MS in food analysis [46].

In this study, many types of pesticides possessing a wide range of different physico-chemical properties (volatility, polarity, pK_a) were selected for evaluation of a rapid analytical method for fruit-based baby food at 0.01 mg/kg concentrations. For the sample preparation, both a "conventional" approach that employs EtOAc extraction followed by GPC cleanup, and a "novel" strategy based on the buffered QuEChERS method were selected. The application of programmed temperature vaporiser injection–low-pressure gas chromatography–high-resolution time-of-flight mass spectrometry (PTV–LP-GC–HR-TOF-MS) was chosen for analysis with

the expectation of enhanced speed, high accuracy, and improved selectivity.

2. Experimental

2.1. Chemicals and materials

Pesticide reference standards, all 95% or higher purity, were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). A composite stock standard solution (20 μg/ml) of multiple pesticides (see Table 1) was prepared in EtOAc. A composition stock internal standard solution containing triphenylphosphate (TPP) and ethoprophos at 20 and 40 μg/ml, respectively, was also prepared in EtOAc (*note*: different concentrations of internal standards were used to obtain similar signal responses).

MeCN, EtOAc, and cyclohexane were high purity solvents for pesticide residue analysis from Supelco (Bellefonte, PA, USA), Scharlau (Barcelona, Spain), and Merck (Darmstadt, Germany), respectively, and the glacial HOAc was HPLC grade from Sigma–Aldrich (St. Louis, MO, USA). Dehydrated $MgSO_4$ was from Fluka (Buchs, Germany), Na_2SO_4 and $NaOAc{\cdot}3H_2O$ were obtained from Penta (Chrudim, Czech Republic), and PSA sorbent was from Varian (Harbor City, CA, USA). $MgSO_4$ and Na_2SO_4 were heated for 7 h at 600 °C in a muffle furnace to remove water and phthalates.

An apple baby food sample was used for blanks, fortified samples for recovery assays, and matrix-matched standards for calibration in the experiments.

2.2. Sample preparation

2.2.1. Procedure I—buffered QuEChERS method

For the determination of recovery and repeatability for fortified pesticides in apple-based baby food (method validation) the buffered QuEChERS method [10] was used as follows with slight modifications: (1) weigh 10 g of thoroughly homogenised sample into a 50 ml plastic centrifuge tube; (2) fortify the sample by appropriate volume to achieve 0.01 mg/kg spikes and 0.05 mg/kg for TPP and 0.1 mg/kg ethoprophos (internal standards); (3) add 10 ml 1% HOAc in MeCN (v/v); (4) add 4 g anh. $MgSO_4$ and 1.7 g $NaOAc{\cdot}3H_2O$; (5) shake vigorously for 1 min by hand; (6) centrifuge the tube at 11 000 rpm for 3 min; (7) transfer 1 ml of extract to a 15 ml plastic centrifuge tube containing 50 mg PSA + 150 mg anh. $MgSO_4$; (8) mix the extract with the sorbent/dessicant for 20 s; (9) centrifuge the tube at 11 000 rpm for 2 min.

2.2.2. Procedure II—EtOAc extraction with GPC cleanup

For the determination of recovery and repeatability for fortified pesticides in apple-based baby foods (method validation), the EtOAc extraction with GPC cleanup was used [26]: (1) weigh 25 g of thoroughly homogenised sample into a 250 ml beaker; (2) fortify the sample by appropriate volume to achieve 0.01 mg/kg spikes and 0.05 mg/kg for TPP and 0.1 mg/kg ethoprophos (internal standards); (3) add 100 ml of EtOAc; (4) add (in portions) 75 g of anh. Na_2SO_4 and stir by a glass rod; (5) use an Ultraturrax macerator at 10 000 rpm for 2 min; (6) filter the crude extract though a layer of anh. Na_2SO_4 (approx. 35 g); (7) rinse three times the filter cake with 25 ml of EtOAc; (8) evaporate the combined filtrates to a volume of ca. 25 ml; (9) transfer the extract into a volumetric flask and bring to 50 ml with cyclohexane; (10) purify 2 ml of this extract by GPC—using a PL gel column (600 mm × 7.5 mm, 50 Å) with a mobile phase cyclohexane–EtOAc (1:1, v/v) at a flow rate of 1 ml/min; (11) evaporate the collected "pesticide" fraction (14.5–31.0 ml), gently removing the last volume of solvent with a stream of nitrogen; (12) redissolve the residues in 1 ml of EtOAc.

2.3. Matrix-matched standards preparation

In both procedures, matrix-matched standards were prepared similarly to fortified samples except for addition of spike solution. A volume of 20 μl of appropriate standards (0.125, 0.25, 0.5, 1.25, 2.5, 5 μg/ml pesticide mixture each containing 2.5 μg/ml TPP and 5 μg/ml ethoprophos was added to 980 μl of extracts to obtain the following concentrations of pesticides in matrix-matched standards: 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1 μg/ml (which also correspond to mg/kg in the sample).

2.4. Instrumentation

For PTV–LP-GC–HR-TOF-MS analyses, the system consisted of a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland), a PTV accessory (ATAS GL International, Veldhoven, The Netherlands), an Optic 3 programmable injector (ATAS GL International), an Agilent (Agilent Technologies, Palo Alto, CA, USA) Model 6890 Series GC, and a GCT high-resolution time-of-flight mass spectrometer (Micromass, Manchester, UK).

For characterisation of matrix co-extractives a GC × GC–TOF-MS system Pegasus 4D was used, consisting of an Agilent 6890N gas chromatograph with a split/splitless injector (Agilent Technologies, Palo Alto, CA, USA), an MPS2 autosampler (Gerstel, Mülheim an der Ruhr, Germany), and a high-speed time-of-flight mass spectrometer Pegasus III (Leco, St. Joseph, MI, USA). A dual-stage jet modulator and the secondary oven (Leco) were mounted inside the GC oven. Resistively heated air was used as a medium for hot jets, while cold jets were supplied by gaseous nitrogen cooled by liquid nitrogen.

2.4.1. PTV–LP-GC–HR-TOF-MS analysis

2.4.1.1. PTV injection. Injection volume: 2 μl; vent time: 15 s; vent flow: 20 ml/min; vent pressure: 96.5 kPa; temperature program: 70 °C (15 s), 10 °C/min to 280 °C (54 s), 10 °C/min to 350 °C (held to the end of GC–MS method); helium carrier gas flow: 1 ml/min set using "virtual" column dimensions of 4 m × 0.15 mm; splitless period: 1 min.

2.4.1.2. GC separation. A 10 m × 0.53 mm, 0.5 μm Rtx-5 Sil MS capillary column (Restek, Bellefonte, PA, USA), which was connected to a 3 m × 0.15 mm non-coated restriction column (Restek) at the inlet end. A 0.33/0.74 mm Vu-Union connector (part no. 20418, Restek) was used to connect the analytical column with the non-coated restriction column. An Agilent oven

Table 1
Optimised conditions of the PTV–LP-GC–HR-TOF-MS method and validation results in apple-based baby food ($n = 6$, fortified at 0.01 mg/kg)

Peak no.	Pesticide	t_R (min)	Quantification mass (m/z)	Buffered QuEChERS			EtOAc extraction with GPC		
				Recovery (%)	RSD (%)	LCL (μg/kg)	Recovery (%)	RSD (%)	LCL (μg/kg)
1	Methamidophos	1.68	141.001	61	13	5	85	20	5
2	Dichlorvos	1.72	184.977	102	13	5	83	13	5
3	Biphenyl	2.08	154.078	88	11	5	97	12	5
4	Mevinphos	2.19	127.016	80	8	5	91	11	5
5	Acephate	2.20	136.016	–	–	25	–	–	25
6	Propham	2.26	179.095	109	14	5	95	38	5
7	Methacrifos	2.35	180.001	89	14	5	Saturated TDC		
8	*o*-Phenylphenol	2.39	170.073	78	9	2.5	105	20	2.5
9	Heptenophos	2.51	124.008	72	25	2.5	92	13	2.5
10	Omethoate	2.55	156.001	–	–	25	–	–	25
11	Diphenylamine	2.62	169.089	77	12	2.5	73	13	2.5
12	Ethoprophos (I.S.)	2.63	200.009						
13	Chlorpropham	2.67	213.056	87	9	5	90	8	5
14	Trifluralin	2.69	306.070	74	4	2.5	72	7	2.5
15	Monocrotophos	2.73	127.016	70	6	5	104	7	5
16	α-HCH	2.77	180.938	80	5	2.5	80	5	2.5
17	Hexachlorobenzene	2.78	283.810	80	10	2.5	73	7	2.5
18	Dimethoate	2.82	87.014	81	8	10	83	15	10
19	β-HCH	2.87	180.938	88	9	2.5	88	11	2.5
20	γ-HCH (lindane)	2.89	180.938	90	12	2.5	95	8	2.5
21	Diazinon	2.93	179.120	83	9	5	102	7	5
22, 28	Phosphamidon I + II	2.93 + 3.07	127.016	92	11	5	89	17	5
23	Pyrimethanil	2.94	198.103	86	13	2.5	92	8	2.5
24	Chlorothalonil	2.96	265.879	86	17	2.5	95	8	2.5
25	Etrimfos	2.98	292.065	78	14	2.5	95	4	2.5
26	δ-HCH	2.99	180.938	86	13	2.5	95	5	2.5
27	Pirimicarb	3.02	166.098	–	–	25	–	–	25
29	Chlorpyrifos-methyl	3.10	285.926	81	13	2.5	94	3	2.5
30	Vinclozolin	3.11	212.003	85	15	2.5	94	9	2.5
31	Parathion-methyl	3.11	263.002	78	14	2.5	96	8	2.5
32	Tolclofos-methyl	3.12	264.986	84	13	2.5	101	6	2.5
33	Carbaryl	3.14	144.058	83	14	5	72	12	5
34	Heptachlor	3.14	271.810	85	19	2.5	95	3	2.5
35	Metalaxyl	3.15	206.118	108	12	5	108	13	5
36	Pirimiphos-methyl	3.19	290.073	83	13	2.5	90	19	2.5
37	Fenitrothion	3.20	277.017	69	12	5	100	8	5
38	Methiocarb	3.20	168.061	83	18	10	85	19	10
39	Dichlofluanid	3.23	123.023	74	12	5	105	9	5
40	Malathion	3.24	173.081	85	14	2.5	93	17	2.5
41	Chlorpyrifos	3.26	313.957	84	13	2.5	104	6	2.5
42	Aldrin	3.27	262.852	84	14	2.5	96	10	2.5
43	Fenthion	3.28	278.020	82	14	2.5	63	13	2.5
44	Parathion	3.29	291.033	86	11	2.5	91	12	2.5
45	Triadimefon	3.29	208.029	91	6	5	108	14	5
46	Tetraconazole	3.30	336.053	87	8	2.5	72	12	2.5
47	Fipronil	3.36	350.949	88	7	2.5	19	11	2.5
48	Cyprodinyl	3.37	224.119	87	9	2.5	88	17	2.5
49, 54	Chlorfenvinphos I + II	3.37 + 3.41	266.938	75	6	2.5	123	18	2.5
50	Imazalil	3.39	215.003	86	16	10	80	26	10
51	Penconazole	3.39	248.095	87	11	2.5	85	13	2.5
52	Tolylfluanid	3.40	237.966	72	18	2.5	97	6	2.5
53	Mecarbam	3.41	131.004	78	10	5	88	17	5
55	Quinalphos	3.43	146.048	89	13	2.5	93	11	2.5
56	Captan	3.43	79.055	Degraded			–	–	25
57	Thiabendazole	3.44	201.036	83	6	2.5	73	22	2.5
58	Procymidone	3.44	283.017	81	5	2.5	93	14	2.5
59	Folpet	3.46	259.934	Degraded		10	97	15	2.5
60	Methidathion	3.48	145.007	78	15	2.5	100	5	2.5
61	*o*,*p*′-DDE	3.48	246.000	86	8	2.5	104	6	2.5
62	Hexythiazox	3.48	155.980	99	7	5	Saturated TDC		
63	Endosulfan I	3.53	240.905	Saturated TDC			92	17	2.5

Table 1 (*Continued*)

Peak no.	Pesticide	t_R (min)	Quantification mass (*m/z*)	Buffered QuEChERS			EtOAc extraction with GPC		
				Recovery (%)	RSD (%)	LCL (μg/kg)	Recovery (%)	RSD (%)	LCL (μg/kg)
64	*p,p′*-DDE	3.59	246.000	83	10	2.5	103	7	2.5
65	Buprofezin	3.60	172.100	91	8	5	99	18	5
66	Dieldrin	3.61	262.852	79	8	2.5	109	4	2.5
67	Myclobutanil	3.61	179.038	91	7	2.5	106	9	5
68	*o,p′*-DDD	3.62	235.008	85	9	2.5	102	9	2.5
69	Bupirimate	3.62	273.102	88	8	2.5	96	14	5
70	Kresoxim-methyl	3.63	116.035	86	21	5	100	12	5
71	Endrin	3.68	262.852	82	5	2.5	102	15	2.5
72	Endosulfan II	3.72	240.905	83	19	2.5	98	15	2.5
73	Ethion	3.73	230.974	84	20	2.5	96	13	2.5
74	*p,p′*-DDD + *o,p′*-DDT	3.74	235.008	86	12	2.5	103	6	2.5
75	Oxadixyl	3.74	163.100	104	8	5	108	8	5
76	Triazophos	3.79	161.059	101	7	2.5	109	12	2.5
77	Benalaxyl	3.81	148.113	80	10	2.5	102	10	2.5
78	Trifloxystrobin	3.83	222.077	89	5	5	97	5	5
79	Endosulfan sulfate	3.84	271.819	78	13	2.5	97	8	2.5
80	*p,p′*-DDT	3.86	235.008	81	13	2.5	105	10	2.5
81	Tebuconazole	3.90	250.075	86	8	2.5	109	7	2.5
82	TPP (I.S.)	3.91	326.071						
83, 84	Propargite I + II	3.91 + 3.95	173.097	78	17	5	96	4	5
85	Iprodione	3.98	314.010	80	11	2.5	89	9	2.5
86	Bifenthrin	4.01	181.102	82	12	2.5	88	5	2.5
87	Bromopropylate	4.01	340.900	85	10	2.5	102	7	2.5
88	Phosmet	4.01	160.040	76	17	2.5	94	8	2.5
89	Fenoxycarb	4.02	186.068	98	10	2.5	100	15	5
90	Tetradifon	4.10	158.967	88	12	2.5	104	7	2.5
91	Phosalone	4.12	182.001	80	12	2.5	101	8	2.5
92	Azinfos-methyl	4.14	160.051	77	14	5	112	12	5
93, 94	λ-Cyhalothrin I + II	4.15 + 4.18	181.066	87	7	5	106	27	5
95	Dicofol	4.22	138.995	77	15	5	90	17	5
96	Fenarimol	4.22	219.033	82	8	2.5	100	23	5
97	Azinfos-ethyl	4.24	132.045	76	10	5	103	12	5
98, 99	Permethrin I + II	4.32 + 4.35	183.081	88	7	5	105	8	5
100	Pyridaben	4.36	147.117	84	6	2.5	110	3	2.5
101, 102	β-Cyfluthrin I + II	4.44 + 4.49	226.067	86	14	5	156	48	5
103, 104	Cypermethrin I–IV	4.52 + 4.56	163.008	84	9	5	116	7	5
105, 106	Fenvalerate I + II	4.77 + 4.84	167.063	86	17	10	105	20	10
107, 108	Difenoconazole I + II	4.94 + 4.96	323.024	81	4	2.5	102	4	2.5
109, 110	Deltamethrin I + II	4.97 + 5.05	252.905	86	14	2.5	105	9	2.5
111	Azoxystrobin	5.14	344.104	83	7	2.5	105	4	2.5

insert (part no. G2646-60500) was used to reduce the effective size of the oven, permitting more rapid and reproducible GC temperature control and decreasing the time to cool the GC oven. Oven temperature program: 90 °C (1 min), 60 °C/min to 280 °C (2.83 min).

2.4.1.3. MS detection. The MS instrument was manually tuned using 2,4,6-tris-fluoromethyl-[1,3,5]-triazine. The mass resolution was calculated from continuum data using the highest mass from reference compound (*m/z* 285) and the full width at half maximum (FWHM) of this peak. Generally, the mass resolution was more than 7000 FWHM in all experiments. For exact mass calibration, nine fragments (obtained in an electron ionisation mode) of this reference compound in centroid display were used. Once this calibration was made the *m/z* 284.9949 was used as an internal reference mass (lock mass). The exact mass calibration was considered successful with maximum differences between measured and theoretical masses of 1.0 mDa. In both cases, 120 final spectra (*i.e.* 120 points) were used for calculation of mass resolution and for exact mass calibration during the tuning procedure. The reference compound was continuously introduced into the ion source also during the real analysis at recommended intensity. MassLynx 3.5, MassLynx 4.1, QuanLynx 4.1, and ChromaLynx 4.1 software and NIST 2002 mass spectral library were employed for the data processing. Acquisition rate: 4 spectra/s; pusher interval: 33 μs (30 303 raw spectra/s); inhibit push value: 17; time-to-digital converter: 3.6 GHz; mass range: *m/z* 65–700; ion source temperature: 220 °C; transfer line temperature: 280 °C; detector voltage: 2750 V.

2.4.1.4. Quantification. The quantification ions for each analyte are shown in Table 1. The responses (peak areas) of pesticides were normalised to the sum of peak areas for TPP and ethoprophos.

2.4.1.5. Time requirements. A total cycle run time of 10.75 min included a 1-min pre-injection step, a 0.25-min venting period, a 7-min GC run, and a 2.5-min cool-down of the GC oven.

2.4.2. Conventional GC–HR-TOF-MS analysis

2.4.2.1. Hot splitless injection. Injection volume: 1 μl; injector temperature 250 °C; helium carrier gas flow: 1 ml/min; splitless period: 1.5 min.

2.4.2.2. PTV injection. Injection volume: 1 μl; vent time: 7 s; vent flow: 20 ml/min; vent pressure: 96.5 kPa; temperature program: 70 °C (7 s), 10 °C/min to 280 °C (54 s), 10 °C/min to 350 °C (held to the end of GC–MS method); helium carrier gas flow: 1 ml/min; splitless period: 1 min.

2.4.2.3. GC separation. A 30 m × 0.25 mm, 0.25 μm Rtx-5 Sil MS capillary column (Restek, Bellefonte, PA, USA) was used for analyte separation. Oven temperature program for hot splitless injection: 60 °C (1.5 min) for EtOAc, 80 °C (1.5 min) for MeCN, 90 °C (1.5 min) for toluene, 20 °C/min to 180 °C, 5 °C/min to 230 °C, 25 °C/min to 280 °C (12 min). Oven temperature program for PTV (MeCN) injection: 90 °C (1 min), 20 °C/min to 180 °C, 5 °C/min to 230 °C, 25 °C/min to 280 °C (12 min).

2.4.2.4. MS detection. Acquisition rate: 2 spectra/s; other MS parameters were the same as described in Section 2.4.1.3.

2.4.3. GC × GC–TOF-MS analysis

2.4.3.1. Hot splitless injection. Injection volume: 1 μl; injector temperature: 250 °C; helium carrier gas flow: 1.3 ml/min; splitless period: 1.5 min.

2.4.3.2. GC separation. A 30 m × 0.25 mm, 0.25 μm DB-5ms capillary column (Agilent) was used as a first dimension column and a 1.1 m × 0.10 mm, 0.10 μm BPX-50 capillary column (SGE, Austin, TX, USA) as a second dimension column. Oven temperature program: 80 °C (1.5 min) and 60 °C (1.5 min) for the injections in MeCN and EtOAc, respectively, 20 °C/min to 180 °C, 5 °C/min to 230 °C, 25 °C/min to 290 °C (17.1 min), secondary oven was held 10 °C above the main oven; modulation time: 5 s (hot pulse 1 s); modulation temperature offset: 30 °C.

2.4.3.3. MS detection. Acquisition rate: 100 spectra/s; mass range: *m/z* 45–500; ion source temperature: 220 °C; detector voltage: −1750 V. ChromaTOF 2.23 software and NIST 2002 mass spectral library were used for the data processing.

3. Results and discussion

3.1. Optimisation of injection conditions

In earlier studies, hot splitless injection, PTV, and DMI (the last two injection techniques in a large volume injection mode with solvent vent) were reported for injection of the QuEChERS extracts [8,10,47–49]. In our preliminary experiments, however, we found that application of hot splitless injection was not suitable for further experiments when coupled to HR-TOF-MS. After injection of the buffered QuEChERS extract (in MeCN) onto the GC column, higher column bleed was observed. This caused the saturation of the time-to-digital converter (TDC), which is used to mark the arrival times of particular ions, and consequently, it was impossible to detect the target analytes at ultratrace concentrations at high GC oven temperature. We believe that the increased column bleed was a result of injecting MeCN containing HOAc because the same phenomenon was not encountered if EtOAc or toluene were injected, as shown in Fig. 1. In the figure, *m/z* 208 was selected as an indicator of column bleed because it was not as influenced by partial saturation of the TDC as *m/z* 207, the most abundant ion of the column bleed. Unfortunately, the column erosion was not a random phenomenon; three other GC columns obtained from different vendors showed similar deterioration of performance after injection of buffered QuEChERS extracts in hot splitless mode. In addition, HOAc containing in buffered QuEChERS extracts eluted as a broad peak over the chromatogram (Fig. 2).

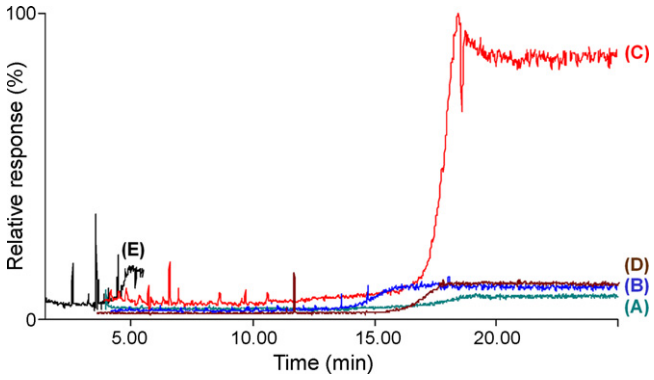

Fig. 1. Column bleed profiles (*m/z* 208) depending on sample preparation strategy and injection technique. (A) EtOAc extraction followed by GPC cleanup, hot splitless injection (1 μl) of EtOAc; (B) EtOAc extraction followed by GPC cleanup, hot splitless injection (1 μl) of toluene; (C) buffered QuEChERS, hot splitless (1 μl) injection in MeCN; (D) buffered QuEChERS, PTV solvent vent injection (2 μl) in MeCN; (E) buffered QuEChERS, PTV solvent vent injection (2 μl) in MeCN. All data acquired at 2 spectra/s. Final temperature of GC oven at 280 °C used in all experiments. (A)–(D) 30 m × 0.25 mm, 0.25 μm Rtx-5 Sil MS column, and (E) 10 m × 0.53 mm, 0.5 μm Rtx-5 Sil MS column were used.

Column bleed was not a limitation in a previous study using full spectra acquisition mode with the original method in which buffering was not employed [49], and the two laboratories that used full spectra acquisition MS (ion trap and HS-TOF) in the AOAC International collaborative study of the buffered QuEChERS method achieved good results (*note:* both labs used "advanced" GC injection techniques for MeCN extracts; for hot splitless injection, collaborators concentrated the extracts in toluene prior to injection) [50]. The original QuEChERS method was modified to use buffering mainly to improve stability and recoveries for a small number of pH-dependent pesticides analysed mainly by LC [10]. Otherwise, higher column bleed is not often a limitation in SIM or MS/MS operation in quadrupole mass analysers, and HS-TOF and ion trap instruments are less affected by the column bleed than HR-TOF. In a novel MS approach, supersonic molecular beam MS greatly minimises

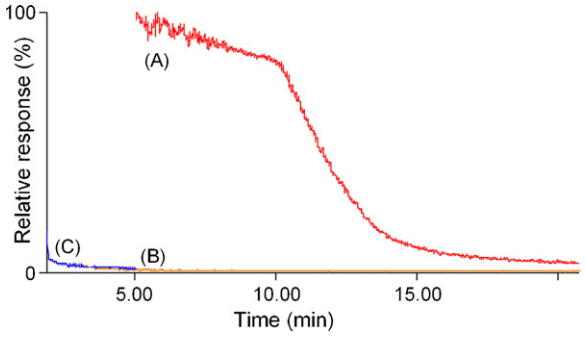

Fig. 2. Acetic acid elution profiles (*m/z* 60.021) depending on injection technique. (A) Hot splitless injection (1 μl) in conventional GC; (B) PTV solvent vent injection (1 μl) in conventional GC; (C) PTV solvent vent injection (2 μl) in LP-GC. Buffered QuEChERS procedure blanks used in experiments. All data acquired at 2 spectra/s.

column bleed through cold ionisation, higher flow rates, and lower analyte elution temperatures [51], and the latest developments take into account many sources of noise to increase analyte detectability in very complicated chromatograms [52].

We had to devise an approach to work around the column bleed problem, and we chose to pursue PTV injection using solvent venting. In this way, the extract is transferred into a cold injector followed by the elimination of the solvent via split exit at a temperature below the boiling point of the solvent, and vaporisation of residues by very rapid heating. Moreover, the PTV injection is suitable if more than 1 μl of MeCN QuEChERS extract has to be injected for obtaining lower limits of detection (LODs), if matrix interferences are not the limiting source of noise. MeCN has a high expansion volume and overflow of the liner can occur during hot splitless injection if larger injection volumes are used.

PTV injection requires careful and time-consuming performance optimisation since many parameters are involved (initial and final injector temperature, inlet heating rate, venting time, flow and pressure, and transfer time) [53]. We started the optimisation procedure employing higher venting flow (50 ml/min) and longer vent time (1 min) to avoid deterioration of the GC column. During the analysis, a source pressure was also monitored, which was a valuable indicator how much of the solvent had been transferred into the column. For 2 μl injection, a vent flow of 20 ml/min for 15 s at 70 °C was found to be optimal. Under these conditions, only a small amount of MeCN entered the column, and no significant column bleed was observed (Fig. 1). A slight decrease of responses and also peak deformations of early eluting compounds (*e.g.* dichlorvos, biphenyl, mevinphos) was observed at higher starting injection temperatures (>70 °C). The final inlet temperature was set to 280 °C at which the last eluting analyte, azoxystrobin was transferred quantitatively onto the column.

During injection of real-world samples, co-injected matrix components may negatively influence the transfer of analytes due to the formation of new active sites. To bake out most of the non-volatile deposits after finishing the splitless period, the PTV inlet temperature was set to 350 °C in the following experiments. With respect to the inlet heating rate, no significant differences in peak shapes or responses were observed when employing the heating rates in the range of 5–10 °C/s, even for rather thermally labile carbamates (*e.g.* pirimicarb, carbaryl, methiocarb, fenoxycarb). Although a relatively rapid heating (10 °C/s) was utilised in the final method, the risk of liner overflow with sample vapours was not an issue of concern since the most of the solvent was eliminated during the venting. A 1 min transfer (splitless) time was sufficient for quantitative transfer of analytes onto the GC column. The same PTV conditions could be used for injection of both MeCN and EtOAc, since the boiling points of MeCN and EtOAc are very similar (81.6 and 77 °C, respectively, at 101.325 kPa).

3.2. Optimisation of LP-GC conditions

Mastovska et al. previously found that the constant column inlet pressure of 137.9 kPa was optimal for obtaining the highest peak heights of the tested pesticides using a combination of a 10 m × 0.53 mm analytical column and a 3 m × 0.15 mm restriction column [16,22]. Unfortunately, the Optic 3-S control unit does not allow using constant column inlet pressure. We had to input different "virtual" column configurations (3, 4, and 5 m × 0.15 mm; 1 ml/min flow) to achieve pressures of approximately 137.9 kPa (only a slight decrease in responses was observed if the column inlet pressure ranged between 103.4 and 189.6 kPa [16]). Using a virtual configuration of 4 m × 0.15 mm and 1 ml/min flow, the inlet pressures at 90 °C (initial oven temperature), 130 °C (elution temperature of methamidophos and dichlorvos), and 280 °C (final temperature) were 96.5, 110.3, and 165.5 kPa, respectively. Under these experimental conditions, the highest peak heights were obtained for early as well as late eluting compounds compared to other tested column configurations. If the initial GC oven temperature was set higher than 90 °C, then peak broadening and distortion occurred; therefore the temperature of 90 °C was used in experiments. It is noteworthy to realise that lower initial GC oven temperature leads to longer equilibration and cool-down times, and maximal sample throughput needs both fast oven temperature programming and rapid cool-down.

3.3. Optimisation of mass spectrometric conditions

Although the GCT instrument allows acquisition rates up to 10 spectra/s, an acquisition rate of 4 spectra/s was sufficient as an acceptable compromise between the number of points (4–15) per chromatographic peak and signal intensity. Moreover, compared to fast GC using microbore columns with very narrow chromatographic peaks, LP-GC separations on a short 0.53 mm column provides relatively broad peaks, thus even lower data collection rate was possible [22].

3.4. Mass spectrometric detection in fast GC

A key advantage in using MS detectors in fast GC is the possibility to spectrometrically resolve coeluting peaks supposing they are not isomers, and thereby compensate for the lower chromatographic resolution. Non-scanning mass analysers, such as

TOF, can provide fast acquisition rates, which is very useful to achieve spectral collection frequency ranging between 2.5 and 12.5 Hz needed to give five points across full peak width [12]. The data acquisition system of the GCT uses the TDC for registration of particular ions. Between each collected spectrum, an inter-scan delay is needed during which no spectra are collected. The acquisition rate is therefore the reciprocal of the sum of "scan time" and "inter-scan delay" (Eq. (1)):

$$\text{spectral acquisition rate (Hz)} = \frac{1}{\text{scan time (s)} + \text{inter-scan delay (s)}} \quad (1)$$

For the spectral acquisition rate of 4 Hz used in this study both the "scan time" of 0.20 s and "inter-scan delay" of 0.05 s (the lowest recommended value) had to be entered into the instrument settings (*note*: the term "scan" is not fully correct in the present case since the TOF-MS represents non-scanning instrument; however, this term is incorporated into the MassLynx software). The "true" sum of raw spectra per each point was 6060 in particular case, as calculated according to Eq. (2):

$$\text{number of raw spectra per point} = \frac{1\,000\,000}{\text{pusher interval (}\mu\text{s)}}\text{scan time (s)} \quad (2)$$

In this equation the "pusher interval" represents the time needed for obtaining one raw (primary) spectrum (33 μs setting for the mass range used in this study).

As mentioned in Section 1, one of the main advantages of TOF-MS is the acquisition of full mass spectra even at very low concentrations compared to the quadrupole mass analyser operated in SIM or ion trap in MS/MS mode. Mastovska et al. evaluated the (fast) LP-GC–MS approach for analysis of 20 representative pesticides in food matrices. In a follow-up study, a large number of pesticides (57) in food extracts were investigated. In both cases, a quadrupole mass analyser was used for detection of target analytes, but this limited not only the number of ions (two to three) that could be monitored for each analyte, but also the total number of analytes that could be analysed to obtain acceptable detectability in ultra-trace analysis. Martinez Vidal et al. reported the application of (fast) LP-GC coupled to tandem MS (triple quadrupole analyser) in the analysis of 130 multiclass pesticide residues [54]. In this approach, initial identification of pesticide residues was based on MS/MS screening that monitored a single transition of each target compound followed by the repeated analysis of potentially positive samples again using MS/MS to monitor two to three transitions for each compound. A disadvantage of this approach is the need to optimise MS/MS conditions, re-analyse the positive samples, and creation of many time segments in the method (as in SIM, too).

On the other hand, using the TOF-MS system enabled us to analyse a larger number of pesticides in a short time. We should note that quadrupole analysers can also acquire full mass spectra at an acquisition rate of 4 spectra/s as in HR-TOF-MS, but with much lower sensitivity. On the contrary, the ion trap analysers operated in full mass spectra can reach detectability comparable to quadrupole analysers operated in SIM mode. However, since both mentioned analysers represent unit mass resolution instruments, there is an increased risk of coelutions of target analyte masses with matrix co-extracts during the fast GC analysis.

Another benefit of using HR-TOF-MS is the possibility to eliminate background interferences (chemical noise originating mainly from matrix co-extractives) through the use of narrow mass window setting for extracting target ions thereby increasing selectivity. As shown for phosalone in Fig. 3, using a 1 Da mass window gave peak-to-peak signal-to-noise ratio (S/N) of 6, but setting the mass window to 0.1 Da or even as low as 0.02 Da led to a S/N of 25 and 74, respectively, in baby food extract.

3.5. Influence of matrix on detection in HR-TOF-MS

In this study, we compared two common extraction solvents in multiresidue analysis of pesticides, EtOAc and MeCN, which have somewhat different selectivity in terms of co-isolation of analytes from matrix [8]. For characterisation of matrix co-extractives, GC × GC coupled to high-speed (HS) TOF-MS was used. While the GC × GC provided greater peak capacity, the HS-TOF-MS offered a wider linear dynamic range than HR-TOF-MS, thus gave better examination of co-extracted com-

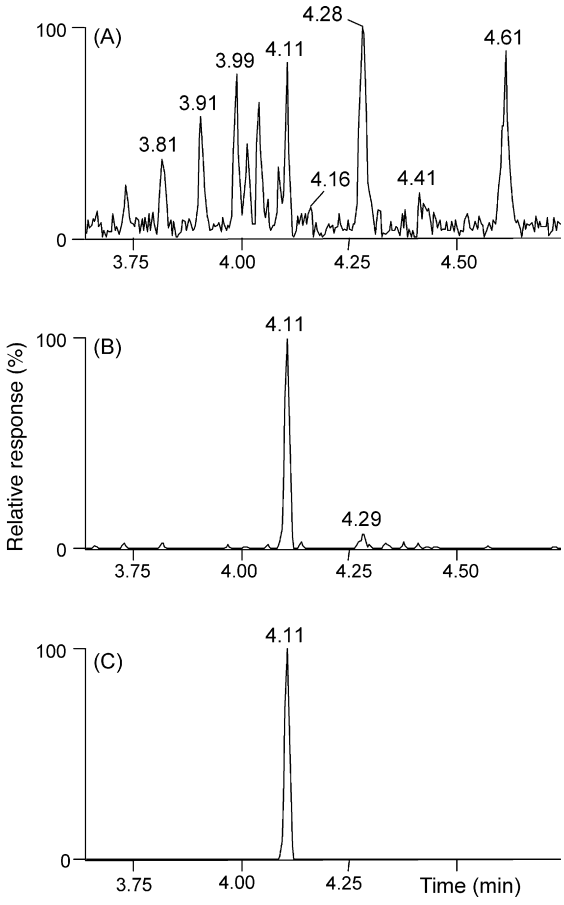

Fig. 3. Influence of mass window setting for detection of 0.01 mg/kg phosalone (t_R = 4.11) in apple baby food extract prepared with the buffered QuEChERS method. Target ion *m/z* 182.001 extracted using a mass window of (A) 1 Da, (B) 0.1 Da, and (C) 0.02 Da.

pounds. Fig. 4 shows results from this approach for different groups of matrix components identified in purified baby food extracts: (i) *fatty acids* (palmitic acid, linoleic acid, stearic acid, eicosanoic acid); (ii) *dicarboxylic acids* (fumaric acid, malic acid); (iii) *hydrocarbons* (fernesene, heptacosane, nonacosane); (iv) *esters of fatty acids* (butyl palmitate, butyl stearate); (v) *other compounds* (5-(hydroxymethyl)furan-2-carbaldehyde, octane-1,3-diol, octadeca-9,12-dienal, sterols). *Note*: Longer modulation period (5 s) than usual (2–3 s) was used in the GC × GC analysis to avoid wrap-around of late eluting sterols.

In this experiment, significantly higher occurrence of 5-(hydroxymethyl)furan-2-carbaldehyde, malic acid, hydrocarbons, and sterols was observed in the case of EtOAc extraction with GPC cleanup compared to the buffered QuEChERS method. Independent of potential coelutions and saturation of the TDC (in HR-TOF-MS), ruggedness, and accuracy are other important considerations which relate to the accumulation of non-volatile compounds in the inlet or front part of the column Although the EtOAc extraction with GPC co-extracted more (semi)volatiles, compared to the buffered QuEChERS method, the total amount of co-extractives was higher in the latter case (0.18 mg/g vs. 0.43 mg/g, respectively).

In both cases, fatty acids represented the most abundant co-extractives. These compounds elute from GPC in the collected "pesticide" fraction, and in the case of dispersive-SPE only a fraction of the fatty acids were removed by the PSA sorbent. As already stated, the original QuEChERS method yields cleaner extracts than the buffered version, and the addition of 50 mg C_{18} with PSA in the dispersive-SPE step has also been shown to improve cleanup without loss of pesticides [55]. Plant sterols elute from the GC column after the last eluting pesticide (azoxystrobin, t_R = 5.14 min), thus they do not interfere with the detection process, but they determine the GC run time (7 min).

For HR-TOF-MS, the most critical matrix components interfering in the detection process were fatty acids and hydrocarbons. These compounds yield mass spectra containing a broad range of low *m/z* masses, which can saturate the trans-

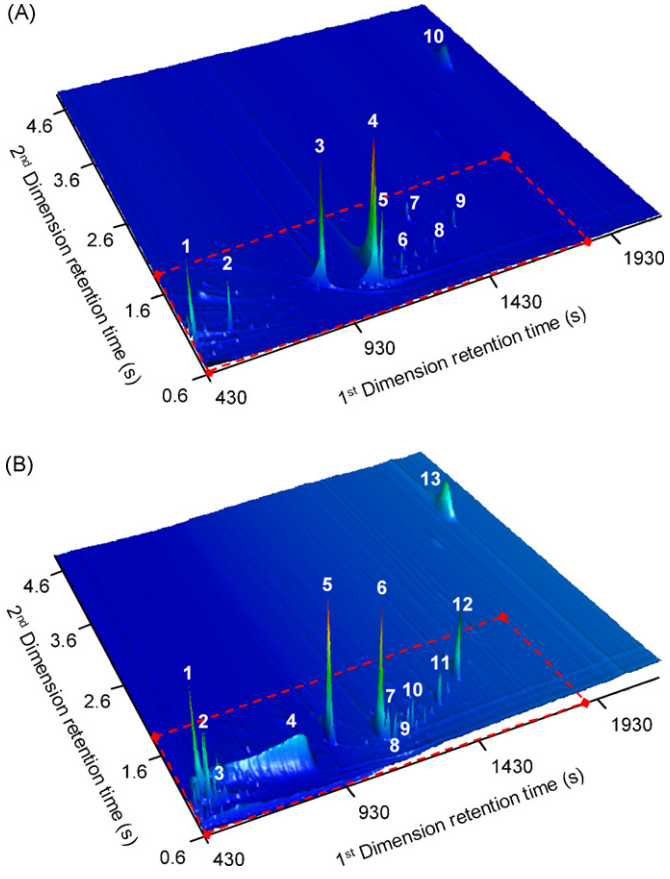

Fig. 4. GC × GC–HS-TOF-MS chromatograms of apple baby food extracts prepared by various sample preparation strategies. The dash lines denote the elution area of pesticides. (A) Buffered QuEChERS with dispersive-SPE cleanup; identified compounds: (1) octane-1,3-diol; (2) fernesene; (3) palmitic acid; (4) linoleic acid; (5) stearic acid; (6) eicosanoic acid; (7) octadeca-9,12-dienal; (8) heptacosane; (9) nonacosane; (10) sterols; (B) EtOAc extraction with GPC cleanup; identified compounds: (1) 5-(hydroxymethyl)furan-2-carbaldehyde; (2) octane-1,3-diol; (3) fumaric acid; (4) malic acid; (5) palmitic acid; (6) linoleic acid; (7) stearic acid; (8) butyl palmitate; (9) eicosanoic acid; (10) butyl stearate; (11) heptacosane; (12) nonacosane; (13) sterols.

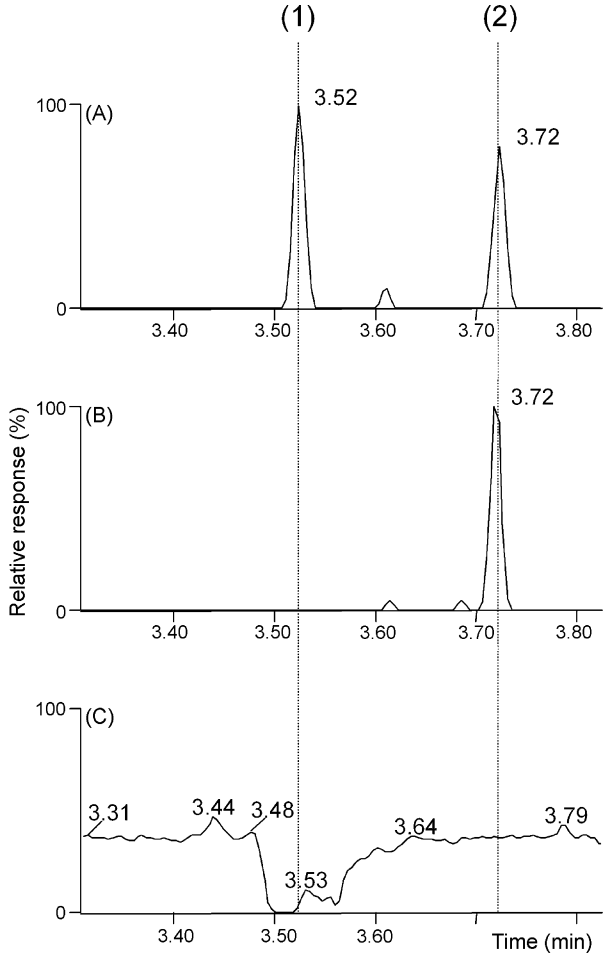

Fig. 5. (A) *m/z* 240.905 corresponding to (1) endosulfan I and (2) endosulfan II in standard (20 pg injected), (B) *m/z* 240.905 corresponding to (1) endosulfan I and (2) endosulfan II in buffered QuEChERS matrix-matched standard (0.01 mg/kg; 20 pg analyte) illustrating signal suppression during elution of an abundant matrix component (fatty acid), and (C) *m/z* 284.995 corresponding to lock mass. The saturation of the HR-TOF-MS is visible from the signal decrease of the lock mass ion, which correlates with signal suppression of other ions (mass window of 0.02 Da in the experiment).

fer buffer of the TDC or even the multichannel plate detector as illustrated in Fig. 5. In these circumstances, the detection of any analytes present at low concentration is suppressed. In practice, this phenomenon can be observed by monitoring the lock mass intensity, which should remain at constant intensity. When saturation occurs, a drop of this ion is typically observed, indicating that signal of other ions is also diminished.

Using MRMs, it is impossible to achieve wide analytical scope for all analytes and matrices, and it is inevitable that high-level interferences will occur in GC–MS. To minimise this saturation phenomenon, it is useful to introduce "spectral skew" for lower masses during the manual tuning of the instrument, which can, however, slightly change ion ratios of particular analytes and lead to worse spectral match if library searching is conducted. Another way to decrease the saturation of the TDC is to increase the "inhibit push" value in its setting because a higher value promotes registration of only higher *m/z* ions by the TDC (*e.g.* for an inhibit push setting of 15, 17, 20, and 25, the ions *m/z* <50, 65, 90, and 140, respectively, are not registered) although the fragment ions still hit the multichannel plate detector [33]. In our study, both spectral skew for lower masses (*m/z* < 60) and an inhibit push of 17 were used to overcome most of the potential saturation phenonomena.

Recently, Green and Bateman demonstrated removal of ions in selected *m/z* regions by modifying the transfer optic of the HR-TOF-MS [56]. By changing the relationship between opening the ion gate and applying voltage to the orthogonal acceleration electrode, the system may be set to only permit ions over a set *m/z* to be transmitted into the TOF analyser (*i.e.* low *m/z* cut off).

3.6. Separation of isomeric compounds

A drawback of LP-GC is its lower chromatographic resolution compared to conventional GC approaches. Although the MS can compensate in many cases, the potential coelution of isomeric compounds can occur. Fig. 6 shows separation of selected isomeric compounds in LP-GC using a short megabore column (10 m × 0.53 mm, 0.5 μm) and conventional GC employing a standard narrow-bore column (30 m × 0.25 mm, 0.25 μm).

The calculated chromatographic resolutions are (not surprisingly) higher for conventional GC. The chromatographic resolution of two closely eluted isomers of HCH (*i.e.* β-HCH and γ-HCH) reached a resolution of 4.6 in conventional GC compared to 1.2 in LP-GC. The isomeric pair *p,p′*-DDD and *o,p′*-DDT represented compounds with a low resolution even in conventional GC (0.96) and in the case of LP-GC analysis coelution of these two compounds occurred. Similarly, difenoconazole (two isomers), with a resolution of 1.3 in conventional GC was almost fully coeluted in LP-GC ($R = 0.3$).

The preferred approach for reporting of isomeric compounds is to integrate the peaks separately, and the responses are then summed to yield the total residue. This approach should be applied whenever possible to avoid quantification errors, especially for those compounds such as λ-cyhalothrin and deltamethrin, where the extent of conversion to their isomers depends on the injection solvent, sample matrix type, sample preparation strategy, and contamination of the GC system [57].

3.7. Method validation

The combination of different sample preparation methods and optimised PTV–LP-GC–HR-TOF-MS was evaluated in a validation study, involving analysis of six replicates of apple baby food spiked at 0.01 mg/kg with 100 pesticides. Table 1 provides mean recoveries, relative standard deviations (RSDs), and lowest calibration levels (LCLs) obtained from the analysis of both types of extracts. For most pesticides, average recoveries

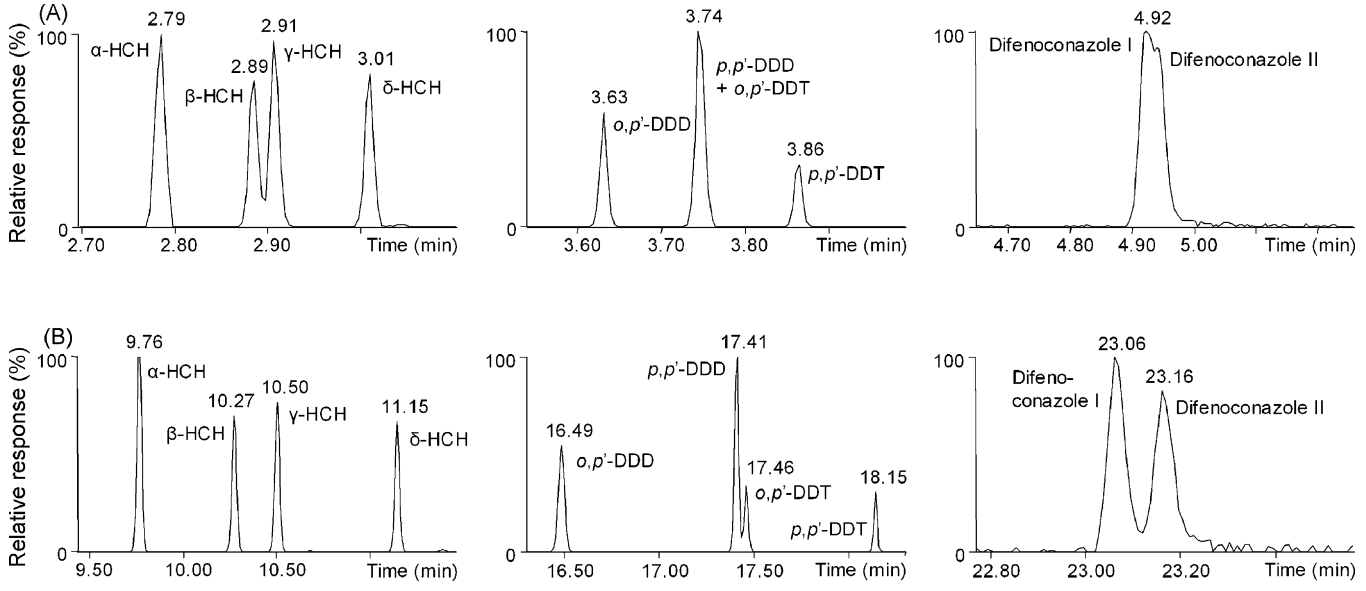

Fig. 6. GC separation of HCH-isomers (*m/z* 180.938), DDD- and DDT-isomers (*m/z* 235.008), and difenoconazole-isomers (*m/z* 323.024) at a concentration of 0.05 mg/kg under the conditions of (A) LP-GC (3 m × 0.15 mm restriction capillary coupled to 10 m × 0.53 mm, 0.5 μm Rtx-5 Sil MS column), and (B) conventional GC (30 m × 0.25 mm, 0.25 μm Rtx-5 Sil MS column). A mass window of 0.02 Da used in the experiment.

were in line with criteria that sets acceptable mean recovery to be between 70 and 120% with RSD ≤20% [58]. For the majority of the analytes, the LCLs were ≤0.01 mg/kg (mostly 0.0025 mg/kg, see Table 1). Thus for these pesticides, the PTV–LP-GC–HR-TOF-MS method can be used for control at the 0.01 mg/kg maximum residue limit established in the EU for pesticides in baby food [5]. Higher LCLs (0.025 mg/kg) were achieved regardless sample preparation procedures for pesticides such as acephate, omethoate, and pirimicarb. In the case of captan, high LCL (EtOAc + GPC method) and degradation (QuEChERS) were observed. Degradation of folpet was also observed in the QuEChERS method whereas the EtOAc + GPC method gave acceptable recovery (97%) and RSD (15%). The use of buffering in the MeCN extraction was found to improve the results for the pesticides, chlorothalonil (86 ± 17% for QuEChERS vs. 95 ± 8% with EtOAc) and tolylfluanid (72 ± 18% vs. 97 ± 6%, respectively).

Poor detection due to the saturation phenomenon was observed for endosulfan I with QuEChERS and for methacrifos and hexythiazox with EtOAc extraction and GPC cleanup. The lower recovery (19%) of fipronil can be attributed to the GPC, which partially eliminated this pesticide of relatively high-molecular weight (MW = 437).

3.8. Ruggedness testing

Co-extracted non-volatile matrix compounds may cause serious problems in routine trace GC analysis of pesticide residues. The long-term stability of analytes in the extracts and instrument robustness in each method was evaluated by repeated injection of matrix-matched standards/blanks. Fig. 7 shows the long-term stability of selected pesticides after the several injections of the extracts prepared using the two different sample preparation methods. In this experiment, 230 injections of extracts from each sample preparation method were carried out. The long-term stability of analyte responses showed similar robustness for both procedures up to ≈175 injections, after which the signal intensity became more varied from the buffered QuEChERS method, mostly due to the formation of new active sites from co-extracted matrix components. Therefore, we recommend replacing the liner after approx. 150 injections of sample extracts.

3.9. Exact mass deconvolution and automated peak reporting

Mass spectral deconvolution software provides an effective tool for automated resolving of coeluting peaks. Peak location/detection, and generation of "clean" mass spectra assigned to coeluting peaks followed by search against commercially available libraries are conducted. This approach makes compound identification in GC–MS more convenient, reliable, and faster.

The capability of this software (ChromaLynx) to identify and report pesticide residues contained in matrix-matched standards (0.01 and 0.1 mg/kg) when employing an acquisition rate of 4 spectra/s was tested for both types of extracts.

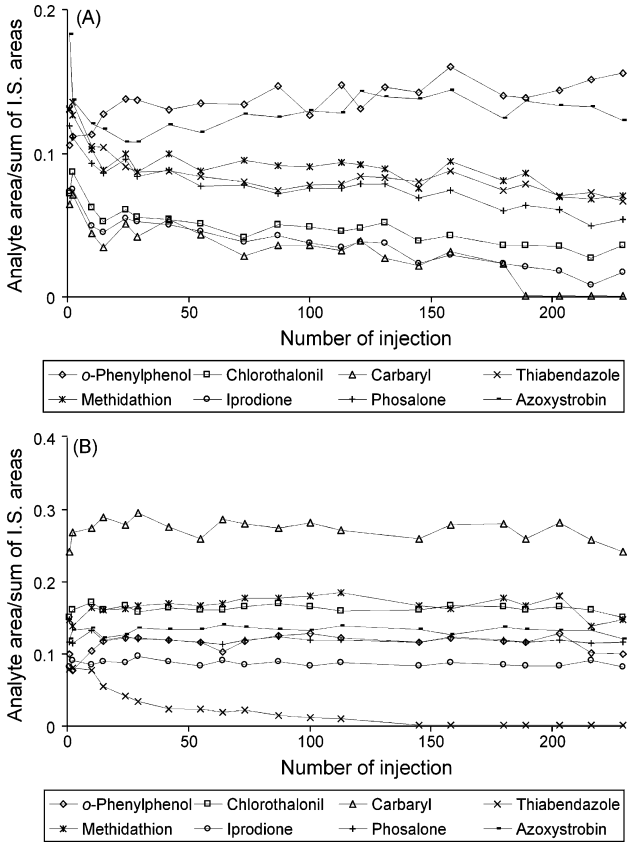

Fig. 7. Long-term stability of PTV–LP-GC–HR-TOF-MS system with injection of extracts prepared under different sample preparation strategies: (A) buffered QuEChERS method; (B) EtOAc extraction with GPC cleanup. Each point corresponds to the injection of a matrix-matched standard at 0.01 mg/kg concentration in a sequence.

Instead of 111 peaks/compounds theoretically reported, only 10 (EtOAc procedure) and 6 (QuEChERS) pesticides at a level of 0.01 mg/kg were assigned. The number of positively reported analytes increased when tested a level of 0.1 mg/kg, in which case 38 (EtOAc procedure) and 50 (QuEChERS) pesticides were reported. As an example Fig. 8 shows identification of a pesticide iprodione. Although most of the pesticide peaks were not automatically identified by the deconvolution software even at the 0.1 mg/kg level (probably due to the complete overlap of many of the detected pesticides), it can be assumed that the software can successfully report presence of pesticide residues in practice because commonly only a single residue (if any) is present in the investigated samples.

3.10. Time and material requirements

Table 2 outlines the approximate time requirements for the analysis of a batch of 12 samples and six matrix-matched standards using both sample preparation methods in combination with fast GC–MS. The number of samples used for this calculation is based on an automated GPC system that is able to run a maximum batch of 18 samples overnight. As a consequence, a sequence of 18 injections would have to be set up in the GC step,

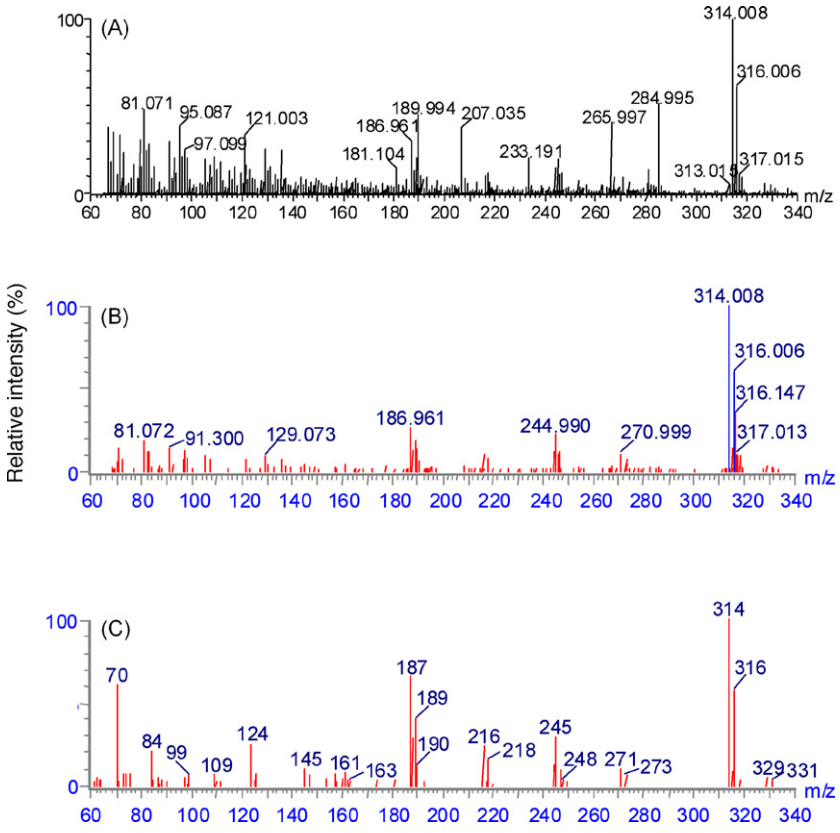

Fig. 8. Iprodione in buffered QuEChERS baby food extract at a level of 0.1 mg/kg. (A) Mass spectrum of elution area of iprodione without deconvolution; (B) deconvoluted mass spectrum of iprodione (library match with a reverse factor of 794); (C) NIST library mass spectrum of iprodione.

too. With the QuEChERS approach, the total time needed for the processing of 18 samples can be reduced by a factor of ≈5, and moreover, extraction and cleanup of the extracts are conducted in parallel in QuEChERS, thus much larger batch sizes can be prepared to take full advantage of the much higher sample throughput of the fast GC–MS analytical step. Finally, the analysis cost can be significantly reduced when using QuEChERS method by a factor of ≈6 (Table 3).

Table 2
Time requirements for the analysis of the batch of 12 samples and 6 matrix-matched standards using the different sample preparation strategies in combination with LP-GC.

Analytical step	Time	
	EtOAc extraction with GPC	Buffered QuEChERS method
(A)		
Sample preparation	5.75 h Extraction: 12 samples and one blank sample for preparation of six matrix-matched standards (12 × 15 min + 1 × 15 min) Evaporation: 13 samples per three vaporisers (4 × 30 min + 1 × 30 min)	0.58 h Two batches per six samples and one blank sample for preparation of six matrix-matched standards (2 × 15 min + 1 × 5 min)
Cleanup	11.5 h Cleanup: 18 samples (18 × 35 min) Evaporation: 18 samples per three vaporisers (6 × 10 min)	0.25 h Two batches per six samples and one sample for preparation of matrix-matched standards (2 × 5 min + 1 × 5 min)
(B)		
Total GC run	3.23 h (18 × 10.75 min)	3.23 h (18 × 10.75 min)
(C)		
Total analysis	20.5 h	4 h
Total analysis during the working hours[a]	10 h	4 h

[a] Time (10.5 h) required for the automated GPC cleanup not taken into consideration.

Table 3
Material requirements and material cost of the analysis

	Sample preparation method	
	EtOAc extraction with GPC	Buffered QuEChERS
Material per sample	EtOAc: ≈193 ml Cyclohexane: ≈42 ml Na_2SO_4: ≈110 g	MeCN: 10 ml HOAc: 0.1 ml $MgSO_4$: 4.15 g $NaOAc{\cdot}3H_2O$: 1.7 g Primary secondary amine: 50 mg
Material cost per sample (€)	7.8	1.3

4. Conclusions

PTV–LP-GC–HR-TOF-MS was demonstrated to be a challenging solution in analysis of multiple pesticide residues in baby food; performance characteristics obtained within validation study comply with quality control requirements stated in SANCO/2007/3131 document [58]. With only a few exceptions, limits of quantification (LOQs) were ≤0.01 mg/kg thus reliable control of "baby food limits" is possible. As compared to the method based on the EtOAc extraction and GPC cleanup, the application of the buffered QuEChERS method significantly reduced the sample preparation time and costs. Thus, the QuEChERS method should be the method of choice for routine pesticide residue monitoring. Using the QuEChERS method in combination with the PTV–LP-GC–HR-TOF-MS technique, 100 pesticide residues can be analysed in a batch of 18 samples within 4 h.

The superiority of PTV–LP-GC–HR-TOF-MS over system employing a unit mass resolution quadrupole mass analyser operated in SIM can be characterised as follows:

(i) Obtaining of full spectral information even at very low concentrations of target analytes.
(ii) Unbiased identification and reliable quantification of target analytes through the application of a narrow mass window (0.02 Da) for extracting analyte ions.
(iii) Avoiding of rather time-consuming formation of time segments for the monitoring of selected ions (SIM mode), which is required for obtaining low determination levels.

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References

[1] D.B. Barr, L.L. Needham, J. Chromatogr. B 778 (2005) 5.
[2] J. Siebers, R. Hänel, in: P.W. Lee (Ed.), Handbook of Residue Analytical Methods for Agrochemicals, vol. 1, Wiley, Chichester, 2003, p. 13.
[3] Council Directive 76/895/EEC of 23 November 1976 relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables, Off. J. Eur. Commun. L340 (1976) 26.
[4] Commission Directive 1999/39/EC of 6 May 1999 amending Directive 96/5/EC on processed cereal-based foods and baby foods for infants and young children, Off. J. Eur. Commun. L124 (1999) 8.
[5] Commission Directive 2003/13/EC of 10 February 2003 amending Directive 96/5/EC on processed cereal-based foods and baby foods for infants and young children, Off. J. Eur. Commun. L41 (2003).
[6] W.G. Fong, H.A. Moye, J.N. Seiber, J.P. Toth, Pesticide Residues in Foods: Methods, Techniques, and Regulations, Wiley, Chichester, 1999.
[7] S.L. Reynolds, in: A.R. Fernandez-Alba (Ed.), Chromatographic–Mass Spectrometric Food Analysis for Trace Determination of Pesticide Residues (Comprehensive Analytical Chemistry, vol. 43), Elsevier, Amsterdam, 2005, p. 75.
[8] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412.
[9] M. Anastassiades, E. Scherbaum, in: A.R. Fernandez-Alba (Ed.), Chromatographic–Mass Spectrometric Food Analysis for Trace Determination of Pesticide Residues (Comprehensive Analytical Chemistry, vol. 43), Elsevier, Amsterdam, 2005, p. 113.
[10] S.J. Lehotay, K. Mastovska, A.R. Lightfield, J. AOAC Int. 88 (2005) 615.
[11] P. Korytar, H.-G. Janssen, E. Matisova, U.A.Th. Brinkman, Trends Anal. Chem. 21 (2002) 558.
[12] K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1000 (2003) 153.
[13] J. de Zeeuw, J. Peene, H.-G. Janssen, X. Lou, J. High. Resolut. Chromatogr. 23 (2000) 677.
[14] A.F.L. Godoi, W. Vilegas, R.H.M. Godoi, L. Van Vaeck, R. Van Grieken, J. Chromatogr. A 1027 (2004) 127.
[15] P.E. Joos, A.F.L. Godoi, D. de Jong, J. de Zeeuw, R. van Grieken, J. Chromatogr. A 985 (2003) 191.
[16] K. Mastovska, S.J. Lehotay, J. Hajslova, J. Chromatogr. A 926 (2001) 291.
[17] C.A. Cramers, G.J. Scherpenzeel, P.A. Leclercq, J. Chromatogr. A 203 (1981) 207.
[18] J. Hajslova, J. Zrostlikova, J. Chromatogr. A 1000 (2003) 181.
[19] M. Careri, F. Bianchi, C. Corradini, J. Chromatogr. A 970 (2002) 3.
[20] O. Nunez, E. Moyano, M.T. Galceran, Trends Anal. Chem. 24 (2005) 683.
[21] R. Stepan, J. Ticha, J. Hajslova, T. Kovalczuk, V. Kocourek, Food Addit. Contam. 22 (2005) 1231.
[22] K. Mastovska, J. Hajslova, S.J. Lehotay, J. Chromatogr. A 1054 (2004) 335.
[23] J.L. Martinez Vidal, F.J. Arrebola, M. Mateu-Sanchez, Rapid Commun. Mass Spectrom. 16 (2002) 1106.
[24] J.F. Garcia-Reyes, C. Ferrer, M.J. Gomez-Ramos, A.R. Fernandez-Alba, J.F. Garcia-Reyes, A. Molina-Diaz, Trends Anal. Chem. 26 (2007) 239.
[25] H.-J. Stan, in: A.R. Fernandez-Alba (Ed.), Chromatographic–Mass Spectrometric Food Analysis for Trace Determination of Pesticide Residues (Comprehensive Analytical Chemistry, vol. 43), Elsevier, Amsterdam, 2005, p. 269.
[26] T. Cajka, J. Hajslova, J. Chromatogr. A 1058 (2004) 251.
[27] M. Guilhaus, D. Selby, V. Mlynski, Mass Spectrom. Rev. 19 (2000) 65.
[28] J. Zrostlikova, J. Hajslova, T. Cajka, J. Chromatogr. A 1019 (2003) 173.
[29] J. Dallüge, R.J.J. Vreuls, J. Beens, U.A.Th. Brinkman, J. Sep. Sci. 25 (2002) 201.

[30] J. Dallüge, M. van Rijn, J. Beens, R.J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 965 (2002) 207.
[31] J.-F. Focant, G. Eppe, M.-L. Scippo, A.-C. Massart, C. Pirard, G. Maghuin-Rogister, E. De Pauw, J. Chromatogr. A 1086 (2005) 45.
[32] J.W. Cochran, J. Chromatogr. Sci. 40 (2002) 254.
[33] T. Cajka, J. Hajslova, R. Kazda, J. Poustka, J. Sep. Sci. 28 (2005) 601.
[34] J.-F. Focant, E.J. Reiner, K. MacPherson, T. Kolic, A. Sjödin, D.G. Patterson Jr., S.L. Reese, F.L. Dorman, J. Cochran, Talanta 63 (2004) 1231.
[35] E. Hoh, K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1145 (2007) 210.
[36] D. Zou, K. Liu, W.-P. Pan, J.T. Riley, Y. Xu, J. Chromatogr. Sci. 41 (2003) 245.
[37] P. Korytar, L.L.P. van Stee, P.E.G. Leonards, J. de Boer, U.A.Th. Brinkman, J. Chromatogr. A 994 (2003) 179.
[38] L. Dunovska, T. Cajka, J. Hajslova, K. Holadova, Anal. Chim. Acta 578 (2006) 234.
[39] T. Veriotti, R. Sacks, Anal. Chem. 73 (2001) 4395.
[40] M.T. Robets, J.-P. Dufour, A.C. Lewis, J. Sep. Sci. 27 (2004) 473.
[41] M. Adahchour, J. Wiewel, R. Verdel, R.J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 1086 (2005) 99.
[42] T. Cajka, J. Hajslova, J. Cochran, K. Holadova, E. Klimankova, J. Sep. Sci. 30 (2007) 534.
[43] S.M. Song, P. Marriott, A. Kotsos, O.H. Drummer, P. Wynne, Forensic Sci. Int. 143 (2004) 87.
[44] J. Dallüge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[45] W. Welthagen, R.A. Shellie, J. Spranger, M. Ristow, R. Zimmermann, O. Fiehn, Metabolomics 1 (2005) 65.
[46] T. Cajka, J. Hajslova, LC GC Eur. 20 (2007) 25.
[47] T. Cajka, K. Mastovska, S.J. Lehotay, J. Hajslova, J. Sep. Sci. 28 (2005) 1048.
[48] K. Patel, R.J. Fussell, D.M. Goodall, B.J. Keely, J. Sep. Sci. 29 (2005) 90.
[49] S.J. Lehotay, A. de Kok, M. Hiemstra, P. Van Bodegraven, J. AOAC Int. 88 (2005) 595.
[50] S.J. Lehotay, J. AOAC Int. 90 (2007) 485.
[51] M. Kochman, A. Gordin, P. Goldshlag, S.J. Lehotay, A. Amirav, J. Chromatogr. A 974 (2002) 185.
[52] A.B. Fialkov, U. Steiner, S.J. Lehotay, A. Amirav, Int. J. Mass Spectrom. 260 (2007) 31.
[53] M. Godula, J. Hajslova, K. Mastovska, J. Krivankova, J. Sep. Sci. 24 (2001) 355.
[54] J.L. Martinez Vidal, F.J. Arrebola Liebanas, M.J. Gonzalez Rodriguez, A. Garrido Frenich, J.L. Fernandez Moreno, Rapid Commun. Mass Spectrom. 20 (2006) 365.
[55] S.J. Lehotay, K. Mastovska, S.J. Yun, J. AOAC Int. 88 (2005) 630.
[56] M.R. Green, R.H. Bateman, in: Proceedings of the 53rd ASMS Conference, San Antonio, TX, USA, 5–9 June, 2005, paper: Method of Removing Ions in Selected M/Z Regions in Orthogonal Time of Flight Mass Spectrometers.
[57] K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1040 (2004) 259.
[58] Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, European Commission document SANCO/2007/3131, Brussels, 2007.