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Method validation of resistive heating—gas chromatography with flame photometric detection for the rapid screening of organophosphorus pesticides in fruit and vegetables

Katan Patel[a,b], Richard J. Fussell[a,*], Roy Macarthur[a], David M. Goodall[b], Brendan J. Keely[b]

[a] *Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK*
[b] *University of York, Heslington, York, YO10 5DD, UK*

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Abstract

A rapid method for the screening of organophosphorus (OP) pesticides in fruit and vegetables is reported. Sample extracts were analysed using resistive heating-gas chromatography (RH-GC) with flame photometric detection (FPD). A CarboFrit insert in the GC liner allowed injection of crude extracts onto the GC system. Separation of up to 20 pesticides was achieved in 4.3 min with excellent retention time stability. Signal-to-noise ratios of 5:1 or better were obtained for the majority of the pesticides at the lowest calibrated level (LCL), 0.01 $\mu g\,ml^{-1}$, with excellent linearity over the range 0.01–0.5 $\mu g\,ml^{-1}$ (0.004–0.2 $mg\,kg^{-1}$ equivalent). Average recoveries between 70 and 116% were obtained for pesticides spiked at 0.01 and 0.1 $mg\,kg^{-1}$ with associated R.S.D. values $\leq$20% in the majority of cases. Estimates of relative reproducibility standard deviation ($R.S.D._R$), made by combining observed R.S.D. values with estimates of uncertainty associated with mean recovery allowed the determination of HORRAT values which confirmed that the method is capable of producing results which are fit for purpose. The validated method was then used to screen peaches, grapes and sweet peppers for a total of 37 pesticides. Incurred residue results obtained using RH-GC–FPD were in good agreement with the results from analysis of the same samples using MS confirmation.
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1. Introduction

Organophosphorus (OP) pesticides are used on a wide variety of crops, and residues in foods are commonly found. The majority of OP pesticides are cholinesterase inhibitors and exposure to high levels can lead to acute food poisoning [1]. In the interests of consumer safety the European Commission introduced a rapid alert system for food and feed (RASFF) to notify member states when a food or feed presenting a potential risk to consumer safety is detected in the market place. In 2002–2003, a significant number of alerts resulted from the detection of relatively high levels of methamidophos, acephate and monocrotophos. On account of the potential risk to the consumer there is a clear need for the development of a rapid screening method for OP pesticides in fruit and vegetables.

Methods for the analysis of a limited number of OP pesticides in foods have been reported in the literature [1–6]. These methods usually involved the use of a clean-up step with chromatographic run times in the order of 20–30 min. One such way to dramatically speed up the GC analysis is by the use of resistive heating-gas chromatography (RH-GC) with flame ionisation detection (FID) [7-8]. RH-GC typically uses a short column (5 m) encased within a steel tube, which is

* Corresponding author. Tel.: +44 1904 462000; fax: +44 1904 462111.
E-mail address: r.fussell@csl.gov.uk (R.J. Fussell).

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connected to a power supply and heated resistively. The steel tube has high thermal conductivity and relatively low thermal mass allowing rapid ramping of temperatures, up to a maximum rate of 1200 °C min^{-1}, and also allows rapid cooling, for fast GC cycle times. Maštovská et al. [9] demonstrated that RH-GC was superior to fast temperature programming of short fused silica capillary columns, housed in a conventional GC oven. In the same study, 15 OP pesticides in cleaned-up sample extracts of wheat were analysed by RH-GC with nitrogen–phosphorous detection (NPD). There are relatively few reports of the use of RH-GC for the routine screening of pesticides in fruits and vegetables, probably because of the high risk of contaminating the RH-GC column. The RH-GC column is usually contained within a steel tube, hence if contaminated it cannot be trimmed as is routinely performed with conventional capillary columns. The usual approach to prevent contamination of the column is to undertake a thorough clean-up of sample extracts, in order to remove non-volatile matrix components before chromatographic analysis, but this negates the advantages of the speed of RH-GC. However, it has also been shown that a CarboFrit insert (porous carbon plug) in the GC liner [10] can be used to avoid the necessity for clean-up steps, thus making the method more suitable for rapid routine analysis by pesticide residue laboratories.

One disadvantage of the use of RH-GC with single channel detectors is the need for additional confirmation, usually by mass spectrometry, when a potential residue has been detected. The combination of RH-GC with quadrupole mass spectrometry has been optimised [11], however this required a compromise between scan rate and scan range. Ideally the time-of-flight mass spectrometer would be more suitable as it can provide sampling frequencies of up to 500 Hz [12], but are still very expensive and have not yet been implemented in many laboratories for routine analysis. In any case, confirmation of the identity of the very polar pesticides, methamidophos and acephate, using GC–MS has been found to be difficult [13]. Quantification of these polar OP pesticides has been reported recently using a method which requires ethyl acetate extracts to be solvent exchanged into methanol:water prior to LC–MS/MS analysis [14]. Because a clean-up step is not needed the extracts can be analysed directly. However, chromatographic cycle times of 20–30 min are required, therefore the LC–MS/MS method is more suitable for confirmatory analysis rather than rapid screening.

The aim of the present work was to optimise and validate RH-GC–FPD equipped with a liner containing a CarboFrit insert for rapid and routine screening of OP pesticides with MS confirmation of residues as necessary.

2. Experimental

2.1. Reagents and materials

Methanol (HPLC grade) and ethyl acetate (analytical reagent grade) were obtained from Fisher Scientific (Loughborough, UK). Anhydrous sodium sulfate, sodium hydrogen carbonate and ammonium acetate (all analytical grade) were also purchased from Fisher Scientific. CarboFrit inserts (for liner i.d. size >4 mm) were purchased from Thames Restek (Saunderton, UK).

Standards of organophosphorus pesticides (purity >98.0%) were purchased from Qm_x (Thaxted, UK) and LGC-Promochem (Teddington, UK). Triphenyl phosphate (purity >99.0%), used as internal standard, was obtained from Qm_x.

2.2. Standard solutions

Individual stock standard solutions (1000 μg ml^{-1}) were prepared in ethyl acetate. Two working standard mixtures (Mix 1 and Mix 2, refer to Table 1), containing 1 or 10 μg ml^{-1} of each pesticide in ethyl acetate, were prepared for use as spiking solutions.

2.3. Samples

Samples of organically-produced peach and sweet pepper were comminuted in the presence of dry ice and grape samples were comminuted at ambient temperature. The homogenised samples were subsequently used as blanks and in the preparation of spiked samples and matrix-matched standards, for recovery assays and calibration, respectively. Matrix-matched calibration standards were prepared by adding known quantities of standard (Mix 1 or Mix 2) to the corresponding blank sample extracts to prepare calibration standards at concentrations of 0.01, 0.025, 0.05, 0.1, 0.25, 0.375 and 0.5 μg ml^{-1}.

2.4. Extraction

A 30 g portion of homogenised sample was weighed into a 250 ml Duran Schott bottle and ethyl acetate (60 ml), anhydrous sodium sulphate (30–40 g) and sodium hydrogen carbonate (5–6 g) were added. For estimation of recovery, blank samples were spiked with 300 μl of a 1 or 10 μg ml^{-1} spiking solution. The bottles were placed in a water bath at 30 ± 3 °C for a minimum of 20 min, after which the samples were homogenised for 30 s using an ultra turrax homogeniser. The organic layer was filtered through solvent-washed cotton wool. Concentrated extracts were prepared by reducing the volume of an aliquot (5 ml) of the extract to <1 ml, under a stream of oxygen-free nitrogen. Triphenyl phosphate (TPP) internal standard (25 μl of a 10 μg ml^{-1}) was added and then the volume adjusted to 1 ml with ethyl acetate (to give a crop concentration of 2.5 g ml^{-1}) prior to FPD analysis. An aliquot (2 μl) of the extract was analysed by RH-GC–FPD and matrix-matched calibration standards were employed for all quantifications.

Ethyl acetate extracts (0.5 g crop ml^{-1}) were concentrated five-fold and solvent exchanged to methanol-water (50:50, v/v) prior to analysis using LC–MS/MS. No internal standard

Table 1
Recoveries and relative standard deviations of the pesticides at the two fortification levels in fruit and vegetable products by RH-GC–FPD for Mix 1 and Mix 2

	Pesticide	Peak ID no.	t_R (min)	Recovery (%) (R.S.D., %)					
				Spiking level (mg kg^{-1})					
				Peach[a]		Grapes[b]		Sweet peppers[b]	
				0.1	0.01	0.1	0.01	0.1	0.01
Mix 1	Dichlorvos	1	0.837	84 (4)	83 (9)	72 (7)	75 (9)	85 (4)	79 (5)
	Methamidophos	2	0.950	76 (5)	74 (6)	76 (11)	71 (7)	78 (4)	73 (5)
	Acephate	3	1.248	77(5)	76 (8)	75 (10)	70 (6)	76 (4)	73 (5)
	Cadusafos	4	1.339	87 (3)	80 (5)	77 (10)	75 (9)	94 (3)	91 (3)
	Omethoate	5	1.521	87 (10)	77 (11)	75 (10)	75 (9)	85 (5)	77 (7)
	Fonofos	6	1.574	89 (3)	81 (6)	78 (8)	76 (9)	93 (3)	88 (3)
	Monocrotophos	7	1.749	92 (6)	80 (10)	81 (7)	87 (13)	92 (3)	81 (11)
	Dimethoate	8	1.799	100 (8)	96 (19)[b]	81 (8)	78 (13)[d]	90 (3)	–
	Tolclofos-methyl	9	1.882	92 (3)	84 (5)	81 (7)	80 (8)	95 (2)	91 (5)
	Parathion-methyl	10	2.053	90 (2)	85 (5)	76 (9)	73 (11)	95 (2)	88 (4)
	Malathion	11	2.111	91 (4)	85 (5)	79 (9)	76 (10)	96 (2)	89 (6)
	Parathion-ethyl	12	2.271	93 (3)	85 (5)	81 (8)	78 (9)	94 (2)	90 (3)
	Quinalphos	13	2.370	92 (3)	85 (5)	80 (9)	78 (9)	99 (3)	87 (4)
	Prothiofos	14	2.516	94 (3)	88 (5)	87 (5)	86 (6)	97 (2)	88 (3)
	Methidathion	15	2.628	92 (2)	84 (4)	77 (9)	77 (9)	96 (3)	86 (5)
	Ethion	16	2.893	94 (3)	87 (5)	86 (6)	83 (7)	98 (3)	89 (6)
	Pyridaphenthion	17	3.364	91 (3)	84 (10)	79 (9)	81 (11)	100 (7)	81 (12)
	Azinphos-methyl	18	3.915	89 (7)	91 (15)[c]	75 (9)	82 (10)	–	–
Mix 2	Mevinphos	19	1.069	90 (4)	95 (9)	80 (3)	78 (5)	96 (4)	86 (3)
	Methacrifos	20	1.110	87 (3)	83 (5)	81 (4)	82 (5)	74 (6)	86 (5)
	Heptenophos	21	1.246	89 (4)	87 (7)	83 (3)	84 (6)	82 (6)	88 (3)
	Ethoprophos	22	1.292	90 (3)	87 (8)	84 (3)	85 (6)	81 (6)	91 (4)
	Diazinon	24	1.519	92 (2)	88 (6)	86 (3)	85 (6)	79 (6)	92 (5)
	Dicrotophos	25	1.557	92 (5)	87 (8)	81 (4)	85 (6)	91 (5)	93 (7)
	Etrimfos	26	1.614	92 (3)	89 (9)	86 (4)	85 (5)	79 (5)	92 (3)
	Chlorpyrifos-methyl	27	1.817	90 (4)	93 (20)[b]	84 (4)	91 (10)[d]	116 (14)	92 (6)
	Pirimiphos-methyl	28	1.916	94 (3)	89 (5)	86 (3)	86 (6)	84 (7)	91 (3)
	Chlorpyrifos	29	2.016	95 (2)	92 (6)	87 (3)	89 (6)	88 (5)	92 (3)
	Pirimiphos-ethyl	30	2.094	95 (2)	90 (6)	87 (3)	89 (5)	87 (6)	93 (2)
	Fenitrothion	31	2.164	95 (5)	88 (6)	85 (4)	86 (5)	83 (5)	88 (5)
	Bromophos-ethyl	32	2.348	95 (2)	93 (8)	88 (3)	91 (5)	94 (3)	89 (5)
	Chlorfenvinphos	33	2.382	95 (3)	90 (6)	86 (4)	87 (6)	85 (6)	87 (6)
	Tetrachlorvinphos	34	2.592	92 (5)	88 (10)	81 (7)	88 (9)	93 (14)	80 (7)
	Ethion	16	2.893	95 (2)	91 (5)	88 (3)	89 (5)	97 (8)	87 (9)
	EPN	35	3.360	92 (5)	92 (8)	88 (2)	92 (8)	91 (15)	83 (14)
	Phosmet	36	3.442	90 (10)	87 (12)	83 (2)	87 (13)	89 (9)	83 (9)
	Phosalone	37	3.630	90 (7)	93 (10)	85 (3)	90 (7)	86 (12)	81 (11)
	Pyrazophos	38	3.705	90 (8)	95 (10)	85 (3)	92 (6)	90 (10)	82 (12)

Note: Peak ID number 23 (expected t_R, 1.479 min) refers to naled, which was converted to dichlorvos.
[a] Mean of 24 determinations unless otherwise superscripted.
[b] Mean and R.S.D. of 12 determinations unless otherwise superscripted.
[c] Mean and R.S.D. of 18 determinations.
[d] Mean and R.S.D. of 6 determinations.

was added and, as for GC, matrix-matched calibration standards were employed for quantification.

2.5. Instrumental conditions

2.5.1. RH-GC–FPD

RH-GC experiments were performed using the Thermedics Detection EZ Flash upgrade kit installed in the oven of an Agilent 6890 gas chromatograph equipped with electronic pressure control (EPC), a split/splitless injector, a flame photometric detection (FPD) system and an Agilent-7683 autosampler (Agilent, Paulo Alto, CA, USA). A single gooseneck splitless liner with an internal diameter of 4 mm containing a single CarboFrit insert was used for all GC analyses. The data was processed using Agilent GC Chemstation Software, Version 8.03. The EZ Flash upgrade kit (ThermoElectron, MA, USA) comprised of a control module, EZ Flash GC column (5 m × 0.25 mm, 0.25 μm film thickness, RTX-1701 phase) and interface heaters for the injector and detector.

The following conditions were used for all RH-GC experiments: helium carrier gas at constant pressure (3.80 psi), equating to an average linear velocity of $48\,cm\,s^{-1}$, inlet temperature 200 °C, injection volume 2 μl (splitless), splitless time of 0.5 min, FPD detection (250 °C; air $100\,ml\,min^{-1}$, hydrogen $75\,ml\,min^{-1}$, make-up (nitrogen) $15\,ml\,min^{-1}$; data acquisition rate 20 Hz). The EZ Flash column temperature programme was: 60 °C initial, at 53 s, ramped at $158\,°C\,min^{-1}$ to 200 °C, at 153 s, $24\,°C\,min^{-1}$ to 240 °C, at 170 s, $141\,°C\,min^{-1}$ to 280 °C, hold for 88 s. The GC oven was ramped from 60 to 90 °C at $10\,min^{-1}$ and held for 1.3 min.

The GC temperature programme for analysis using a conventional oven and a DB-1701, 30 m × 0.53 mm i.d. column with a film thickness of 1 μm film thickness (J&W Scientific) was: initial temperature 100 °C followed by $20\,°C\,min^{-1}$ ramp to 200 °C (held for 3 min), $5\,°C\,min^{-1}$ ramp to 240 °C (held for 2 min) and a final ramp of $5\,°C\,min^{-1}$ to 280 °C (held for 8 min).

2.5.2. LC–MS/MS conditions

A Sciex API 2000 triple-quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada) was used with TurboIonspray™ (TIS) in positive mode for polar OP confirmation analysis. The ionisation source-specific parameters were: curtain gas, 50 arbitrary units (a.u.); ionspray voltage, 5000 V; heater gas, 380 °C; nebulizer gas (GS1), 40 a.u.; auxiliary or turbo gas (GS2), 80 a.u. Nitrogen was used as curtain gas, nebulizer gas, collision-activated dissociation (CAD) gas and auxiliary or turbo gas. Exhaust and curtain gas regulators were each set at 3.5 bar and the GS1/GS2 regulator was set at 6.5 bar.

SRM transitions were as follows (declustering potential (DP/V) and collision energy (CE/V) for all transitions are given in brackets): acephate 184 > 143 (DP 20, CE 15), 184 > 125 (DP 25, CE 25); methamidophos 142 > 125 (DP 50, CE 20), 142 > 94 (DP 50, CE 20); monocrotophos 224 > 193 (DP 20, CE 10), 224 > 98 (DP 20, CE 18); omethoate 214 > 183 (DP 20, CE 15), 214 > 155 (DP 20, CE 25); heptenophos 251 > 215 (DP 20, CE 15), 251 > 127 (DP 20, CE 20); mevinphos 225 > 193 (DP 20, CE 10), 225 > 127 (DP 20, CE 20); chlorpyrifos-methyl 322 > 125 (DP 30, CE 26), 322 > 290 (DP 30, CE 21) and chlorpyrifos 350 > 198 (DP 47, CE 24), 350 > 322 (DP 47, CE 16). The CAD gas was set at 3 a.u. and focussing potential at 350 V. Dwell times were 50 ms for each transition. A Hypurity Aquastar C18, 150 mm × 2.1 mm (5 μm particle size) column (Thermo Hypersil-keystone, Runcorn, UK) was used with a guard column (Phenomenex, Macclesfield, UK) and gradient elution (mobile phase A was 10 mM aqueous ammonium acetate, mobile phase B methanol). The mobile phase composition, initially 5% mobile phase B, was linearly increased to 95% B over 10 min, and then held for 2 min before returning to the initial conditions. Re-equilibration time was 3 min, flow rate $0.2\,ml\,min^{-1}$, and injection volume was 15 μl.

2.6. Method performance and quality control

Accuracy and precision of the extraction and RH-GC–FPD method were established by determination of six replicate recoveries at two spiking levels (0.01 and $0.1\,mg\,kg^{-1}$) for each of the three different crops. Only the peach validations included extraction on two different days. All extracts were analysed using duplicate injections and all validations were carried out on different days thus providing data for; peach day 1, peach day 2, grape day 3 and sweet pepper day 4. Ethion was included in Mix 1 and Mix 2 to cross check for variation in response and retention time on the four different days. In accordance with DG SANCO guidelines [15] the validation was considered acceptable if the mean recoveries were in the range 70–110% and the relative standard deviations (R.S.D.s) ≤20%. In addition to spiked samples a total of 18 samples (6 samples per matrix), some known to contain incurred residues, were analysed in duplicate.

2.6.1. Estimation of parameters describing method performance

An estimate of the relative standard deviation in reproducibility ($R.S.D._R$) for the RH-GC–FPD method was obtained by combining estimates of between-batch variation with estimates of the uncertainty associated with the apparent mean recovery, using methods described in the Eurachem Guide to Quantifying Uncertainty in Analytical Measurement [16] and the Harmonised Guidelines for Single Laboratory Validation of Methods of Analysis [17]. Measurement uncertainty is defined by ISO [18] as "a parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand". If, in this case, the measurand is treated as being equal to the quantity of pesticide that is extracted from the matrix using the procedures defined in the method (i.e. the usual practice of not correcting measurements of pesticide concentration for recovery is followed [19]) then the estimation of $R.S.D._R$ could also be treated as an estimation of relative measurement uncertainty. However, in order to avoid misinterpretation of results it is better to describe uncertainty estimates associated with the results of empirical methods simply as estimates of $R.S.D._R$ and to reserve the term 'measurement uncertainty' for cases where the measurand is the true (bias corrected) value of the quantity of analyte.

2.6.2. Estimation of between run standard deviation and uncertainty associated with mean recovery

Results from the analysis of pesticides in Mix 1 and Mix 2 (four batches, i.e. peach, day 1; peach, day 2; grape, day 3 and sweet pepper, day 4, thus three matrices represented, see Section 2.6) were analysed by ANOVA (with some pesticides removed; see Section 3.3) in order to gain an estimate of the size of the between-batch standard deviation associated with the measurement method. The between-batch standard deviation estimate also includes a contribution from between-matrix variation.

The uncertainty associated with the mean recovery was estimated using the assumption that if the study were repeated, the range of mean recoveries across analytes would remain constant, but the mean recovery associated with a particular analyte would be liable to change within that range. Hence, the uncertainty associated with mean recovery was estimated to be described by a flat distribution with minimum and maximum values given by the minimum and maximum observed mean recovery across analytes [16].

2.6.3. *Estimation of R.S.D.$_R$*

An estimate of the R.S.D.$_R$ associated with measurement of analytes in each mix at each concentration was obtained by combining the between-batch standard deviation associated with the measurement of each analyte with the uncertainty associated with the mean recovery (converted to a standard uncertainty [16]) for the analytes in each mix using the equation:

$$\text{R.S.D.}_R = \sqrt{s_b^2 + \frac{1}{12}(\bar{r}_{\max} - \bar{r}_{\min})^2}$$

where s_b is the R.S.D. associated with the results of the measurement of an analyte, and $\bar{r}_{\max}$ and $\bar{r}_{\min}$ are the maximum and minimum values of mean recovery displayed by the analytes in the mix (the divisor '12' comes from the conversion of a flat distribution to a standard uncertainty).

The fitness for purpose of the measurement method was assessed by producing HORRAT values from the R.S.D.$_R$ estimates using the modified Horwitz equation [20]. A commonly used criterion is that fit for purpose methods produce results with a HORRAT value less than 2.

3. Results and discussion

3.1. *RH-GC–FPD system optimisation*

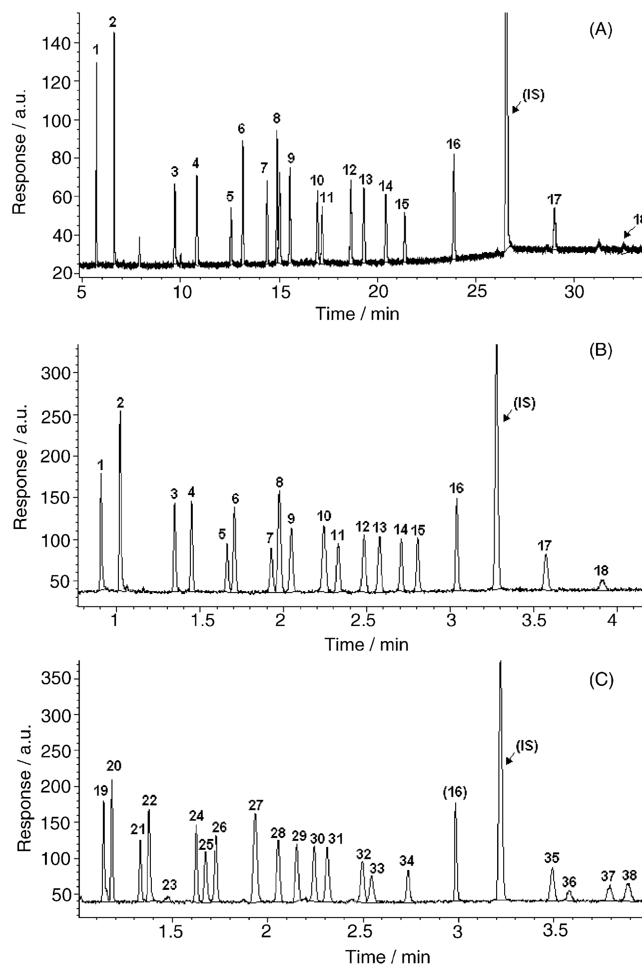

Fig. 1. GC–FPD chromatograms of peach: (A) conventional GC–FPD chromatogram; (B) RH-GC chromatogram, Mix 1 and (C) RH-GC chromatogram, Mix 2. Spiking concentration is 0.025 μg ml^{-1} (equivalent to 0.01 mg kg^{-1}) for each analyte, injection volume 2 μl of a 2.5 g crop ml^{-1} sample. IS refers to the internal standard. For peak identification refer to peak ID numbers column in Table 1.

The RH-GC system parameters were adjusted to achieve sufficient separation with rapid chromatographic times from Section 2.5.1. During optimisation it was observed that if all of the pesticides (Table 1) were included into a single mix, many were not baseline separated and lower temperatures gradients did not improve the separation, thus peak integration was not reliable. From a practical perspective to avoid partial co-elutions the 38 pesticides were subsequently divided into two standard mixtures, Mix 1 and Mix 2, containing 18 and 20 compounds respectively. In these mixtures, individual pesticides were fully resolved during RH-GC analysis (Fig. 1B and C). Four pesticides in Mix 1 have similar retention times to components in Mix 2 (peak ID numbers in parenthesis; see Table 1), acephate (3) and heptenophos (21), omethoate (5) and diazinon (24), quinalphos (13) and chlorfenvinphos (33) and pyridaphenthion (17) and EPN (35) and have been classified as critical pairs whereby their quantification by RH-GC–FPD are validated only when confirmed by MS. Chromatograms for Mix 1 using a conventional capillary GC–FPD system (maximum heating rate 20 °C min^{-1}; Fig. 1A) give the same elution order as RH-GC (maximum heating rate 158 °C min^{-1} see Section 2.5.1; Fig. 1B). Adequate resolution but with a significant decrease in the chromatographic run time is achieved; 34 min reduced to 4.3 min. Similar resolution is obtained for Mix 2 using the same RH-GC conditions (Fig. 1C). The cycle time (time between two injections) of the RH-GC–FPD method is still partly dependant on the oven cool down time (90–60 °C). A small gradient temperature oven program is recommended to prevent the GC oven shutting down as oven temperature increases due to heating from the RH-GC column. After the RH-GC column temperature program has finished the RH column uses the oven temperature to reach the start temperature (60 °C) for the next injection, thus a need to wait for the GC oven to stabilise. Nevertheless, the cycle time was approximately 6 min for RH-GC–FPD, whereas for conventional analysis this time was approximately 38 min.

In order to maintain stable retention times and chromatographic resolution it is important to prevent contamination of the GC system with non-volatile matrix co-extractives. The retention gap (∼4 cm) necessary to connect the RH-GC column to the injector offers only minimal protection. The effectiveness of a CarboFrit insert, or silanised glass wool placed in the injection liner, to retain non-volatiles was evaluated. A single CarboFrit insert permitted ∼70 injections (2 μl) of crude extracts (2.5 g crop ml^{-1}) in a single sequence of a 0.025 μg ml^{-1} concentration standard with no significant drift in retention time or any observable deterioration in chromatographic peak shape over the sequence. A glass wool plug proved less satisfactory, with retention time drift of >1 s observed for many of the pesticides. The build up of matrix components on the glass wool could account for the drift, resulting in longer transfer of the pesticides on to the column in later injections. The CarboFrit has a larger surface area to absorb matrix co-extractives and does not seem to suffer from such effects. The long-term stability of the retention times can be maintained by changing the CarboFrit insert for each new sequence (one sequence containing ∼70 injections). In addition, to maintain stable retention times for each new sequence the positioning of the CarboFrit insert needs to be consistent from liner to liner.

The two most critical factors in achieving satisfactory response and peak shape were found to be the injector temperature and initial column temperature (see Section 2.5.1 for conditions). The initial temperature of the column and injector temperature were optimised by observing the peak height and response as a function of temperature at 10 °C increments over the range 50–100 °C for the former and at 25 °C increments over the range 150–250 °C for the latter. For the early eluting pesticides, for example, dichlorvos and methamidophos, poor peak response and shape were observed when the initial RH-GC column temperature was set between 70 and 100 °C. The injector temperature was found to provide the highest responses, in terms of peak height, at 200 °C. Thus, an initial RH-GC column temperature of 60 °C and isothermal injector temperature of 200 °C were selected.

3.2. Calibration

The use of standards in solvent was evaluated to access if a single set of calibration standards could be utilised for several different matrices. Therefore eliminating the need to prepare different matrix-matched standards for each commodity type. The RH-GC calibration plot for methamidophos in solvent over the range 0.01–0.5 μg ml^{-1} (Fig. 2) shows slightly higher responses for matrix standards than for standards in solvent, consistent with previous reports [21]. Acephate behaved satisfactorily when injected in matrix, but gave no response when injected at low concentrations (<0.1 μg ml^{-1}) in solvent. This was not surprising as interaction of polar compounds with active sites on the inner walls of the glass liner and possibly the CarboFrit insert can be prevented by the build up of contaminants in the injection port and by the presence of matrix inhibiting access to these active sites. Calibration curves for matrix-matched standards for RH-GC and for LC–MS/MS were linear over the range 0.01–0.5 μg ml^{-1} (equivalent to 0.004–0.2 mg kg^{-1}), with correlation coefficients >0.980.

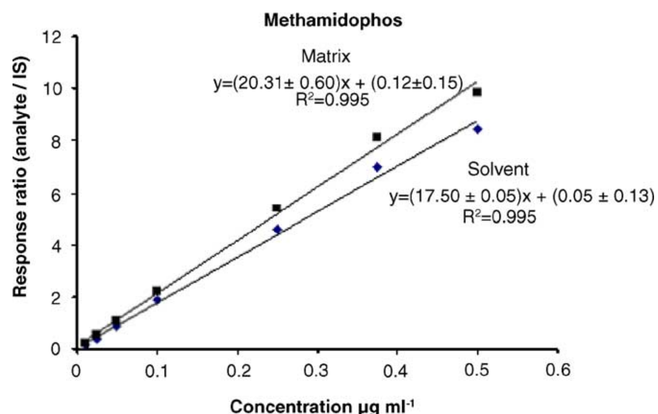

Fig. 2. RH-GC calibration plots over the range 0.01–0.5 μg ml^{-1}, (equivalent to 0.004–0.2 mg kg^{-1}) for methamidophos in matrix and in solvent standards.

3.3. Validation of the method

The mean recoveries of 37 pesticides at the two spiking levels determined using RH-GC–FPD with a CarboFrit insert are presented in Table 1. Extraction with ethyl acetate followed by a five-fold concentration allowed a reporting limit (RL) of 0.01 mg kg^{-1} for all 37 OP pesticides in peach and grape commodities and for 36 of the 37 OP pesticides in sweet peppers. Mean recoveries obtained by RH-GC–FPD for Mix 1 ranged from 70 to 100%, with R.S.D.s between 2 and 19%. Mean recoveries for Mix 2 ranged from 74 to 116%, with R.S.D.s between 2 and 20%. Thus, the DG SANCO criteria were met for the majority of pesticide-commodity combinations analysed. For the purposes of assessing method performance, results for dimethoate and chlorpyrifos-methyl were rejected when they suffered from the intermittent presence of an interfering compound, thus preventing these results being used in statistical analysis. The presence of such a compound should be detected by normal quality control procedures (analysis of blank sample alongside unknown samples) or problems in quantitation. Hence, such results would not normally be reported.

Adequate separation of the OP pesticides was achieved with good peak shapes and a signal-to-noise ratio of 5:1 or better for most of the pesticides at the lowest calibrated level (LCL). One exception was azinphos-methyl, which is known to be difficult to quantify at low mg kg^{-1} levels as the recovery is dependent on the pH of the extract and the chromatographic response on the condition of the GC inlet liner. Notably, the response for azinphos-methyl was consistently higher using RH-GC compared to GC–FPD (cf. Fig. 1), and the 0.01 mg kg^{-1} level was achieved for the peach and grape matrix, though not sweet peppers. The improvement in re-

sponse can be explained by the reduction in chromatographic peak width, which gave an increase in the peak height allowing azinphos-methyl to be quantified.

Naled, which has been reported to be unstable, was converted completely to dichlorvos using RH-GC, which is in agreement with previous reports [4]. The polar pesticides methamidophos and acephate gave reproducible but lower recoveries (∼70–80%) than the other pesticides (80–100%). Since linearity and response for these pesticides was good the lower recoveries are most likely due to the extraction method. The extent of partitioning of polar pesticides between aqueous and ethyl acetate phases during extraction was found to be highly temperature dependent. An increase in equilibration time from ∼20 to ∼30 min gave improved recoveries for acephate and methamidophos (85–90%).

The validation experiments also show the presence of an intermittent interferent, which co-eluted in Mix 1 with dimethoate and in Mix 2 with chlorpyrifos-methyl. The interferent did not always appear in the blank but occasionally resulted in exaggerated peak responses for the pesticides at the 0.01 mg kg^{-1} level. At the higher validation level, the interference was insignificant, and good recoveries were achieved for the two pesticides. This intermittent interference is possibly a result of contamination of labware or solvents. It has also been observed in previous work in our laboratories during analysis of these compounds using conventional GC–FPD systems. The evidence is consistent with the presence of a single contaminant which is difficult to eliminate. The method performance parameters reported for dimethoate and chlorpyrifos-methyl were calculated after the removal of affected results and are applicable to results of measurements for which the interference problem is not present.

For a sequence consisting of 20 injections (14 injections of standards and 6 recovery samples) within a peach validation batch, retention time repeatability expressed as a standard deviation was between 0.03 and 0.18 s for pesticides in Mix 1. For these pesticides, peak widths at half height (W_h) ranged from 0.57 s (dichlorvos) to 1.42 s (pyridaphenthion). The excellent retention time repeatability can be attributed to the efficient control of heating associated with resistive heating in combination with the use of a CarboFrit insert. With regard to the performance of the CarboFrit inserts an average of 60 (2 μl) injections of crude extracts (2.5 g crop ml^{-1}) in one sequence was performed per validation experiment (12 in total). No maintenance of the chromatographic system, apart from routine changing of the GC liner and septum, between chromatographic runs was necessary. Thus, the performance of the CarboFrit indicates that it is applicable for use in routine GC analysis of a large series of crude extracts.

The LC–MS/MS method was validated at 0.01 and 0.1 mg kg^{-1} for only the potential residues (Table 4) and selected others (Section 2.5.2) to show that the method was capable of providing confirmation in case required. All pesticides were in the range 70–110% with R.S.D.s <17% ($n = 5$), with the exception of chlorpyrifos and chlorpyrifos-methyl. Nevertheless, the LC–MS/MS method allowed the injection of ∼25 crude extracts for each validation analysis (6 in total) showing that it is capable of analysis of crude extracts, however the long term stability needs to be further evaluated. The important requirement of providing a confirmation method for acephate and methamidophos, which are difficult to quantify by GC–MS, was achieved.

3.3.1. *Results of method performance study*

Estimates of between-batch R.S.D.s (including between matrix variation), $R.S.D._R$s and resulting HORRAT ratios for the measurement of Mix 2 pesticides at approximately 0.01 mg kg^{-1} show the most variable results produced in the study (Table 2). Mean recoveries lie between 80 and 113%. Between-batch relative standard deviation lay between 0.9% (fenitrothion) and 21.7% (chlorpyrifos methyl). Estimated values of $R.S.D._R$ lie between 9.6% (fenitrothion) and 23.7% (chlorpyrifos methyl), give HORRAT value estimates between 0.44 and 1.08. Table 3 shows a summary of the method performance parameters for the pesticides in each mix at each concentration. Estimates for HORRAT values calculated from the estimates of $R.S.D._R$ show that the method is capable of producing results that are fit for purpose.

3.3.2. *Contributions to uncertainty not represented in the measurement results*

Measurement results were produced by the analysis of fortified samples. Hence, some contributions to uncertainty associated with the measurement method are not represented in the variation displayed by the results. The size of this contribution to uncertainty could be estimated (if it were significant) by comparing the variation associated with the measurement of fortified samples with the variation associated with the measurement of samples with incurred pesticides.

3.4. *Application of the method*

A number of samples from recent surveillance exercises were analysed to evaluate the performance of the optimised RH-GC method. Samples reported to contain no residues were included to check that the RH-GC method did not generate false positive results. The samples were screened against the two standard mixes, typically using the following sequence; Mix 1 followed by Mix 2, unknown samples, recovery at reporting limit (RL) for Mix 1, re-injection of unknown samples, recovery at RL for Mix 2, and finally re-injection of Mix 1 and Mix 2. The RH-GC screening method gave positive results for the pesticides summarised in Table 4. There was full agreement with LC–MS/MS in the samples found to contain acephate and/or methamidophos. For acephate, the mean difference between RH-GC and LC–MS/MS results was 0.005 mg kg^{-1} and the highest difference in any single result was 0.024 mg kg^{-1}. For methamidophos, the mean difference between RH-GC and LC–MS/MS results was 0.003 mg kg^{-1} and the highest difference in any single result was 0.009 mg kg^{-1}. This suggests that even though the GC method is designed for rapid qualitative screening,

Table 2
Estimates of between-batch relative standard deviation (including between matrix variation), $R.S.D._R$ and resulting HORRAT ratio for the measurement of Mix 2 pesticides at approximately 0.01 $mg\,kg^{-1}$

	R.S.D. (%)	Concentration ($mg\,kg^{-1}$)	Mean recovery (%)	$R.S.D._R$ (%)	HORRAT value[a]
Mevinphos	10	0.0092	91	13.8	0.63
Methacrifos	5	0.0080	80	10.7	0.49
Heptenophos	3.3	0.0084	85	10.1	0.46
Ethroprophos	4.8	0.0084	85	10.7	0.49
Diazinon	6	0.0084	85	11.3	0.51
Dichrotophos	5.7	0.0088	87	11.1	0.51
Etrimfos	8.5	0.0084	85	12.8	0.58
Chlorpyrifos-methyl	21.7	0.0104	113	23.7	1.08
Pirimiphos methyl	3.6	0.0088	87	10.2	0.46
Chlorpyrifos	2.9	0.0092	90	10.0	0.45
Pirmiphos ethyl	4.8	0.0088	89	10.7	0.49
Fenitrothion	0.9	0.0088	86	9.6	0.44
Bromophos-ethyl	2	0.0092	93	9.7	0.44
Chlorfenvinphos	4.3	0.0088	88	10.4	0.47
Tetrachlorvinphos	17.9	0.0088	89	20.3	0.92
Ethion	10.3	0.0092	92	14.0	0.64
EPN	18.5	0.0092	92	20.8	0.94
Phosmet	10.8	0.0088	87	14.4	0.65
Phosalone	9.7	0.0092	91	13.6	0.62
Pyrazophos	8.3	0.0092	93	12.6	0.57

[a] Modified Horwitz relative standard deviation at 0.01 $mg\,kg^{-1}$ = 22% HORRAT value = $R.S.D._R$/22.

Table 3
Summary of the method performance parameters for the pesticides in each mix at each concentration

Mix	Concentration ($mg\,kg^{-1}$)	Between-batch R.S.D. (%)		Mean recovery (%)		$R.S.D._R$ (%)		HORRAT value	
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
1	0.01	1.5	14.4	73	90	5.1	15.0	0.2	0.69
1	0.1	5.2	14.8	76	93	7.2	16.0	0.3	0.71
2	0.01	0.9	23.7	80	113	9.6	24.0	0.4	1.08
2	0.1	3.4	8.1	85	93	4.1	8.4	0.2	0.38

Table 4
Summary of results obtained from screening samples containing incurred residues by RH-GC–FPD and LC–MS/MS

Commodity	Sample[a]	Pesticide			
		Acephate Mix 1 RH-GC–FPD (LC–MS/MS[c]) Detected level ($mg\,kg^{-1}$)	or	Heptenophos[b] Mix 2	Methamidophos Mix1
Peach	1	0.020 (0.017)	or	0.037 (-)	0.007 (0.007)
	2	0.112 (0.088)	or	0.203 (-)	0.038 (0.040)
	3	0.080 (0.060)	or	0.146 (-)	0.032 (0.034)
	4	–		–	–
	5	0.033 (0.027)	or	0.057 (-)	0.012 (0.017)
	6	0.051 (0.046)	or	0.091 (-)	0.025 (0.025)
Grapes	2	0.043 (0.043)		0.065 (-)	–
Lettuce	1	–		–	–
	2	0.037 (0.050)	or	0.050 (-)	0.021 (0.030)
	3	0.015 (0.023)	or	0.016 (-)	0.010 (0.014)

Responses are quantitatively correct only if a single pesticide of the pair is present, as shown by LC–MS/MS.
[a] Six samples per commodity analysed, only samples with positives reported.
[b] Acephate (Mix 1) and heptenophos (Mix 2) have identical retention times.
[c] LC–MS/MS results are given in parenthesis to RH-GC–FPD results.

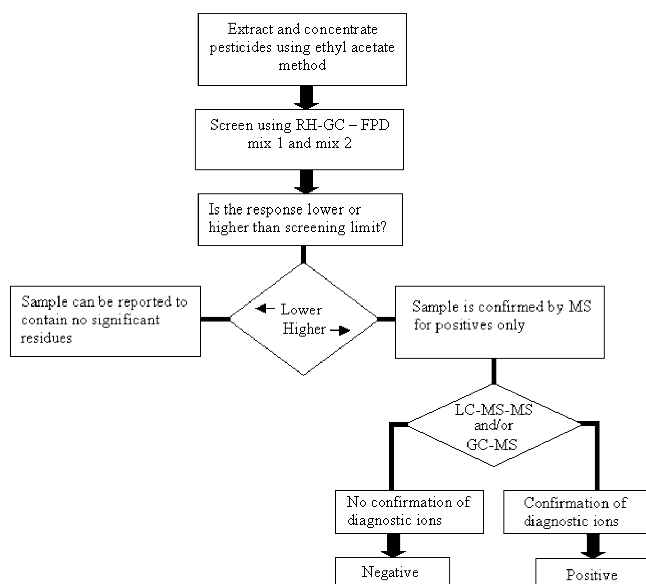

Fig. 3. Flowchart for the determination of the presence of positive residues by a combination of RH-GC–FPD screening and confirmation.

it also has the potential for quantification at levels near the RL. Results obtained by RH-GC for heptenophos are accounted for by acephate and heptenophos being a critical pair. Thus, if acephate is present in the sample a positive result will also be obtained for heptenophos. Consequently, the LC–MS/MS confirmation method needs to include both pesticides. Positive results for chlorpyrifos obtained by RH-GC (grape samples 1, 2 and 3, not shown in Table 4 at 0.035, 0.015 and 0.049 mg kg^{-1}) could not be confirmed by LC–MS/MS (poor method performance), but were in good agreement with GC–MS confirmatory analysis; 0.033, 0.0094 and 0.050 mg kg^{-1}. A flow-chart of the screening procedure is presented in Fig. 3. The results found above the limits permitted in the screening analysis and corresponding LC–MS/MS confirmation data is summarised in Table 4. An example of an incurred sample and a sample containing no residues above the 0.01 mg kg^{-1} level is shown in Fig. 4. The results from RH-GC and confirmation by MS are in good quantitative agreement.

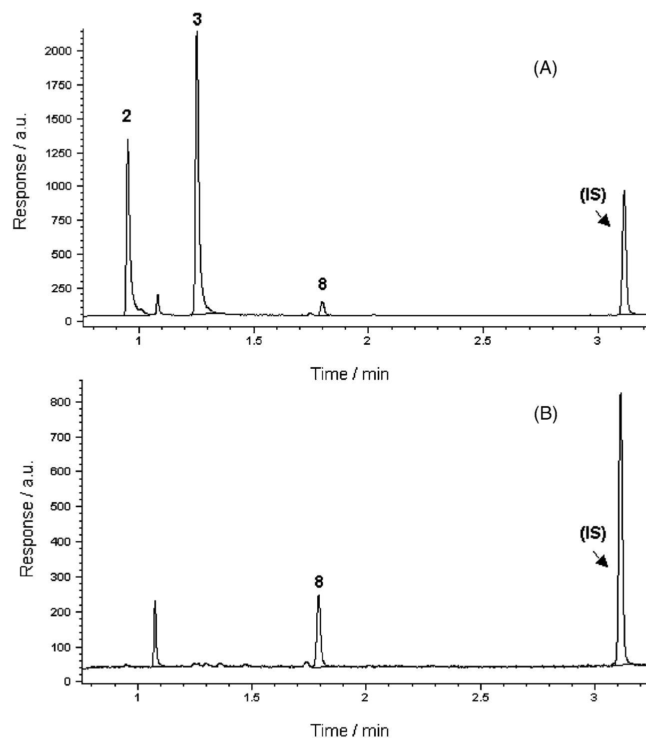

Fig. 4. Chromatogram (A) showing incurred residues (peach sample 3 from Table 4); acephate (peak ID 2) at 0.073 mg kg^{-1} and methamidophos (peak ID 3) at 0.030 mg kg^{-1}. Sample with no incurred residue at or above the 0.01 mg kg^{-1} level is shown in chromatogram (B) (peach sample 4 from Table 4): dimethoate (peak ID 8). *Note*: Peak at 1.09 min does not correspond to any OP pesticide t_R in this study.

4. Conclusion

A rapid and robust screening method for 37 OP pesticides has been validated for representative commodities. Statistical treatment shows that the method is capable of producing results that are fit for purpose and the method has been applied to the analysis of blank samples and those containing incurred residues. The robustness is attributed in large part to use of a CarboFrit insert to protect the RH-GC column. The use of two standard mixtures essentially allows for a more comprehensive screening method and there is also the possibility of using other standard mixes. Using this method 20 samples can be screened in ~3 h. Other advantages include the reduced requirements for clean-up, for solvent, for carrier gas and for laboratory space, meaning that laboratories can implement savings in capital and consumable costs. Using the EZ Flash upgrade kit, any conventional GC–FPD system can be easily adapted to allow RH-GC analysis. As long as a relevant method is available for confirmation of positive samples, either LC–MS/MS or GC–MS, positive results from the RH-GC screening method can be verified.

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