Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 982 (2002) 225–236
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Multi-residue method for rapid screening and confirmation of pesticides in crude extracts of fruits and vegetables using isocratic liquid chromatography with electrospray tandem mass spectrometry

Michael J. Taylor, Kenneth Hunter*, Kirsty B. Hunter, David Lindsay, Soazig Le Bouhellec

Scottish Agricultural Science Agency, East Craigs, Craigs Road, Edinburgh EH12 8NJ, Scotland, UK

Received 29 May 2002; received in revised form 30 September 2002; accepted 30 September 2002

Abstract

A LC–MS–MS method capable of the quantitative determination of a range of pesticide residues present in crude extracts from a variety of fruit and vegetables has been developed. Isocratic LC conditions have been used in conjunction with electrospray ionisation tandem mass spectrometry to detect and identify up to 38 pesticides presented as various mixtures in different matrices. The utility of the method is demonstrated by the analysis of crude extracts, with no sample clean up, from grape, kiwi fruit, strawberry, spinach, lemon, peach and nectarine. Mean recoveries ranging from 63 to 96% with relative standard deviations <20% were obtained for 30 of the 38 pesticides following analysis of organic produce fortified at concentrations between 0.01 and 0.8 mg/kg. Detected residues were quantified from interpolation against calibration data generated using matrix-matched standards that covered analyte concentration ranges between 0.005 and 0.8 μg/ml. Conditions suitable for the qualitative and quantitative confirmation of residues detected in samples are specified.

Keywords: Fruits; Vegetables; Food analysis; Pesticides

1. Introduction

Pesticides are applied to fruit and vegetables at various stages of cultivation and during post-harvest storage to provide protection against a range of pests, before they become available to the consumer. The formulations used have been developed with specific pesticidal properties that are dependent upon the inherent chemical functionality and physical characteristics of constituent chemicals. Consequently, pesticides from a range of compound classes are used in various combinations and perhaps at different times to impart the desired control effects. Statutory maximum residue levels for pesticides in foodstuffs have been defined in most countries to guarantee consumer safety and to regulate international trade [1,2]. The determination of pesticide residues in foodstuffs is then a requirement to support enforcement of legislation, to ensure trading compliance, and in the conduct of surveillance programmes to monitor residues in regional and national dietary components [3]. Analytical methodologies employed must be capable of residue measurement at very low

*Corresponding author.
E-mail address: ken.hunter@sasa.gsi.gov.uk (K. Hunter).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01610-2

levels and must also provide unambiguous evidence to confirm both the identity and the magnitude of any residues detected.

Extraction of pesticide residues from fruit and vegetables produces complex mixtures that have often required sample purification and preparation procedures to isolate the targeted pesticides for analysis. In addition, a multi-technique analytical strategy is often necessary to facilitate the quantitative determination of each pesticide residue due to the differences between their chemical and physical properties and incompatible detection techniques. The costs of labour and materials, and long turnaround times could be significantly reduced if sample preparation and clean-up procedures were eliminated and if a widely applicable chromatographic method was available. This is particularly true for the diverse range of pesticides that are not readily amenable to gas chromatography, where a common end-point would also allow more efficient use of high specification instrumentation such as triple stage mass spectrometers.

The use of high-performance liquid chromatography (HPLC) in hyphenation with atmospheric pressure ionisation mass spectrometry (API–MS) [4] has shown a sustained growth in pesticide residue analysis over recent years. Fernandez et al. [5] compared electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) in conjunction with single ion monitoring (SIM) for the determination of five fungicides in crude extract from oranges using gradient separation. APCI, matrix solid-phase dispersion and gradient HPLC separation have been combined for the multi-residue determination of five pesticides [6] and 13 carbamates [7] in a variety of matrices. Barnes et al. [8] combined the same ionisation technique, SIM and isocratic separation for the determination of various pesticides in crude extracts from strawberry and plum and for the determination of fenbutatin oxide in tomato, banana and cucumber [9]. Kim et al. [10] combined APCI and SIM with gradient separation for the simultaneous determination of carbamate and organophosphorus pesticides. The use of electrospray tandem mass spectrometry (ESI–MS–MS) and size exclusion chromatography to overcome adverse matrix effects associated with the determination of thiabendazole, carbendazim and phenylurea pesticides in fruit matrices was reported by Bester et al. [11]. Hogenboom et al. [12] used large volume injection of crude sample extract from carrot and potato, gradient separation and ESI–MS–MS for the quantitative determination of pesticides of different polarity.

Our laboratory had been routinely using reversed-phase gradient separations with tandem mass spectrometric detection for multi-residue analysis of crude extract, screening for a range of pesticides considered likely to be used on fruit and vegetables. The pesticide/commodity combinations involved in these particular studies represented part of the UK pesticide residue surveillance programme for 2001. However, it was often necessary to adapt the gradient separation or even develop novel gradients due to the different pesticide/matrix combinations encountered. In addition, the potential advantage of faster turnaround times, facilitated through the screening of crude extracts from large numbers of samples, could be compromised simply due to the fact that some gradients required significant equilibration times (up to 20 min between runs). This report presents details of a simplified approach that combines isocratic HPLC separation with electrospray tandem mass spectrometry for multi-residue analysis of crude extracts from grapes, kiwi fruit, lemons, peaches, nectarines, spinach and strawberries.

2. Experimental

2.1. High performance liquid chromatography

HPLC was performed using an 1100 Series liquid chromatograph system (Agilent Technologies, Stockport, UK). A 3-μm Hypersil C_{18} BDS column (100×4.6 mm), fitted with a guard cartridge (Security Guard, both Phenomenex, Macclesfield, UK), was operated at 35 °C and at a flow-rate of 0.5 ml/min. A post-column flow-splitting device was incorporated to deliver the column effluent into the mass spectrometer at approximately 20 μl/min [polyether ether ketone (PEEK) zero dead volume T-piece]. This was found to reduce ion-source maintenance

requirements, which are inevitable following analysis of large numbers of ''dirty'' samples over prolonged periods. The isocratic elution conditions employed used 30% solvent A and 70% solvent B, where A=10 m*M* aqueous ammonium acetate solution and B=methanol (solution pH 7.1 at 25 °C). Sample injection volumes of 5 or 10 μl were used throughout.

Alternative HPLC methods employed for confirmation purposes involved the use of a Hypersil HyPURITY Elite C_{18} 100×4.6 mm, 5 μm analytical column (ThermoHypersil-Keystone, Runcorn, UK) and the above guard cartridge, injection volumes and flow-splitting regime. Isocratic conditions used for the confirmation of (i) carbendazim and (ii) 2-phenylphenol residues were; (i) acetonitrile–water (50:50, v/v), column temperature of 35 °C, flow-rate of 0.6 ml/min and (ii) acetonitrile–water (70:30, v/v), column temperature of 25 °C and a flow-rate of 0.5 ml/min, respectively.

2.2. Mass spectrometry

API–MS detection was achieved using the Quattro Ultima tandem mass spectrometer (Micromass, Manchester, UK). The instrument was operated in positive or negative ion electrospray mode with up to 16 MS–MS transitions monitored during a typical isocratic separation using multiple reaction monitoring (MRM) and time-schedule sequencing. The single ion recording (SIR) scan function was used to monitor for 2-phenylphenol and to confirm residues of carbaryl in kiwi fruit. A dwell time of 0.5 s and a span corresponding to 0.2 dalton were used. Direct infusion of analytes for optimisation procedures was achieved using a syringe pump (Harvard Apparatus, Kent, UK). Argon of 99.9% purity (BOC, Manchester, UK) was used as collision-gas. A nitrogen generator (Peak Scientific, Renfrew, UK) and compressor system (Atlas Copco Compressors, Cumbernauld, UK) were used to supply nitrogen as the nebuliser, cone and desolvation gas. The optimum cone voltage and collision energy values were determined for each analyte (see Table 1). The most favorable gas pressures were set at universally applied values of approximately 500 l/h for desolvation gas flow, 80 l/h cone gas and $1.4 \cdot 10^{-3}$ mbar for the argon collision gas. The ion source was operated at 150 °C, the desolvation temperature was 350 °C and the capillary voltage was maintained at 3 kV.

2.3. Materials

Pesticide reference materials were obtained from Qm_x Labs, UK Ethyl acetate (Super Purity, Romil Chemicals, Loughborough, UK), methanol (HPLC grade, Rathburn, Walkerburn, UK) and high purity laboratory water (prepared in the laboratory) were used. Sodium hydrogen carbonate was obtained from Fischer Scientific (Loughborough, UK), and JT Baker (Milton Keynes, UK) supplied anhydrous sodium sulfate. Gelman PTFE HPLC syringe filters (0.45 μm) were obtained from Fischer Scientific.

2.4. Sample preparation

On initial receipt, each commodity was processed to generate a homogeneous and representative analytical sample that was frozen until required. The defrosted and thoroughly mixed sample (8 g) was combined with 70 g of sodium sulfate, 2 g of sodium hydrogen carbonate and 50 ml of ethyl acetate in a 150-ml beaker. An aliquot (1 or 4 ml) of appropriate standard mixture solution was added to an 8-g portion of organic produce before being combined with the sodium salts for subsequent determination of recovery. The mixture was homogenised for 2 min using an UltraTurrax T25 tissue disperser, the liquid layer was decanted through a Whatman No. 1 filter paper (18.5 cm) and the filtrate collected in a round-bottom flask (150 ml). Another 50 ml of ethyl acetate were added to the residual material in the beaker. This was re-homogenised for a further 2 min, filtered and the filter-cake rinsed with ethyl acetate. All filtrates were collected in the round-bottom flask.

The crude extract was evaporated to low volume (~2 ml) by rotary evaporation (water bath temperature did not exceed 30 °C). Methanol (10 ml) was added to the flask and the solution was re-evaporated to ~2 ml. A further 10 ml of methanol was added, the solution again evaporated to ~2 ml, then transferred quantitatively to a 20-ml volumetric flask and made up to volume with methanol (≡ a concentration of 0.4 g sample per ml). An aliquot of this

Table 1
MS–MS transitions used for screening and instrument conditions

Pesticide (Precursor ion assignment)	MSMS transition	Cone voltage (V)	Collision energy (eV)
2,4-D $[M-H]^-$	219→161	30	10
Aldicarb $[M+Na]^+$	213→89	25	25
Aldicarb sulfone $[M+NH_4]^+$	240→86	25	20
Aldicarb sulfoxide $[M+H]^+$	207→89	25	25
Azoxystrobin $[M+H]^+$	404→372	20	15
Bendiocarb $[M+H]^+$	224→167	34	10
Butocarboxim $[M+Na]^+$	213→75	30	15
Butocarboxim sulfone $[M+Na]^+$	245→130	25	20
Butocarboxim sulfoxide $[M+H]^+$	207→75	30	15
Carbaryl $[M+H]^+$	202→145	20	15
Carbendazim $[M+H]^+$	192→160	35	15
Carbofuran $[M+H]^+$	222→165	27	15
Carbofuran 3-hydroxy $[M+H]^+$	238→181	10	15
Dichlofluanid[a] $[M+H]^+$	333→224+335→226	20	15
Diethofencarb $[M+H]^+$	268→226	19	7
Ethiofencarb $[M+H]^+$	226→107	26	10
Fenhexamid $[M+H]^+$	302→97	25	25
Furathiocarb $[M+H]^+$	383→195	26	15
Imazalil $[M+H]^+$	297→159	42	30
Isoprocarb $[M+H]^+$	194→95	26	11
Kresoxim-methyl[a] $[M+H]^+$	314→206+314→222	23	10
Methiocarb $[M+H]^+$	226→109	25	10
Methiocarb sulfone $[M+H]^+$	258→226	25	10
Methiocarb sulfoxide $[M+H]^+$	242→185	25	10
Methomyl $[M+H]^+$	185→128	27	10
Metolcarb $[M+H]^+$	166→109	34	10
Myclobutanil $[M+H]^+$	289→70	25	20
Oxamyl $[M+H]^+$	242→72	21	20
Penconazole $[M+H]^+$	284→159	30	30
2-Phenylphenol $[M-H]^-$	169 (SIR)	21	n/a
Propiconazole $[M+H]^+$	342→159	25	30
Pymetrozine $[M+H]^+$	218→105	24	28
Pyrimethanil $[M+H]^+$	200→107	30	28
Tebuconazole $[M+H]^+$	308→70	30	50
Thiabendazole $[M+H]^+$	202→175	30	20
Thiodicarb $[M+H]^+$	355→88	25	20
Thiophanate-methyl $[M+H]^+$	343→151	28	24
Trifloxystrobin $[M+H]^+$	409→186	20	25

[a] Summed transitions; n/a=not applicable.

solution was then filtered into an autosampler vial using a disposable 1-ml syringe and a HPLC syringe filter.

2.5. Standard preparation

Stock solutions of individual pesticides were prepared in methanol with aliquots taken to compose mixtures specific to the various pesticide/commodity combinations at concentrations ranging between 1 and 20 μg/ml. Serial dilutions using methanol produced a range of standard mixture solutions with pesticide concentrations ranging between 0.01 and 1.6 μg/ml. Aliquots of these solutions were subsequently admixed with blank matrix solution (obtained following extraction of organic produce prepared at a concentration of 0.5 g sample per ml) to produce a range of matrix-matched standards at

pesticide concentrations equivalent to 0.5, 1, 2 and 4 times the target reporting level, i.e 0.01–0.8 mg/kg. Fortification was achieved by the addition of 1 or 4 ml of the 0.01–1.6 μg/ml standard mixture solution to a portion (8 g) of organic produce to obtain concentrations equivalent to 0.01–0.8 mg/kg. This was done in duplicate or in triplicate at each level for evaluation of method accuracy.

Two standard mixture solutions were prepared to separate thiodicarb and methomyl sought in kiwi fruit in order to avoid interference from these interrrelated compounds during method validation. Thiodicarb was part of a mixture containing butocarboxim, butocarboxim sulfone, butocarboxim sulfoxide, ethiofencarb and kresoxim methyl.

3. Results and discussion

Target reporting levels are generally set below the available MRL of each pesticide in a particular commodity or at the level at or about the limit of determination as specified by the Codex Alimentarius Commission and in the UK Statutory Instrument for Pesticides Maximum Residue Levels [1,2]. These levels vary according to the pesticide/commodity combination. For example, the MRL is 0.02 mg/kg for imazalil in spinach and 5 mg/kg for the same compound in lemon. The reporting levels set by the UK Pesticide Residue Committee for the 2001 UK pesticide residue monitoring programme ranged from 0.01 to 0.2 mg/kg for the particular pesticide/commodity combinations and defined the scope of this study. The lowest calibration level (LCL) of each pesticide was prepared at a concentration equivalent to 50% of the relevant reporting level and did not necessarily correspond to the limit of detection for a particular pesticide/commodity combination. All of the pesticides listed in Table 1, excluding 2,4-D (free acid) and 2-phenylphenol, yielded ions characteristic of the molecular mass of the neutral molecule (M) i.e. $[M+H]^+$, $[M+NH_4]^+$ or $[M+Na]^+$ in positive ion electrospray mode. Both 2,4-D and 2-phenylphenol yielded molecular anions, i.e. $[M-H]^-$, in negative ion electrospray mode. Structurally diagnostic product-ions were produced for all compounds when subjected to collision-induced dissociation (CID) using argon, with the exception of the candidate 2-phenylphenol pre-cursor ion, which remained intact. Consequently, it was necessary to use SIR to screen for this compound. Screening of all other compounds present in each mixture was achieved by the simultaneous monitoring of characteristic precursor ion→product-ion transitions (i.e. MRM).

The LC–MS–MS ion-chromatograms of 16 pesticides sought in peach, at concentrations ranging from 0.04 to 0.80 μg/ml, are shown in Fig. 1 and are typical of the data obtained using this method. The time-scheduled data acquisition sequence involved three sets of 2, one set of 4 and one set of 6 MRM channels. These chromatograms demonstrate how the enhanced selectivity afforded by MS–MS detection allowed discrimination between target pesticides that were marginally separated under isocratic conditions. Interference from co-extracted plant material can also be reduced significantly in comparison with single ion recording (SIR). This is conveniently illustrated in more detail in Fig. 2, which compares SIR and MRM monitoring of thiabendazole and carbaryl in lemon matrix. A particular feature of MS–MS detection, which was exploited in these studies, was the capability to differentiate between isobaric analytes, especially those that co-elute. Differences in the product-ion mass spectra yielded by common pre-cursor ions were decisive in the unhindered determination of the following pairs of pesticides: (i) aldicarb and butocarboxim (ii) aldicarb sulfoxide and butocarboxim sulfoxide and (iii) carbaryl and thiabendazole. The product-ion mass spectra of carbaryl and thiabendazole are shown in Fig. 3 and illustrate this feature.

Calibration curves, derived from ion-chromatogram peak area measurements from matrix-matched standards, were obtained for all analytes and displayed good linearity over the selected concentration range with linear regression correlation coefficients (r) better than 0.99 achieved routinely ($n=4$). The use of matrix-matched calibration standards was necessary to compensate for signal suppression/enhancement of target analytes in matrix solution compared to their response in pure solvent [8]. Several workers minimised such effects via the addition of different buffers to the mobile phase, avoiding column overload and two-dimensional LC [13,14] or by optimisation of eluent flow-rate [15].

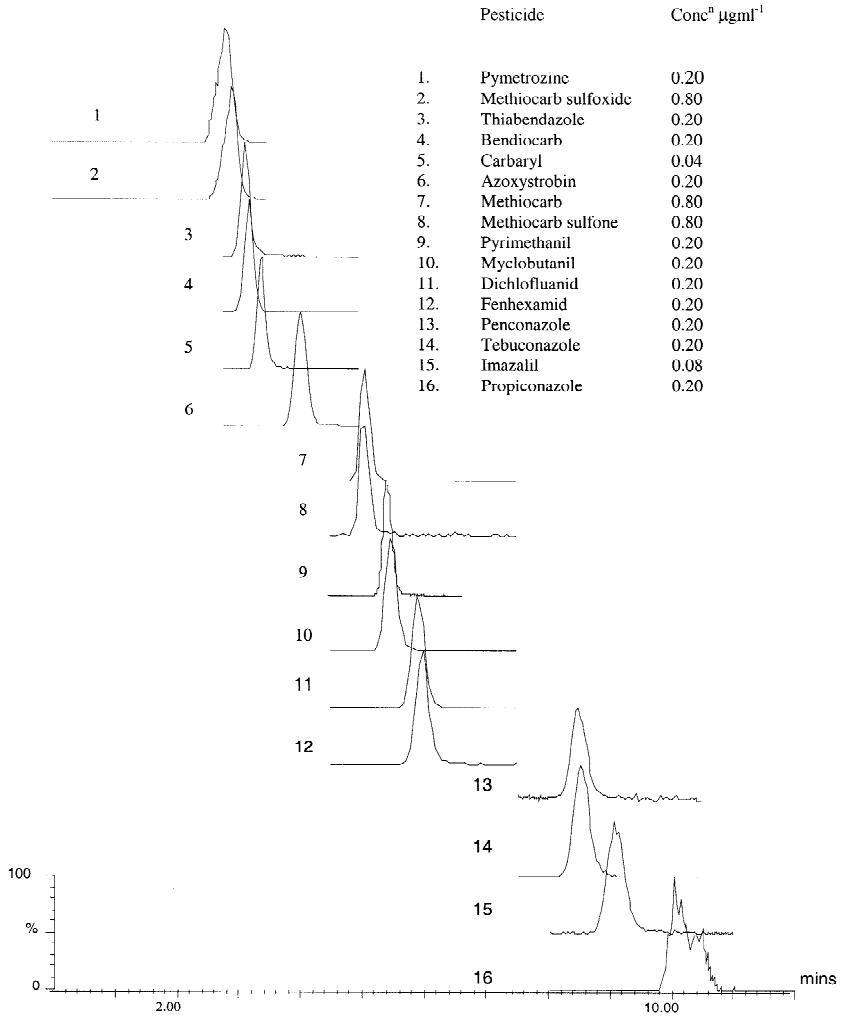

Fig. 1. LC–MS–MS ion chromatograms of pesticides sought in peach. Matrix-matched standard containing 16 pesticides at concentrations between 0.04 and 0.8 μg/ml. One set of 4, one set of 6 and three sets of 2 MRM channels.

Signal suppression of target analytes observed in this study however, did not inhibit their detection at the LCL. It was also important to recognise that the dynamic range in electrospray ionisation can be limited and generally depends on the properties of the analyte itself and the presence of other ionisable material [4]. Consequently, standards were prepared at concentrations that covered ranges of linearity and most importantly, which encompassed targeted reporting levels. For example, it was necessary to perform a second order regression (quadratic) to best fit calibration points for imazalil and thiabendazole over concentration ranges of 0.05–2.0 and 0.0125–5.0 μg/ml, respectively, and slightly curved calibration lines were generated.

Table 2 shows detailed recovery data for 16 pesticides targeted in organic peach that had been fortified at levels equivalent to 0.01–0.8 mg/kg. Mean recoveries were higher than 70% with the exception of methiocarb sulfone, which was 64%. In addition, the relative standard deviation of 43.2% determined for methiocarb sulfoxide was indicative of the irregular recoveries obtained for methiocarb and its' metabolites throughout this study. Prelimin-

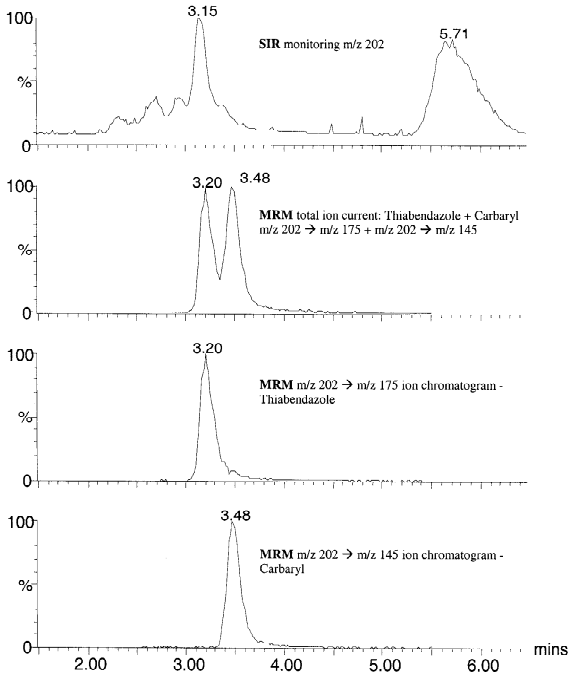

Fig. 2. Example of the enhanced selectivity afforded by tandem mass spectrometry (MRM) over the SIR technique using isocratic conditions. Signals obtained from a lemon matrix-matched standard containing 0.20 μg/ml thiabendazole and 0.04 μg/ml carbaryl (isobaric pre-cursor ions at m/z 202).

ary HPLC–UV, LC–MS (full-scan) and LC–MS–MS investigations however, indicated the presence of phenolic derivatives in aqueous mobile phase standard stock solutions of methiocarb sulfone and that this instability could be responsible for erroneous recovery measurements. Phenolic derivatives have been used to determine the amount of methiocarb and metabolites in rice plant using GC–flame photometric detection [16]. Further evaluation of the stability of methiocarb and metabolites in aqueous solutions and inclusion of phenolic derivatives could facilitate their determination by isocratic LC–ESI–MS–MS. An individual recovery result was excluded when it was significantly beyond the 60–140% range [17] and where subsequent investigations had revealed an underlying cause. Consequently, in Table 2, $n=5$ for methiocarb and its metabolites (complete degradation in solution suspected) and $n=5$ for dichlofluanid (interference from electronic spike).

Ethyl acetate was selected as extraction solvent because it had been used successfully in our laboratory to extract many of the pesticides involved here from other fruit and vegetables for analysis by gradient LC–MS–MS. It was also in routine use to extract pesticides traditionally analysed using GC-based techniques, e.g. organochlorines, organophosphates or pyrethroids. This meant that the procedure could be readily incorporated as part of an integrated analytical strategy. Acceptable matrix-matched calibration of aldicarb, butocarboxim, methiocarb and their metabolites, 2,4-D free acid, and thiophanate-methyl was readily achieved using this method however, the combination of extraction protocol, analyte instability in solution, degradation and possible matrix effects contrived to give inferior or irregular recoveries for these analytes under prevailing conditions. This behavior was prohibitive for the determination of ethiofencarb in kiwi fruit and for 2,4-D in lemon and investigation of alternative extraction procedures is required for application of this method. Attempts to measure the recovery of free thiophanate-methyl and as carbendazim were unsuccessful, although deliberate manipulation of experimental conditions to promote/control the conversion of thiophanate-methyl to carbendazim was not performed. Consequently, ion chromatographic data was considered as qualitative only for these particular compounds.

The recovery of aldicarb (72%), aldicarb sulfone (54%) and aldicarb sulfoxide (68%) from fortified organic lemon appeared to be related to instability in solution although associated RSDs, of 6.8, 4.2, and 22.2%, respectively, were considered satisfactory. Higher recoveries were obtained for each compound when the analysis of fortified extracts, included in subsequent analysis of sample batches to assess the analytical performance, was performed within hours of sample preparation although data supporting this behaviour was limited. The recoveries of butocarboxim, butocarboxim sulfone and butocarboxim sulfoxide from fortified organic lemon were greater than 70% but higher RSDs (15–26%) indicated the variability involved and similar instability in solution with aldicarb and metabolites was observed. This apparent analysis-time dependency will be investigated in more detail.

Summarised recovery and precision data for the remaining pesticides/commodity combinations are

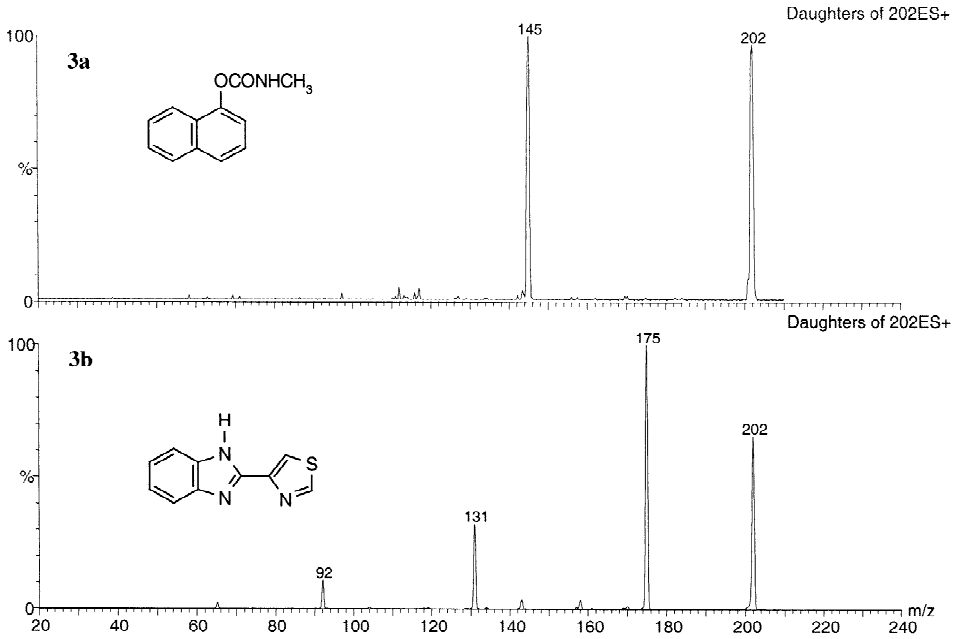

Fig. 3. Product-ion mass spectra of carbaryl (a) and thiabendazole (b). Highlighting the differences used to discriminate between isobaric species that were marginally separated under isocratic conditions. Carbaryl is characterised by the loss of methylisocyanate, CH_3NCO, (Δ57) and thiabendazole is characterised by the loss of HCN (Δ27) from the common precursor ions at m/z 202 to yield relatively intense product-ions at m/z 145 and m/z 175, respectively.

Table 2
Recovery and precision data obtained for 16 pesticides sought in fortified organic peach

Pesticide	Fortification levels (mg/kg)	Mean recovery[a] (%)	RSD	n	Range
Azoxystrobin	0.20 and 0.05	78	9.7	6	70–86
Bendiocarb	0.20 and 0.05	79	8.7	6	70–86
Carbaryl	0.04 and 0.01	78	7.7	6	68–87
Dichlofluanid	0.20 and 0.05	84	9.7	5	60–93
Fenhexamid	0.20 and 0.05	79	5.9	6	72–84
Imazalil	0.08 and 0.02	77	7.5	6	67–87
Methiocarb	0.80 and 0.20	71	10.1	5	59–78
Methiocarb sulfone	0.80 and 0.20	64	17.5	5	55–76
Methiocarb sulfoxide	0.80 and 0.20	96	43.2	5	41–147
Myclobutanil	0.20 and 0.05	77	11.4	6	67–86
Penconazole	0.20 and 0.05	77	12.1	6	66–88
Propiconazole	0.20 and 0.05	80	9.9	6	71–91
Pymetrozine	0.20 and 0.05	73	6.1	6	66–79
Pyrimethanil	0.20 and 0.05	84	8.4	6	77–88
Tebuconazole	0.20 and 0.05	78	11.3	6	68–89
Thiabendazole	0.20 and 0.05	78	9.5	6	68–86

[a] Mean recovery determined from all six fortifications except when $n=5$ (incl. 2×lowest level). RSD = relative standard deviation (%).

shown in Table 3. Mean recoveries for the majority of pesticide/commodity combinations were greater than 70%, or in the case of kresoxim-methyl (69%) and imazalil (68%) in spinach, methomyl (67%) and metolcarb (69%) in kiwi fruit just below this value. Lower mean recoveries were recorded for carbendazim in lemon and kiwi fruit (both 66%), trifloxystrobin in spinach (65%) and 2-phenylphenol in lemon (63%). Associated RSDs were less than 20% with the exception of aldicarb sulfoxide and butocarboxim in lemon (22.2 and 25.7%). In general, the recovery and repeatability data are in accordance with EU guidelines for pesticide residue analysis [18]. Many of the recoveries were within 70–80% which suggests some systematic error. Nunes et al. [19] noted the influence of the detection technique upon the determination of recovery following a comparison between LC–APCI–MS and LC–fluorescence detection. The latter technique gave superior recoveries following analysis of the same chromatographic extracts during studies involving the determination of residues of aldicarb and metobolites in potato, orange and tomato. Reasons for this behaviour however, were not discussed. It is possible that refinement of extraction procedures employed in our studies, such as reducing the amount of sodium sulfate used, could identify the source(s) of any systematic error.

Table 3
Recovery data for remaining pesticide/commodity combinations

Grape[a]	Kiwi fruit[a]	Lemon[b]	Spinach[b]	Strawberry[b]
Azoxystrobin	Azoxystrobin	Aldicarb	Azoxystrobin	Azoxystrobin
(83, 9.3)	(78, 6.3)	(72, 6.8)	(74, 8.0)	(75, 11.2)
Dichlofluanid	Bendiocarb	Aldicarb sulfone	Carbaryl	Carbaryl
(78, 4.5)	(73, 8.1)	(54, 4.6)	(78, 7.2)	(80, 10.1)
Imazalil	Carbaryl	Aldicarb sulfoxide	Imazalil	Imazalil
(73, 11.2)	(81, 16.8)	(68, 22.2)	(68, 4.9)	(73, 11.2)
Thiabendazole	Carbendazim	Azoxystrobin	Kresoxim-methyl	Kresoxim-methyl
(92, 17.7)	(66, 13.2)	(72, 11.5)	(69, 8.8)	(79, 8.2)
	Carbofuran	Butocarboxim	Myclobutanil	Myclobutanil
	(70, 2.9)	(72, 25.7)	(75, 8.1)	(77, 10.5)
	Carbofuran-3OH	Butocarboxim sulfone	Penconazole	Penconazole
	(73, 12.6)	(77, 15.1)	(78, 7.8)	(81, 4.1)
	Diethofencarb	Butocarboxim sulfoxide	2-phenylphenol	2-phenylphenol
	(79, 7.0)	(96, 18.8)	(70, 5.1)	(70, 5.1)
	Furathiocarb	Carbaryl	Pyrimethanil	Pyrimethanil
	(76, 3.8)	(72, 7.5)	(81, 6.8)	(76, 7.0)
	Isoprocarb	Carbendazim	Thiabendazole	Thiabendazole
	(75, 8.1)	(66, 12.9)	(79, 7.2)	(76, 14.7)
	Kresoxim-methyl	Imazalil	Trifloxystrobin	Trifloxystrobin
	(87, 10.1)	(74, 14.6)	(65,7.5)	(70, 8.8)
	Methomyl	2-Phenylphenol		
	(67, 10.9)	(63, 12.1)		
	Metolcarb	Pymetrozine		
	(69, 7.1)	(74, 2.2)		
	Oxamyl	Thiabendazole		
	(86, 16.0)	(72, 8.5)		
	Thiabendazole	Trifloxystrobin		
	(75, 15.7)	(72, 6.3)		
	Thiodicarb			
	(87, 10.2)			
	Trifloxystrobin			
	(76, 2.6)			

Mean recovery and relative standard deviation in parentheses. Fortification range = 0.01–0.8 mg/kg.
[a] $n = 6$ each level in triplicate.
[b] $n = 4$ each level in duplicate.

Table 4
LC–MS parameters used for confirmation of residues indicated following analysis of sample extracts

Pesticide	Screen method	Confirmation method
Azoxystrobin	m/z 404→m/z 372	m/z 404→m/z 344
Carbaryl	m/z 202→m/z 145	SIR m/z 202
Carbendazim	m/z 192→m/z 160	m/z 192→m/z 160[a]
Imazalil	m/z 297→m/z 159	m/z 297→m/z 69
Myclobutanil	m/z 289→m/z 70	m/z 291→m/z 70
Penconazole	m/z 284→m/z 159	m/z 284→m/z 70
2-Phenylphenol	SIR m/z 169	SIR m/z 169[b]
Pyrimethanil	m/z 200→m/z 107	m/z 200→m/z 82
Tebuconazole	m/z 308→m/z 70	m/z 308→m/z 125
Thiabendazole	m/z 202→m/z 175	m/z 202→m/z 131

[a] Isocratic acetonitrile–water (50:50, v/v), C_{18} Elite column 100×4.6 mm, 5 μm.
[b] Isocratic acetonitrile–water (70:30, v/v), C_{18} Elite column 100×4.6 mm, 5 μm.

Samples of each commodity were obtained from various retail outlets, prepared for analysis as described above and screened for the presence of target pesticides using isocratic LC–ESI–MS–MS. Existing internal quality control procedures recommend quantitative and qualitative confirmation of positive results where indicated at or above the target reporting level following screening experiments. This was achieved by the use of alternative MS–MS transitions for confirmation of residues of azoxystrobin, imazalil, myclobutanil, penconazole, pyrimethanil, tebuconazole and thiabendazole detected in various samples.

Since alternative transitions were not of sufficient intensity for confirmation of residues of carbendazim and carbaryl, a different isocratic HPLC method and the same characteristic transition was used for confirmation of carbendazim. Confirmation of carbaryl residues however, was achieved using SIR and the original isocratic HPLC method, which was possible due to the absence of thiabendazole in the sample. SIR was used simply for expediency although it would have been more appropriate to monitor the same transition using different HPLC conditions. Residues of 2-phenylphenol were confirmed by the use of SIR and an alternative isocratic HPLC method. Table 4 contains LC–MS–MS methods used for confirmation of residues detected in samples.

This approach is demonstrated by the determination of azoxystrobin residues in a grape sample. The product-ion mass spectrum of azoxystrobin is shown in Fig. 4 (structure inset). Product-ions at m/z 372 and m/z 344 correspond to the loss of methanol and methylformate neutral moieties, respectively, from the $[M+H]^+$ precursor ion at m/z 404 (relative molecular mass=403, $C_{22}H_{17}N_3O_5$). Fig. 5 contains ion chromatograms generated following screening (m/z 404→m/z 372) and confirmation (m/z 404→

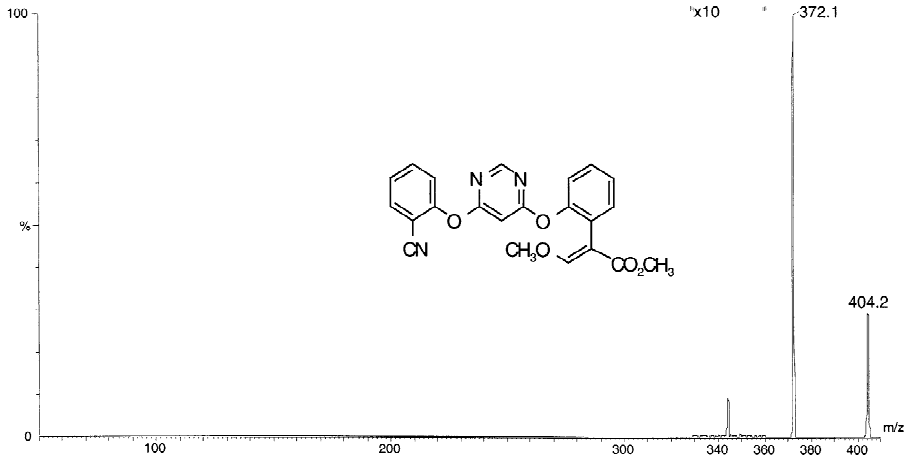

Fig. 4. Product-ion mass spectrum of azoxystrobin. $[M+H]^+$ precursor ion at m/z 404 (structure inset). Product-ions at m/z 372 and m/z 344 correspond to the loss of methanol and methylformate neutrals, respectively.

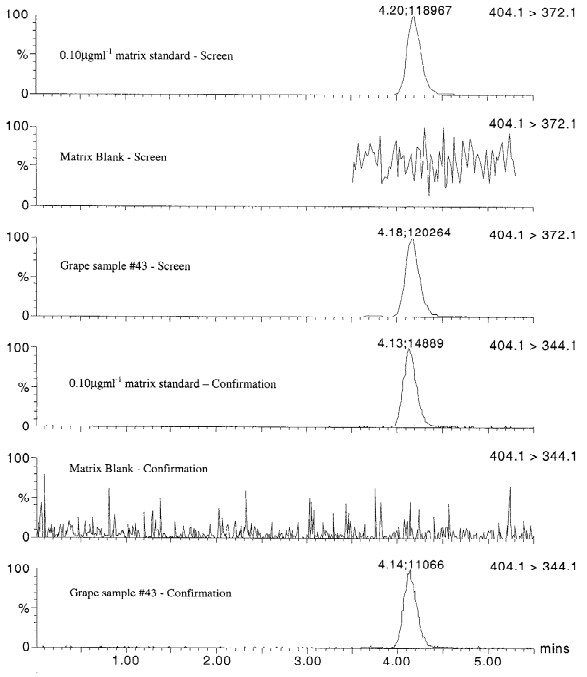

Fig. 5. Determination of azoxystrobin residues in a grape sample. Comparison of ion chromatograms obtained following screen (m/z 404→m/z 372) and confirmation (m/z 404→m/z 344) of ethyl acetate extracts.

m/z 344) experiments of sample extract, matrix blank and nearest matrix-matched calibration standard. The actual residue levels determined were 0.09 μg/ml (≡0.23 mg/kg in produce) and 0.08 μg/ml (≡0.20 mg/kg in produce) for screen and confirmation, respectively. Details of the comparison between screening and confirmation results obtained for different pesticide residues detected in various samples are shown in Table 5 along with the relevant MRL and target reporting level. A single fortified extract was included in the analysis batch to facilitate assessment of analytical performance. The results obtained were within ±20% and satisfied laboratory quality control confirmation criteria for quantitative and qualitative results of residues at these levels.

The method provided substantial efficiency gains particularly when compared to the variety of GC-based techniques, HPLC–UV or HPLC–fluorescence methods used previously in our laboratory to analyse for many of the pesticides involved in this study. The use of a generally applicable isocratic separation combined with MS–MS detection also reduced analysis cycle times compared to gradient LC–MS–MS simply by eliminating the need for post-run column equilibration. For example, previous gradient multiresidue analysis of a pesticide mixture where tebuconazole was the last analyte to elute took 18 min. Analysis of the same mixture takes only 10 min using isocratic conditions. The use of the isocratic method also minimised the frequency of method adaptation/development experienced with gradient separations.

Table 5
Comparison of screening[a] and confirmation[b] results obtained following analysis of a batch of samples using isocratic LC–MS–MS method

Analyte	Commodity	RL/MRL (mg/kg)	Spike (mg/kg)	Recovery (%)		Residue level[c] (mg/kg)	
				Screen	Confirmation	Screen	Confirmation
Azoxystrobin	Grape	0.05/2.0	0.20	72	74	0.23	0.23
Carbaryl	Kiwi fruit	0.01/10.0	0.04	84	68	0.10	0.10
Carbendazim	Lemon	0.10/5.0	0.40	95	101	0.28	0.30
Imazalil	Lemon	0.05/5.0	0.20	78	83	1.40	1.50
Myclobutanil	Strawberry	0.05/1.0	0.20	73	74	0.50	0.53
2-Phenylphenol	Lemon	0.1/10.0	0.40	88	78	2.10	2.10
Penconazole	Strawberry	0.05/0.1	0.20	82	76	0.05	0.05
Pyrimethanil	Strawberry	0.05/na	0.20	74	76	0.18	0.19
Tebuconazole	Peach	0.05/1.0	0.20	81	86	0.10	0.10
Thiabendazole	Lemon	0.05/5.0	0.20	73	83	2.00	2.10

RL = target reporting level; MRL = maximum residue level; na = not available.
[a] Screen batch: 12 samples, single spike, matrix blank, reagent blank and set of matrix-matched standards.
[b] Confirmation batch: samples containing residues, single spike, matrix blank, reagent blank and new set of matrix-matched standards.
[c] Not corrected for recovery.

4. Conclusions

The method described facilitates the quantitative and qualitative multi-residue determination of various combinations of 32 of the 38 pesticides targeted for analysis in crude extracts of grape, kiwi fruit, lemon, peach, nectarine, spinach or strawberry. The limitations of separating complex mixtures using isocratic HPLC conditions, such as analyte co-elution or marginal separation and interference from co-extracted material and background ions, can be overcome by the use of tandem mass spectrometric detection and electrospray ionisation techniques.

The combination of isocratic HPLC and tandem mass spectrometry employed in the method significantly reduced sample analysis times. Savings of at least 25% were achieved compared with gradient LC–MS–MS methods used previously in our laboratories in the analysis of similar pesticide mixtures. The method can however, be readily combined with GC–MS multi-residue methods in the analysis of complex analytical suites through manipulation of a common crude extract thus yielding further efficiency gains.

References

[1] WHO, 2nd ed, Residues of Pesticides in Foods and Animal Feeds, Vol. 2, Codex Alimentarius Commission, 2000.
[2] The Pesticides (Maximum Residue Levels in Crops, Food and Feeding Stuffs) (England and Wales) (Amendment) Regulations, Statutory Instruments, 2001, No. 1113, The Stationery Office, April 2001.
[3] Annual Report of the Pesticide Residues Committee, Pesticide Safety Directorate, York, 2000.
[4] R.B. Cole, Electrospray Ionisation Mass Spectrometry: Fundamentals, Instrumentation and Applications, Wiley–Interscience, New York, 1997.
[5] M. Fernandez, R. Rodriguez, Y. Pico, J. Manes, J. Chromatogr. A 912 (2001) 301.
[6] X. Pous, M.J. Ruiz, Y. Pico, Fresenius J. Anal. Chem. 371 (2001) 182.
[7] M. Fernandez, Y. Pico, J. Manes, J. Chromatogr. A 871 (2000) 43.
[8] K. Barnes, R.J. Fussell, J. Startin, M.K. Pegg, S.A. Thorpe, S.L. Reynolds, Rapid Commun. Mass Spectrom. 11 (1997) 117.
[9] K. Barnes, R.J. Fussell, J.E. Startin, H.J. Mobbs, R. James, S.L. Reynolds, Rapid Commun. Mass Spectrom. 11 (1997) 159.
[10] K. Byungjoo, S. Hun-Young, Bull. Korean Chem. Soc. 21 (2000) 471.
[11] M. Bester, G. Bordin, A. Rodriguez, H. Schimmel, J. Pauwels, G. Van Vyncht, Fresenius J. Anal. Chem. 371 (2001) 550.
[12] A.C. Hogenboom, M.P. Hofman, S.J. Kok, W.M.A. Niessen, U.A.T. Brinkman, J. Chromatogr. A 892 (2000) 379.
[13] B.K. Choi, D.M. Hercules, A.I. Gusev, Fresenius J. Anal. Chem. 369 (2001) 370.
[14] R. Pascoe, J.P. Foley, A.I. Gusev, Anal Chem. 73 (2001) 6014.
[15] A. Asperger, J. Efer, T. Koal, W. Engewald, J. Chromatogr. A 937 (2001) 65.
[16] Y.S. Keum, K.H. Liu, Y.S. Lee, J.S. Lee, B.J. Chung, J.H. Kim, Chromatographia 52 (3–4) (2000) 237.
[17] Guidelines for Residue Monitoring In the European Union, SANCO/3103/2000, 2000.
[18] Guidance document on residue analytical methods, EC SANCO/825/00 rev.6, 2000
[19] G.S. Nunes, R.M. Alonso, M.L. Ribeiro, D. Barcelo, J. Chromatogr. A 888 (2000) 113.