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Determination of priority pesticides in baby foods by gas chromatography tandem quadrupole mass spectrometry

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Abstract

A gas chromatography–tandem quadrupole mass spectrometry (GC–MS/MS) method for the determination of twelve priority pesticides, and transformation products (e.g. metabolites) specified in the EU Baby Food Directive 2003/13/EC is described. Prior to GC–MS/MS analysis, co-extractives were removed from acetonitrile extracts using dispersive solid phase extraction with octadecyl (200 mg) and primary secondary amine (50 mg) sorbents. The clean up proved essential for the satisfactory long-term chromatographic performance during the analysis of a range of representative commercially pre-prepared baby food samples. Extracts spiked with pesticides at 1–8 $\mu g\,kg^{-1}$, yielded average recoveries in the range 60–113% with relative standard deviations less than 28%.

Keywords: Tandem quadrupole mass spectrometry; Gas chromatography; Baby food; Pesticides

1. Introduction

The EU Baby Food Directive 2003/13/EC on processed cereal-based foods and processed foods for infants and young children, which came into force on 6th March 2004, [1] places emphasis on the control of pesticides or transformation products (including metabolites) of pesticides with a maximum acceptable daily intake of 0.0005 $mg\,kg^{-1}$ body weight. Pesticides are either designated as prohibited, and considered not to have been used if their residue does not exceed 3 $\mu g\,kg^{-1}$, or have maximum residue limits (MRLs) set between 4 and 8 $\mu g\,kg^{-1}$. Twelve of the pesticides and breakdown products listed in the Directive (cadusafos, ethoprophos, fipronil, fipronil-desulfinyl, heptachlor, *trans*-heptachlor epoxide, hexachlorobenzene, nitrofen, aldrin, dieldrin, endrin and omethoate) are suitable for multi-residue analysis by gas chromatography–mass spectrometry (GC–MS). The remaining compounds specified in the Directive, because of their physicochemical properties, require analysis either by multi-residue liquid chromatography–mass spectrometry (LC–MS) or by specific single residue methods.

Before the introduction of the Directive very little emphasis was placed on the analysis of pesticides in baby food at levels below 10 $\mu g\,kg^{-1}$. Moore et al. [2] determined four organochlorine pesticides at 10 $\mu g\,kg^{-1}$ in baby food, and Cressey and Vannort [3] determined dieldrin at 2 $\mu g\,kg^{-1}$ in milk-based infant formula by gas chromatography with electron capture detection (GC-ECD). In response to the Directive, Linkerhägner et al. [4] determined 24 of the priority pesticides and breakdown products at levels below 10 $\mu g\,kg^{-1}$ using acetone extraction, liquid-liquid partition and gel permeation chromatography clean-up with detection by GC-ECD and tandem LC–MS analysis (LC–MS/MS). Recoveries for the GC-amenable compounds were not reported.

To meet the requirement for enforcement of EU Directive 2003/13/EC, laboratories require multi-residue methods with lower limits of detection (LOD) than those currently available. This necessitates improvements in the extraction, clean up and detection of pesticides in baby food samples.

A simple extraction with acetonitrile, followed by dispersive solid-phase extraction (SPE) clean up, was reported for

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the analysis of a wide range of pesticides [5]. Acetonitrile extracts generally gives lower quantities of co-extractives compared with other solvents, e.g. ethyl acetate, especially in the analysis of complex matrices like baby foods that often contain many different raw ingredients and additives.

Automated large volume injection (LVI) systems, based on programmable temperature vaporizing (PTV) injection, are employed to improve the limits of detection for pesticides in food matrices [6–9]. The PTV injector can also overcome the disadvantage of the high volume-expansion coefficient of acetonitrile as excess solvent vapour is vented to atmosphere prior to introduction onto the column.

Tandem mass spectrometry (MS/MS) using quadrupole systems has been shown to be a valuable technique in pesticide residue analysis of complex mixtures and complex matrices, with increased selectivity, excellent limits of detection, and improved signal to noise ratio [10–12].

The aim of this work was to develop a simple and rapid method for the analysis of priority pesticides in baby foods, avoiding off-line concentration steps and using LVI–GC–MS/MS to provide quantification and confirmation of residues at levels of 1–8 $\mu g\,kg^{-1}$.

2. Experimental

2.1. Reagents, standards and samples

Pesticide reference standards (purity >98.0%) were purchased from Qm_x (Thaxted, UK) and LGC Promochem (Teddington, UK). Working standard mixtures, containing between 1 and 8 $\mu g\,ml^{-1}$ of each compound, were prepared in acetonitrile for use as spiking solutions. Acetonitrile (HPLC fluorescence grade) and sodium chloride (analytical reagent grade) were purchased from Fisher Scientific UK (Loughborough, UK). Anhydrous magnesium sulfate (analytical reagent grade) was purchased from York Glassware (York, UK). Bondesil-primary secondary amine (Varian PSA; 40 μm) was purchased from Essex Scientific (Hadleigh, UK) and octadecyl (C_{18}) sorbent was taken from Isolute SPE cartridges (part no. 220-0050-B) purchased from Argonaut Technologies (Hengoed, UK).

2.2. Apparatus

Determination was performed using a Varian GC–MS/MS system comprising a CP-3800 gas chromatograph with a 1079 PTV injector, a CP-8400 autosampler and a 1200 triple quadrupole MS (Varian, Walnut Creek, CA, USA). Data acquisition and processing were performed using Varian Star Workstation software (version 6.2). A fused-silica capillary column (Zebron ZB-50, 50% phenyl–50% methylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Phenomenex, USA) was used and protected by a 7 mm CarboFrit insert (Restek, Bellefonte, PA, USA) in the GC liner. The CarboFrit insert was changed for each new sequence of samples.

2.3. LVI–GC–MS/MS conditions

Large volume injections (8 μl) were performed with a vent time of 45 s, split ratio of 30 and an injection speed of 5 $\mu l\,s^{-1}$. The injector temperature was held at 70 °C during the solvent evaporation stage and then ramped to 280 °C at 200 °C min^{-1}. The GC temperature program was 90 °C for 1 min programmed to 200 °C at 20 °C min^{-1} (held for 6 min) and then programmed to 280 °C at 20 °C min^{-1} (held for 5 min). The total GC run time was 21.5 min. Helium (99.997% purity; flow rate of 1 ml min^{-1}) was used as a carrier gas.

The tandem quadrupole mass spectrometer was operated in electron ionisation (EI) mode. The MS/MS detector interface temperature was set at 200 °C, the source temperature at 300 °C, electron energy at 70 eV, filament current at 150 μA and the detector voltage at 1700 V. The solvent delay time was 6 min. The MS/MS conditions in the multiple reaction monitoring (MRM) mode are given in Table 1. Argon (137 kPa)

Table 1
Summary of MRM transitions selected for analysis of the pesticides in EI mode

	Pesticide	First transition (*m/z*)	CE (V)	Second transition (*m/z*)	CE (V)	Quantifying ion	Qual. ion
1	Ethoprophos	158-97	20	200-114	20	97	114
2	Cadusafos	127-99	20	159-97	30	97	99
3	Hexachlorobenzene	284-214	40	284-249	30	249	214
4	Omethoate	110-79	20	156-110	20	110	79
5	Fipronil-desulfinyl	388-333	30	333-231	40	231	333
6	Dimethoate[a]	229-87	10	143-111	20	87	111
7	Heptachlor	272-237	20	274-239	40	237	239
	δ-HCH (VS)	181-145	30	219-183	10	–	–
8	Aldrin	263-191	40	293-257	10	191	257
9	Fipronil	213-143	30	367-213	30	213	143
10	Heptachlor epoxide (*trans*)	253-217	40	289-253	10	253	217
11	Dieldrin	263-193	40	277-241	10	193	241
12	Endrin	263-191	40	281-245	20	191	245
13	Nitrofen	202-139	30	283-162	30	139	162

VS: volumetric standard.
[a] Dimethoate is included because of it can breakdown to omethoate.

was used as the collision gas. The mass spectrometer was calibrated weekly using perfluorotributylamine.

2.4. Samples

Representatives of three different baby foods of fruit and rice, fish and pasta, and potato and pork were used as blanks and for the preparation of spiked samples and matrix-matched calibration standards for validation experiments. A further 10 samples: vegetable lasagne, chicken, leek and sweetcorn risotto, spaghetti with tomatoes and mozzarella, vegetable and chicken risotto, apple and vanilla dessert, mango and banana with yogurt, tropical fruit salad, banana and peach dessert, creamy rice breakfast, and apple and blueberry dessert were used to test the robustness of the method. All samples were labelled as organic and the fat content ranged from 0.05 to 2.8 g per 100 g of sample.

2.5. Extraction procedure and analysis

A sub-sample (10 g) was weighed into a centrifuge tube (40 ml) and an appropriate volume of standard solution added to give a fortification level of 1–8 $\mu g\,kg^{-1}$. Acetonitrile (10 ml), anhydrous magnesium sulfate (4 g) and sodium chloride (1 g) were added and shaken immediately, thus preventing coagulation of $MgSO_4$ [10]. Volumetric standard (delta-hexachlorocyclohexane, δ-HCH) was added (100 μl of a 1 $\mu g\,ml^{-1}$ solution) and the sample was centrifuged at 4300 × *g* for 5 min. An aliquot (1 ml) of the supernatant was transferred to a microcentrifuge vial containing PSA sorbent (50 mg), anhydrous $MgSO_4$ (150 mg) and C_{18} sorbent (100–300 mg). The vial was vortexed for 30 s, centrifuged at 5000 × *g* for 1 min, and the supernatant was analysed by LVI–GC–MS/MS.

2.6. Method performance

The precision and accuracy of the method was tested with spiked samples of fruit and rice, fish and pasta, and potato and pork. Recoveries for seven replicates at 1 and 3 $\mu g\,kg^{-1}$ were determined. Matrix-matched multi-level calibration standards, which bracketed the samples, were used for all analyses. All results were calculated using δ-HCH to correct for volumetric errors.

3. Results and discussion

3.1. Determination of MS/MS parameters

The selection of MS/MS transitions and the acquisition parameters collision energy, number of transitions per time segment and the number of data points across the peak were evaluated for best response under electron ionisation conditions.

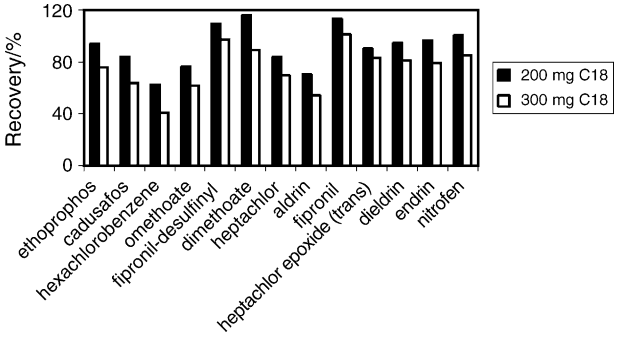

Fig. 1. Recoveries (%) calculated using volumetric standard in potato based baby food using different amounts (200, 300 mg) of C_{18} sorbent in the clean up ($n = 2$). Note: data not shown for experiments using 100 mg of Cig sorbent because calibration curves were not acceptable.

The most intense, highest mass precursor ions were selected from full scan spectra for the development of the MRM method with two transitions for each analyte. Following precursor ion selection, product ion spectra were acquired by collision-induced dissociation (CID) with argon gas. Precursor ions were subjected to collision energy (CE) voltages of 10, 20, 30 and 40 V (potential on quadrupole 2) and the most intense product ions from each precursor ion were selected. The transitions and CE for all the analytes are detailed in Table 1.

A maximum of four transitions were programmed into each time segment to optimise the response and a scan rate of 0.3 s datapoint^{-1} was set to collect a minimum of 7–10 points across a chromatographic peak.

3.2. Selection of method for extraction and clean up

Analysis of crude ethyl acetate extracts of fruit-based baby food gave satisfactory results for the majority of the pesticides of interest (unpublished work). Analysis of ethyl acetate extracts of baby foods with a higher fat content, i.e. those containing fish and pork, revealed excessive matrix effects and contamination of the GC system, hence, this approach was not pursued. By contrast, acetonitrile extraction [5] with dispersive SPE clean up (PSA sorbent and C_{18} sorbent) provided relatively clean extracts.

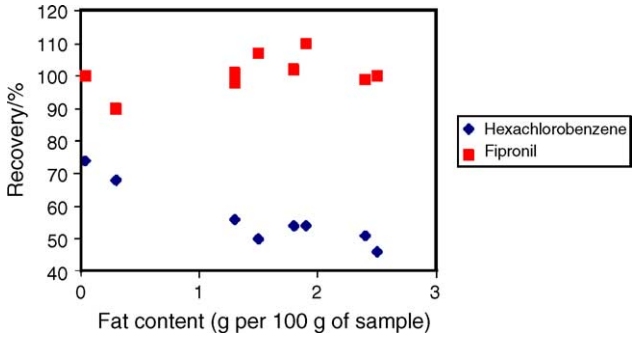

Fig. 2. Recoveries (%) vs. fat content of a baby food sample for hexachlorobenzene and fipronil.

Table 2
Recoveries (%) and relative standard deviations, RSD (%), obtained by GC–MS/MS (MRM mode) analysis of three different baby foods, at two spiking levels of 1 and 3 μg kg^{-1} (n = 7)

Compound	t_R (min)	MRL (mg kg^{-1})	Apple/pear/rice		Salmon/spinach				Potato/pork	
			Spiking level (μg kg^{-1})							
			1		1		3		1	
			Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
Ethoprophos	8.07	0.008	66	28	101	6	78	9	90	5
Cadusafos	8.24	0.006	69	5	85	12	83	26	65	6
Hexachlorobenzene	8.95	0.003	66	15	57	11	65	5	60	12
Omethoate	9.22	0.003	63	13	71	7	71	5	70	9
Fipronil-desulfinyl	11.04	0.002[a]	87	8	106	4	102	3	102	12
Dimethoate	11.33	0.01	91	14	99	11	100	7	113	10
Heptachlor	11.38	0.0015[a]	84	12	90	5	80	5	92	23
Aldrin	12.73	0.0015[a]	93	10	81	9	73	6	94	13
Fipronil	14.10	0.002[a]	82	14	100	4	94	5	87	10
Heptachlor epoxide (*trans*)	14.69	0.0015[a]	75	19	88	10	90	7	99	19
Dieldrin	15.78	0.0015[a]	80	11	88	10	88	8	78	18
Endrin	16.32	0.003	79	10	79	23	84	4	81	25
Nitrofen	16.48	0.003	80	8	87	5	89	4	81	11

VS: internal standard.
[a] MRL for fipronil = 0.004 mg kg^{-1} expressed as sum of fipronil and fipronil-desulfinyl; MRL for heptachlor = 0.003 mg kg^{-1} expressed as sum of heptachlor and heptachlor epoxide (*trans*); MRL for dieldrin = 0.003 mg kg^{-1} expressed as sum of dieldrin and aldrin. For dual component MRLs, the target levels for the individual compounds were set at half the MRL.

The effectiveness of different amounts (100–300 mg) of C_{18} sorbent with a constant amount (50 mg) of PSA sorbent was assessed with regard to the quantity of co-extractives remaining after evaporation of the solvent. This was determined by the effect on the long-term chromatographic performance and recovery data. Quantification using 100 mg of C_{18} sorbent resulted in a lack of reproducibility among the diverse range of matrices, in some cases giving non-linear calibration curves. Lower recoveries were obtained using 300 mg of C_{18} sorbent, whereas 200 mg of C_{18} sorbent gave relatively clean extracts, improved signal to noise ratio, a consistent level of response and satisfactory calibration curves. Thus, the appropriate amount of sorbent lies between 100 and 200 mg of C_{18} sorbent.

Increasing the amount of C_{18} sorbent used in the clean up reduced the recovery of a number of the pesticides (Fig. 1). Recoveries were calculated using a volumetric standard, δ-HCH that was added before the clean up step and was subject to losses of approximately 10%. The losses of the pesticides were consistent across the individual sample types but were variable between different sample types. To achieve accurate results it is necessary to prepare matrix

Table 3
Ion ratio ($A_{qual\ ion}/A_{quant\ ion}$) of matrix calibrants and recovery samples at the MRLs for 10 different baby food samples (each in duplicate)

Compound	Recovery (%)		$A_{qualifying\ ion}/A_{quantifying\ ion}$		
	Average (n = 20)	RSD (%)	Expected ratio from standards average (n = 20)	Observed ratio from samples average (n = 20)	RSD (%)
Ethoprophos	81	2	0.20	0.20	5
Cadusafos	77	8	0.32	0.31	8
Hexachlorobenzene	58	16	1.08	1.05	7
Omethoate	73[a]	9	0.52	0.47	11
Fipronil-desulfinyl	99	4	0.70	0.68	7
Dimethoate	92	7	0.34	0.35	13
Heptachlor	75	11	0.35	0.34	9
Aldrin	67	8	0.35	0.35	11
Fipronil	101	5	0.96	0.93	19
Heptachlor epoxide (*trans*)	84	10	0.61	0.58	15
Dieldrin	81	9	0.65	0.66	12
Endrin	82	10	0.32	0.31	19
Nitrofen	87	6	0.33	0.33	14

Note 1: Same levels apply as specified in Table 2. Note 2: If the expected ratio is: (1) >0.5, the observed ratio should be within ±10%; (2) > 0.2 <0.5, the observed ratio should be within ±15%; (3) > 0.1 <0.2, the observed ratio should be within ±20%.
[a] n = 18.

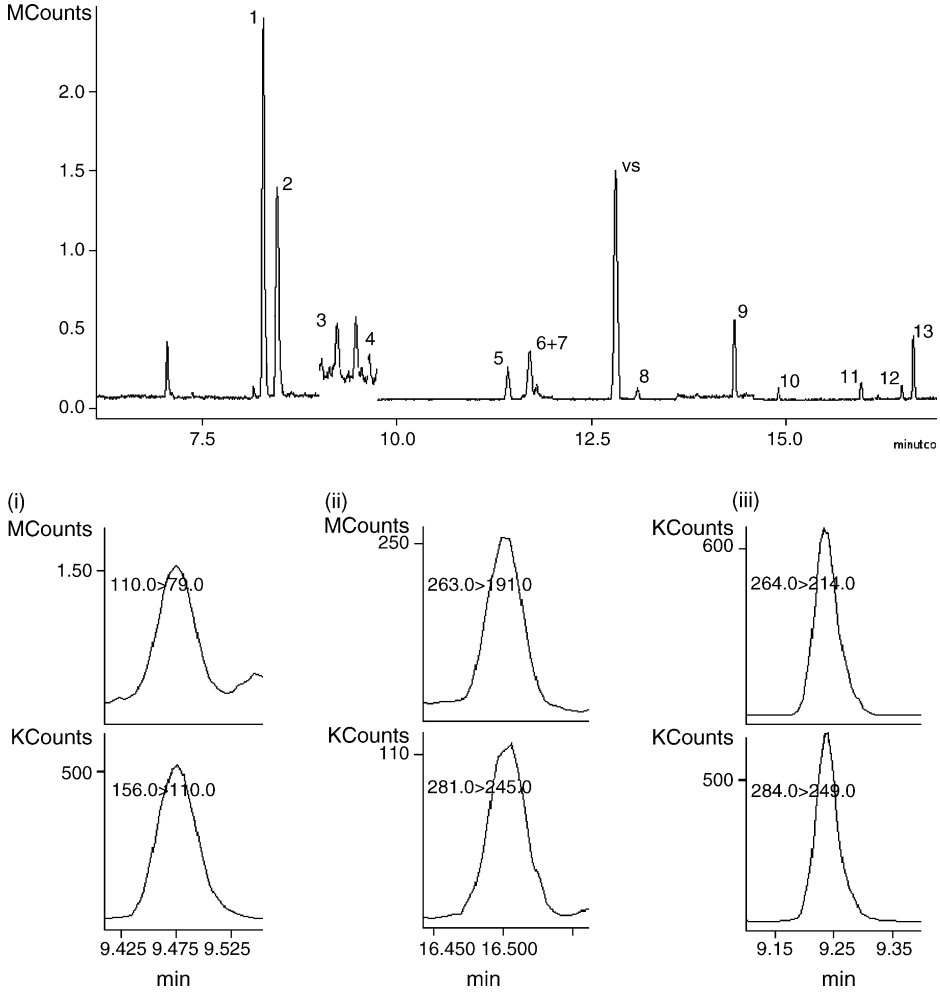

Fig. 3. MRM chromatogram of a calibrant at the MRLs in vegetable lasagne based baby food, and individual peaks (two transitions) at the MRL for: (i) omethoate; (ii) endrin and (iii) hexachlorobenzene.

matched calibration standards for each individual sample type.

The use of C_{18} sorbent in combination with the internal standard proved suitable for the quantification of 12 of the 13 pesticides, in all samples evaluated. The relatively low recoveries (approximately 60%) for hexachlorobenzene, even after correction by internal standard, suggest that hexachlorobenzene is preferentially retained either by the fat or by the C_{18} sorbent. The recovery of hexachlorobenzene decreased with increasing fat content (Fig. 2) as has been reported previously [13]. The C_{18} sorbent removes most of the lipophilic material, hence fat-soluble pesticides such as hexachlorobenzene are removed either in association with the fat or directly by association with the sorbent.

Accurate quantification of residues, especially hexachlorobenzene, will require either the use of internal standards that are matched more closely to the lipophilic analytes, or development of an alternative clean up method. Improvements in the accuracy are feasible using deuteriated pesticides as internal standards, or ^{13}C labelled standards in the case of hexachlorobenzene, due to the low quantities of internal standard required. Gel permeation chromatography (GPC) was not used for the removal of fat from the extracts because it is not suitable for fipronil and fipronil-desulfinyl. Consequently, 50 mg of PSA and 200 mg of C_{18} sorbent were used for all analyses.

3.3. Method performance, recoveries and selectivity

Calibration curves were linear over the range 0.0005–0.01 $\mu g\,ml^{-1}$ (equivalent to 0.5–10 $\mu g\,kg^{-1}$) with correlation coefficients >0.98 for all analytes. With the exception of hexachlorobenzene, satisfactory recoveries (63–113%) were obtained for all the pesticides spiked at the 1 and 3 $\mu g\,kg^{-1}$ levels in three different baby foods. Quantification of ethoprophos, heptachlor and endrin was not reliable at the 1 $\mu g\,kg^{-1}$ level in at least one of the test matrices. At the 3 $\mu g\,kg^{-1}$ level (MRL) there was an improvement in recovery and precision for endrin (Table 2).

The robustness of the method was tested by the analysis of a further 10 baby foods containing a wide variety of ingredients. Each sample type was analysed both as a blank and after

fortification with pesticides and metabolites at the target levels (Table 3). The precision and repeatability of the method was assessed using single level matrix matched calibration standards.

Combining the results of the individual analyses (duplicate recoveries for each of the 10 different baby foods) yielded overall recoveries in the range 58–101% with RSD less than 17% (Table 3). The ability to confirm the identity of each pesticide or degradation product was determined on the basis of the relative abundance of the two selected transitions for each compound. The ratio of the less abundant ion (usually the qualifying ion) over the most abundant ion (usually the quantifying ion) for each pesticide examined was within the expected range obtained for standards (Table 3). The results demonstrate that confirmation of identity was possible for all of the compounds in the majority of the baby foods tested. None of the blank samples analysed contained residues of the compounds tested at or above the MRL.

Fig. 3 illustrates good selectivity and a signal to noise (*S*/*N*) ratio of at least 3:1 for representative target compounds; omethoate (most polar analyte), endrin (lowest response) and hexachlorobenzene (lowest recovery) at the respective MRLs in matrix. The good peak shape for omethoate, especially at such low levels, demonstrates that the GC–MS/MS system is very inert with respect to polar compounds.

4. Conclusions

The LVI–GC–MS/MS multi-residue method developed is simple, rapid and suitable for the screening of 12 priority pesticides in fruit-, fish- and potato-based baby food at $1\,\mu g\,kg^{-1}$, and for quantification and confirmation at their respective MRLs. The method was found not to be suitable for the quantification of hexachlorobenzene. Thus, if hexachlorobenzene is detected an alternative method would be required for accurate quantification. Nevertheless, the extraction and clean up method presented is still a practical solution to the challenge set by the EU Directive, and it requires minimal equipment and utilises low volumes of organic solvents. It is possible to include other GC-amenable pesticides in the analysis to allow for any future amendments to the Directive. To enforce the EU Baby Food Directive, parallel multi-residue analysis for LC–MS amenable pesticides is also required. The extraction method developed should be suitable for both GC and LC analysis and is the subject of ongoing studies.

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