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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: PS2524

2. Project title:
   Development of multi-residue methods for the analysis of pesticides in herbs and spices.

3. Contractor organisation(s):
   Central Science Laboratory
   Sand Hutton
   York

   YO41 1LZ

4. Total Defra project costs: £

5. Project: start date ..... 01 April 2005

   end date ..... 31 March 2006

6. It is Defra's intention to publish this form.
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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Overview

Each year the UK pesticides monitoring programme [co-ordinated by the Pesticide Residues Committee (PRC)] involves the analysis of up to 4000 samples for pesticide residues. These results are used to check for MRL compliance and consumer risk assessments. Despite concerns regarding the chemical contamination (e.g. Sudan and other azo dyes) of spices, the situation regarding contamination by pesticides is unknown due to a lack of data. There has been no UK monitoring of pesticides in dried herbs and spices because of the difficulty of the associated with the analysis of these complex samples. The purpose of this project was to develop and validate robust multi-residue methods for the determination of pesticides in representative commodities (curry and chilli powder). The methods could then be used in for the analysis of spices in future monitoring programmes.

Main objectives

1) To carry out an initial evaluation of extraction and mass spectrometry (MS) techniques and then select analytical an approach for validation.

2) To attempt to validate the selected pesticides in chilli powder and curry powder at 0.5 mg/kg.

The Pesticides Safety Directorate (PSD) requested that carbendazim, methomyl, methamidophos, monocrotophos and procymidone should be included in pesticides evaluated. The other pesticides were selected on the basis of proposed EU and Codex MRLs, and also reported occurrence of residues. A total of 61 pesticides were evaluated in this project.

Methods used

A literature search produced few relevant references, with no published methods based on gas chromatography – tandem mass spectroscopy (GC-MS/MS). Rapid extraction methods based

on acetonitrile procedure [1] were evaluated, since the Quechers multi-residue methodology has already been successfully applied to other dry (i.e. low moisture content) commodities such as wheat and rice [2].

GC-MS/MS and liquid chromatography – tandem mass spectroscopy (LC-MS/MS) using tandem quadrupole instruments were employed for the detection and quantification of pesticides. These instruments provide a high degree of selectivity to distinguish between the pesticides of interest and matrix co-extractives. Most of the previous work reported in the literature was based on gas chromatography – electron capture detection (GC-ECD) which, is prone to interference from matrix co-extractives and does not provide confirmation of identity. At least two MS/MS transitions were used for each pesticide, to enable both quantification and confirmation of identity of residues, in a single analysis.

The methods were validated by analysis of 5 replicate 'blank' samples (of each commodity) fortified with each pesticide at 0.5 mg kg^{-1}. Recovery concentrations were calculated against calibration graphs constructed from multilevel, matrix-matched standards. Mean percentage recovery values and % CVs were calculated for each analyte in each commodity.

Summary of research findings

Analytical methods based on rapid extraction / dispersive SPE cleanup were successfully validated for the quantitative confirmation of the five target pesticides (carbendazim, methomyl, methamidophos, monocrotophos and procymidone) in curry powder and for methomyl, monocrotophos and procymidone in chilli powder at 0.5 mg kg^{-1}. For carbendazim and methamidophos in chilli powder, the recovery data indicated that the methods were suitable for screening purposes.

Key parameters were hydration of the sample prior to extraction, the use of rapid acetonitrile-based extraction and dispersive SPE cleanup with primary secondary amine (PSA) and carbon, and the use of tandem quadrupole instruments for GC-MS/MS and LC-MS/MS. This provided a rapid method with a high degree of detection selectivity to distinguish between the pesticides of interest and matrix co-extractives.

In the case of curry powder an additional 45 pesticides were successfully validated at 0.5 mg kg^{-1}, whereas for chilli powder an additional 37 pesticides were validated. Ethofumesate (proposed EU MRL of 0.5 mg kg^{-1}) was validated in both commodities.

Overall the analytical results indicate significant progress in the development of methods for the determination of pesticides in herbs and spices, and the methodology should be suitable for the analysis of spices in future PRC monitoring programmes.

Project Report to Defra

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   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and

- any action resulting from the research (e.g. IP, Knowledge Transfer).

Objective 1:

To carry out an initial evaluation of extraction and MS techniques and then select an analytical approach for validation.

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Experimental

Extraction

Preliminary experiments were carried to evaluate the use of the buffered Quechers acetonitrile extraction method with dispersive, solid phase extraction (SPE) cleanup steps for GC-MS/MS analytes. The use of PSA/carbon (pre-weighed into centrifuge vials) for the dispersive SPE clean up stage gave good recoveries for most analytes. It was important that volume of acetonitrile extract was limited to a maximum of 0.4 ml to avoid exceeding the clean-up capacity of the fixed amount of SPE material in the centrifuge vials. The addition of toluene (20 %) to the SPE clean-up stage was employed to optimise recoveries of analytes which contained aromatic ring structures (eg carbofuran, HCB, quintozene and pyrethroids). An evaluation of the buffered Quechers procedure with these modifications gave good results for nearly all analytes. Thus the buffered Quechers acetonitrile extraction followed by dispersive, solid phase extraction (SPE) cleanup with magnesium sulphate/PSA/carbon was used for GC-MS/MS validation experiments.

Preliminary experiments were carried out to evaluate the use of the Quechers acetonitrile extraction both with and without the use of PSA clean-up for the LC-MS/MS analytes. A series of calibration standards in the range 0.0125 – 0.25 $\mu g\ ml^{-1}$ (0.05 – 1.0 $mg\ kg^{-1}$) were prepared with and without PSA clean-up. Examination of calibrations and MRM chromatograms showed that the additional PSA clean-up offered no significant improvement to chromatographic performance, and thus was not included in the final LC- MS/MS method.

The final extraction/clean–up methods used for validation experiments were as follows:

Extraction

5 g sample was shaken vigorously with 9 ml water for 1 minute. 10 ml acetonitrile containing 1 % acetic acid was added and shaken vigorously for 1 minute. Then 4 g anhydrous magnesium sulphate and 1.66 g sodium acetate trihydrate was added. The mixture was shaken for 30 seconds, and then centrifuged at 3600 x g for 5 minutes.

Clean up for GC-MS/MS

0.4 ml of sample extract followed by 0.6 ml of 1% acetic acid in acetonitrile/toluene (2:1) was added to a 2 ml centrifuge tube containing 150 mg anhydrous magnesium sulphate, 50 mg PSA and 50 mg carbon. The tube was shaken for 30 seconds, then centrifuged at 6000 rpm for 1 minute. The supernatant was analysed by GC-MS/MS.

Preparation for LC-MS/MS

As described in extraction above, then 0.25 ml of sample extract was diluted with 0.25 ml of 10 mM formic acid (aqueous).

GC-MS/MS

GC-MS/MS analysis was carried out using a Varian 1200 triple quadrupole GC-MS/MS system fitted with a Phenomenex Zebron ZB 50 (30 m x 0.25 mm i.d. x 0.25 um film thickness) capillary column. Splitless injection (1 µl) was carried out using an injector temperature of 250 ^{o}C, and the injector liner was fitted with a carbofrit (carbon) insert. The GC temperature program was 100 ^{o}C for 1 min, programmed to 160 ^{o}C at 15 $^{o}C\ min^{-1}$ (held for 1 min), then to 230 ^{o}C at 2 $^{o}C\ min^{-1}$ (held for 1 min), then to 280 ^{o}C at 5 $^{o}C\ min^{-1}$ (held for 1 min), and then to 315 ^{o}C at 10 $^{o}C\ min^{-1}$ and held for 3.5 mins. The total GC run time was 60 mins. Helium at a flow rate of 1.0 ml min^{-1} was used as carrier gas. The GC-MS/MS system was operated in electron ionisation (EI) mode. The MS detector interface temperature was 300 ^{o}C, the source temperature was 200 ^{o}C, electron energy was 70 eV, filament current 150 µA and the detector voltage was 1700 V.

The most intense, highest mass precursor ions were selected from full scan mass spectra of each analyte. Product ion mass spectra were derived from collision-induced dissociation (CID) with argon gas, of the selected precursor ions. Precursor ions were subjected to various collision energy (CE) voltages of 10 – 40 V, and the most intense product ions from each precursor ion were selected. The selected precursor-product ion combinations were all analysed concurrently in MRM mode for direct comparison, and the MRM transitions giving the best response were used for subsequent GC-MS/MS analysis. Two MS/MS transitions were obtained for each component of each pesticide, allowing both quantification and confirmation of identity of residues in a single analysis.

A run time of 60 minutes was required to allow sufficient time segmentation to optimise the MRM responses for all of the 54 pesticides analysed by GC-MS/MS.

LC-MS/MS

LC-MS/MS was carried out using a Sciex API 2000 system, in positive electrospray mode, with an Atlantis C18 (150 mm x 2.1 mm i.d.) column, using gradient elution with aqueous 10 mM ammonium acetate (A), and methanol (B). The gradient started at 10% B and rising linearly to 90% B over 10 min. The composition was held at 90% B for a further 10 min before being returned to the initial conditions over 1 min, followed by re-equilibration for 9 min, giving a total run time 30 min. The total flow rate was 0.2 ml min^{-1}, and the injection volume was 5 µl. The transitions used were obtained by direct infusion of analyte solutions into the ionspray source, and scanning in positive mode to obtain the positive electrospray mass spectra. Abundant precursor ions consistent with the pseudomolecular $[M+H]^+$ species were obtained for all analytes. The product ion mass spectra were obtained from collision induced fragmentation of the precursor ions. Mass dependent parameters were optimised for each MS/MS transition, and two MS/MS transitions were obtained for each component of each pesticide, allowing both quantification and confirmation of identity of residues in a single analysis.

Objective 2:

To attempt to validate the selected pesticides in chilli powder and curry powder at 0.5 mg kg^{-1}.

Experimental

See details in Objective 1. Spiking was carried out by addition of pesticide standard solutions (in acetonitirile) to the 'dry' samples prior to hydration and extraction of samples.

Results

The results obtained from the validation experiments are given in Table 1. Eight analytes: acephate, azinphos-methyl, carbofuran, dimethoate, methamidophos, monocrotophos, omethoate and phorate, were amenable to both GC and LC, thus data was obtained from both GC-MS/MS and LC-MS/MS methods. Calibrations were constructed after correction for internal standard, with calibration co-efficients of $\geq$0.99 nearly all analytes (the minimum was 0.95). There was little evidence of response drift.

PSD Target Pesticides

The methodology was successfully validated (mean recoveries in the range 70 – 110 %, with CVs of $\leq$20 %) for carbendazim, methamidophos, methomyl, monocrotophos and procymidone, at spike levels of 0.5 mg kg^{-1}.

In chilli powder the methodology was successfully validated for methomyl, monocrotophos and procymidone at 0.5 mg kg^{-1}. The mean recoveries obtained for carbendazim and methamidophos at 0.5 mg kg^{-1} were 63 %, (15 % CV) and 66 % (18 % CV) respectively. As the LCL for these analytes was 0.025 mg kg^{-1}, the methodology was considered suitable for screening for these pesticides.

Additional pesticides

The results for curry powder in Table 1 show that 50 out of a total of 59 quantifiable pesticides were successfully validated (mean recoveries were in the range 70 – 110 %, with CVs of ≤20 %, n = 5) at 0.5 mg kg^{-1} using a combination of GC-MS/MS and LC-MS/MS. Recovery data for a further seven pesticides was considered suitable for screening (mean recoveries were in the range 50 – 70 %, with CVs of ≤20 %). One pesticide (HCB) had low recoveries (<50 %) and another pesticide (trifloxystrobin) could not be determined due to poor calibration.

The corresponding results for chilli powder in Table 1 show that 40 out of the 59 pesticides were successfully validated at 0.5 mg kg^{-1}. A further 11 pesticides had recovery data considered suitable for screening (mean recoveries were either in the range 50 – 70 %, with CVs of ≤20 %, or 70 – 110% with CVs of 20 – 30 %). Six pesticides had low recoveries and another two pesticides (parathion ethyl and pirimicarb) could not be determined on this occasion as the GC-MS/MS retention times drifted outside the SIM time window.

The relatively high % CVs (10 – 20 %) obtained for many of the pesticides, illustrates the analytical difficulties associated with the determination of pesticides in spices.

For some analytes determined by both GC and LC, e.g., acephate, methamidophos, LC usually gave better recovery data as there was no PSA/carbon cleanup, which may have affected recoveries. Thus LC-MS/MS is the preferred analytical approach for these compounds. Conversely, as the results for phorate indicated higher recoveries and lower variation using GC determination, therefore GC-MS/MS is preferable.

Hexachlorobenzene gave low recoveries (14 – 29 %) in both curry and chilli powder. This was probably due to adsorbtion by the carbon used for dispersive SPE clean up. In chilli powder, aldrin, pp-DDE, pentachloroaniline, phenthoate and quintozene also gave low recoveries (29 – 49 %), again probably due to irreversible adsorbtion by the carbon used for dispersive SPE clean up. However chilli powder has a higher fat content than curry powder, which may have also adversely affected recoveries. Iprodione and dicofol (not listed in Table 1) were also included in the spiking standard mix, but gave no quantifiable response by GC-MS/MS, thus no data for these pesticides could be reported for either commodity. All multi-residue methodology represents a compromise, as it is not possible to optimise a single method for each one of a large number of analytes.

The validation data for all analytes, which were either fully validated or considered acceptable for screening at 0.5 mg kg^{-1}, have been entered into the PSD Methods Compendium [3].

Table 1 Validation data for curry and chilli powder at 0.5 mg kg^{-1}, % recoveries and CVs (n = 5).

Pesticide	Suite	Curry Powder		Chilli Powder	
		Mean	CV	Mean	CV
acephate	GC	61	31	69	10
acephate	LC	87	8	74	13
alachlor	GC	94	11	97	10
aldrin	GC	56	6	36	21
azinphos-methyl	GC	nd	nd	80	18
azinphos-methyl	LC	107	1	68	16
carbendazim	LC	98	4	63	15
carbofuran	GC	95	5	88	11
carbofuran	LC	107	1	74	12
carbofuran-3 hydroxy	LC	104	4	72	13
chlorpyrifos	GC	83	9	71	16
chlorpyrifos-methyl	GC	89	10	74	13
cypermethrin	GC	82	10	91	23
DDD-pp	GC	74	6	68	17
DDE-pp	GC	58	10	49	29
DDT-op	GC	64	8	51	18
DDT-pp	GC	67	8	53	19
diazinon	GC	94	9	89	12
dichlorvos	GC	102	10	94	13
dieldrin	GC	82	9	76	12
dimethoate	GC	99	10	93	17
dimethoate	LC	105	2	76	13
endosulfan-alpha	GC	81	13	66	10
endosulfan-beta	GC	83	8	74	12
endosulfan-sulfate	GC	89	12	90	14
ethion	GC	94	6	91	15
ethofumesate	GC	94	6	98	10
fenitrothion	GC	97	7	94	11
fenvalerate	GC	80	9	75	21
HCH-alpha	GC	86	7	74	13
HCH-beta	GC	89	10	75	14
HCH-gamma	GC	86	8	74	15
heptachlor	GC	72	12	57	15
heptachlor-epoxide	GC	84	10	72	15
hexachlorobenzene	GC	29	12	14	23
malaoxon	GC	90	3	84	18
malathion	GC	96	7	93	12
metalaxyl	GC	90	6	104	13
methamidophos	GC	66	14	65	16
methamidophos	LC	78	9	66	18
methomyl	LC	100	5	73	11
methoxychlor	GC	88	7	81	18
mevinphos	GC	98	11	92	14
monocrotophos	GC	79	16	84	19
monocrotophos	LC	107	4	78	17
mycobutanil	GC	88	6	98	19
omethoate	GC	66	23	76	18
omethoate	LC	89	7	68	12
paraoxon-methyl	GC	96	11	94	14
parathion-ethyl	GC	95	5	nd	nd
parathion-methyl	GC	99	7	94	13
pentachloroaniline*	GC	50	9	29	17
permethrin	GC	72	14	66	17
phenthoate	GC	63	10	46	32
phorate	GC	92	8	83	11
phorate	LC	106	6	68	12
phorate-sulfone	LC	105	5	74	14
phorate-sulfoxide	LC	108	3	76	12
phosalone	GC	84	7	79	18
pirimicarb	GC	87	7	nd	nd
procymidone	GC	93	6	92	13
profenofos	GC	83	5	82	17
quintozene	GC	64	10	46	14
thiabendazole	LC	95	5	61	12
thiodicarb	LC	108	2	71	13
trifloxystrobin	GC	nd	nd	95	16
vinclozolin	GC	93	7	92	13

nd = not determined * pentachloroaniline is a metabolite of quintozene

Overall Conclusions

Analytical methods based on rapid extraction / dispersive SPE cleanup were successfully developed and validated for the quantitative confirmation of 50 pesticides in curry powder and 40 pesticides in chilli powder at 0.5 mg kg^{-1}. For most of the remaining pesticides, the recovery data indicated that the methods were suitable for screening purposes. The validation data obtained for the pesticides requested by PSD (carbendazim, methomyl, methamidophos, monocrotophos and procymidone) showed that all were validated in curry powder, and either validated or considered suitable for screening in chilli powder. A combination of GC-MS/MS and LC-MS/MS is required for comprehensive analysis and monitoring of all of the pesticides in spices.

The methods described represent a significant advance in the multi-residue determination of spices and should be suitable for the analysis of pesticides in spices for future PRC monitoring purposes. The results obtained demonstrate that the methodology is suitable for the purpose of enforcing the proposed EU MRL for ethofumesate in spices. The results obtained suggest that the methodology is also suitable for the detection of most of these pesticides at 0.1 mg kg^{-1}, but further work is required to achieve validation of all analytes at or below that level.

The methodology has been shown to give good recovery of most pesticides added to samples of dried spices. Further work is required to test extraction efficiency for samples with incurred residues. The scope of the methods could also be extended to include other pesticides.

An abstract of a poster covering the GC-MS/MS part of the validation work has been accepted for presentation at the European Pesticides Residues Workshop (EPRW) 2006.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

REFERENCES

1. Anastassiades, M and Lehotay, S. J. (2003) J.A.O.A.C Int. 86, 412 – 431.
2. Caldow, M and Fussell, R.J., (2004), CSL FD Report 03/32.
3. http://compendium.csl.gov.uk/search.cfm