Journal of Chromatography A, 1217 (2010) 2056–2064

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Independent evaluation of a commercial deconvolution reporting software for gas chromatography mass spectrometry analysis of pesticide residues in fruits and vegetables

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ARTICLE INFO

Article history:
Received 21 July 2009
Received in revised form
30 December 2009
Accepted 8 January 2010
Available online 2 February 2010

Keywords:
Pesticides
Multiresidue analysis
Fruit
Vegetables
GC–MS
Deconvolution
AMDIS
PTV injection

ABSTRACT

The gas chromatography mass spectrometry (GC–MS) deconvolution reporting software (DRS) from Agilent Technologies has been evaluated for its ability as a screening tool to detect a large number of pesticides in incurred and fortified samples extracted with acetone/dichloromethane/light petroleum (Mini-Luke method). The detection of pesticides is based on fixed retention times using retention time locking (RTL) and full scan mass spectral comparison with a partly customer built automated mass spectral deconvolution and identification system (AMDIS) database. The GC–MS was equipped with a programmable temperature vaporising (PTV) injector system which enables more sample to be injected. In a blind study of 52 real samples a total number of 158 incurred pesticides were found. In addition to the 85 pesticides found by manual interpretation of GC–NPD/ECD chromatograms, the DRS revealed 73 more pesticides (+46%). The DRS system also shows its potential to discover pesticides which are normally not searched for (EPN in long beans from Thailand). A spiking experiment was performed to blank matrices of apple, orange and lettuce with 177 different pesticides at concentration levels 0.02 and 0.1 mg/kg. The samples were analysed on GC–MS full scan and the AMDIS match factor was used as a mass spectral quality criterion. The threshold level of the AMDIS match factor was set at 20 to eliminate most of the false positives. AMDIS match factors from 20 up to 69 are regarded only as indication of a positive hit and must be followed by manual interpretation. Pesticides giving AMDIS match factors at ≥70 are regarded as identified. To simplify and decrease the large amount of data generated at each concentration level, the AMDIS match factors ≥20 was averaged (mean AMF) for each pesticide including the commodities and their replicates. Among 177 different pesticides spiked at 0.02 and 0.1 mg/kg level, the percentage of mean AMF values ≥70 were 23% and 80%, respectively. For 531 individual detections of pesticides (177 pesticides × 3 replicates) giving AMDIS match factor 20 in apple, orange and lettuce, the detection rates at 0.02 mg/kg were 71%, 63% and 72%, respectively. For the 0.1 mg/kg level the detection rates were 89%, 85% and 89%, respectively. In real samples some manual interpretation must be performed in addition. However, screening by GC–MS/DRS is about 5–10 times faster compared to screening with GC–NPD/ECD because the time used for manual interpretation is much shorter and there is no need for re-injection on GC–MS for the identification of suspect peaks found on GC–NPD/ECD.

1. Introduction

Analysis of pesticides in fruit and vegetables has for many years been performed by use of gas chromatography (GC) often in combination with nitrogen phosphorus detector (NPD) and electron capture detector (ECD) [1,2]. The interpretation of the chromatograms is then very time consuming, because chromatograms of samples have to be manually compared with chromatograms of standards. The identity of peaks with matching retention times has further to be confirmed by combined gas chromatography mass spectrometry (GC–MS). The process also requires very experienced analysts. Governmental regulations demand an increasing number of pesticides to be included in the monitoring programmes. This force the laboratories to look for effective methods capable of detecting an increasing number of pesticides with a high degree of certainty.

Databases of electron ionization (EI) mass spectra giving fingerprint information of different organic compounds have existed for a long time. The sensitivity of GC–MS combined with traditional split/splitless injector is, however, too low to get reliable EI spectra of pesticides at low concentrations. Programmable temperature vaporising (PTV) injector enables more sample to be injected on the

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doi:10.1016/j.chroma.2010.01.022

GC–MS system when operating in the solvent vent mode. Injecting more sample means also injecting more matrix, which can cause problems by masking target compounds.

AMDIS provided by the National Institute of Standards and Technology (NIST) [3] has demonstrated the ability to detect target pesticides in matrices with high background of interfering compounds [4]. The usual way to extract background from target spectra is by subtracting a spectrum next to the target peak. This approach can be difficult unless the background is constant (column bleed for example). The AMDIS identifies ion traces that maximize simultaneously to fit a model of a chromatographic peak. The resulting component spectrum is compared with spectra in a database and reported if the quality match factor of the spectra is over a certain preset value. Component spectra not found in the database belong to the background and will not be reported. This deconvolution process works only if there is a small difference in retention time between the target peak and the interferences. Customers can also make their own AMDIS databases for target spectra and link each spectrum to a retention time. This significantly increases the reliability of the identified target compounds.

The deconvolution reporting software (DRS) version A.02.00 from Agilent Technologies incorporates AMDIS, NIST 05 database, retention time locking (RTL) and MS ChemStation software. The retention times of the target pesticides are locked by use of RTL to match the retention times in the AMDIS database. Purified spectra from AMDIS are sent to NIST 05 for confirmation, and with the MS ChemStation software it is possible to quantify the targets. Some reports exist where AMDIS, or equivalent deconvolution software, has been evaluated for pesticide residue analysis [4–8], but no one has published in the peer-reviewed literature results where the DRS from Agilent Technologies has been evaluated as a tool for pesticide residue analysis including a high number of pesticides.

The objective of this study was to evaluate the capability of the DRS to detect pesticide residues in incurred samples and samples spiked with pesticides at 2 concentration levels: 0.02 and 0.1 mg/kg. Blank matrices of apple, orange, and lettuce were spiked with 177 pesticides from two mixtures: The "A" mixture of 93 pesticides with the most common pesticides found in the Norwegian monitoring of fruit and vegetables, and the "B" mixture with 84 pesticides more seldom found. In addition, a blind study on real samples was performed where hits found by manual interpretation of GC–NPD/ECD chromatograms were compared with hits automatically found by GC–MS/DRS.

2. Experimental

2.1. Materials and reagents

The sample materials of organic origin were homogenised in a blender (Malavasi s.r.l., Bologna, Italy) checked for pesticide residues and frozen at −20 °C before use. Acetone, dichloromethane, iso-octane, toluene, and light petroleum (50–60 °C) were of pestipur quality (SDS Valdonne, France) and decane was of purum quality (Fluka, Buchs SG, Schweiz).

Primary standards of pesticides were supplied from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Stock standard solutions were prepared at 1 mg/mL in toluene except simazine, thiabendazole, oxadixyl, fenmedipham, tetraconazole and boscalid which were dissolved in acetone. A mixture of the 93 most commonly found pesticides were made by diluting 2.0 mL of each stock solution to 100 mL in toluene giving a concentration of 20 μg/mL. This mixture, denoted "A", was diluted further with iso-octane:toluene (9:1) to give calibration mixtures at 0.01, 0.05, 0.2 and 1.0 μg/mL. The remaining 83 pesticides were treated in the same way and were denoted mixture "B". 1.0 mL of each calibration mixture was transferred to a GC-injection vial and added to 0.1 μg (100 μL) of a mixture of triphenylphosphate and ditalimfos in toluene (1 μg/mL). Triphenylphosphate was used as an internal standard for quantification, while dithalimphos was a reference compound for the retention time locking.

2.1.1. Sample extraction

20 g of homogenised sample was put in a 250 mL PTFE flask and low and high concentration levels were made by adding 0.02 and 0.1 mL of standard stock solution 20 μg/mL to the homogenised sample giving a concentration of 0.02 and 0.1 mg/kg of each pesticide, respectively. The sample was added to 40 mL of acetone and the mixture was extracted on a Polytron (Kinematica AG) in 30 s with a speed between 9500 and 9700 rpm. The homogenated sample was added to 40 mL of dichloromethane and 40 mL of light petroleum and processed further on the Polytron for 30 s at the same speed [9].

The sample was centrifuged for 5 min at 2000 rpm. The organic layer was decanted and stored in a 50 mL amber glass flask at 5 °C until further treatment.

2.1.2. Sample concentration

200 μL of a solution containing decane in light petroleum (20 g/L) was transferred to a test tube as keeper and added to 0.1 μg of triphenyl phosphate and dithalimphos. 4.0 mL of the sample extract was added, and the mixture was evaporated under a stream of nitrogen to almost dryness. The sample was redissolved in 723 μL iso-oktane:toluene 9:1 to a sample concentration of 1.00 g/mL.

2.1.3. Instrumentation

The measurements were carried out on an Agilent 6890 N gas chromatograph connected to an Agilent 5973 mass spectrometer with an inert ion source. The gas chromatograph was equipped with a Gerstel (Mühlheim Ruhr, Germany) programmable temperature vaporising (PTV) injector with a multibaffle liner. The separation column was a fused silica J&W Scientific HP-5MSI 30 m with 0.25 mm internal diameter and 0.25 μm film thickness. A 2.5 m methyl deactivated pre column (Varian Inc. Lake Forest CA, USA) of same internal diameter was connected to the analytical column. The columns were connected by a press fit connector (BGB Analytik, Schweiz). The precolumn was frequently changed after 25–35 injections, to avoid contamination of the analytical column. By changing the whole precolumn the retention times were more stable, and it was easier to keep the retention time of dithalimphos within the limits set by the pressure versus retention time calibration curve in the RTL program. The temperature program was set according to Ref. [10]; 70 °C held for 2 min, 25 °C/min to 150 °C, held for 0 min, 3 °C/min to 200 °C, held for 0 min, 8 °C/min to 280 °C, held for 10 min, total time 41.87 min. After optimalisation the PTV program was as follows: injection volume 20 μL with an injection speed of 100 μL/min. The solvent vent temperature was kept at 60 °C in 1 min with a solvent vent flow at 5.0 mL/min. After 1.1 min the split valve was closed, and the injector temperature was raised by 720 °C/min to 280 °C and held there for 1.2 min. The mass spectrometer was operated in scan mode from m/z 40 to 550, threshold 50 and 2.86 scans/s. Transfer line temperature was set at 280 °C, ion source temperature at 230 °C and quadrupole temperature at 150 °C.

2.1.4. Software parameters

The DRS version A.02.00 combines AMDIS version 2.62, NIST05 database and MS ChemStation. The AMDIS database contained 567 pesticides and suspected endocrine disrupters according to Ref. [11]. 20 pesticides not originally present in the database were additionally included. The AMDIS match factor was set to 20. A pesticide was reported only when the retention time was within ±20 s of the retention time in the AMDIS database.

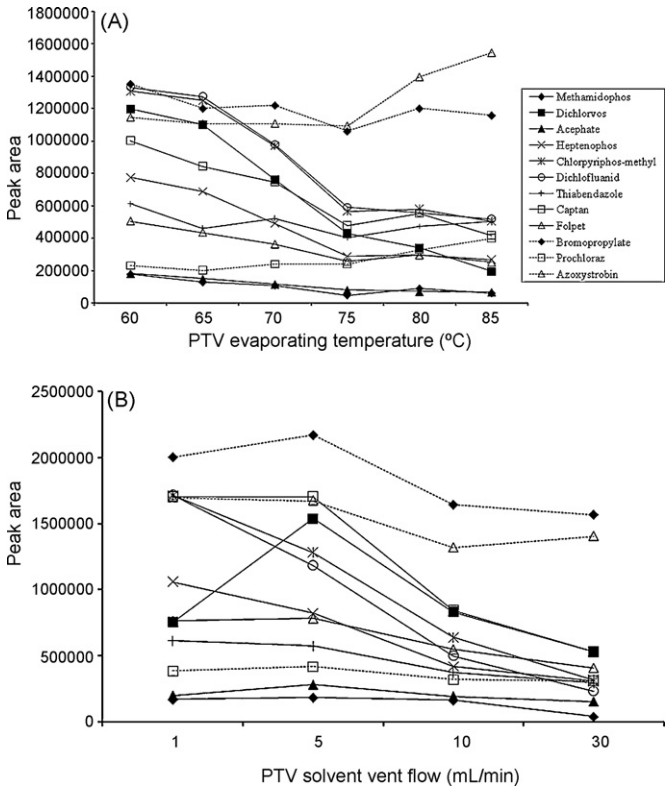

Fig. 1. (A) The effect of solvent vent temperature on peak area of selected pesticides at concentration 0.05 μg/mL in iso-octane:toluene (9:1). Injection volume: 20 μL, split vent time 1.1 min and flow rate 5 mL/min. B. The effect of solvent vent flow on peak area of selected pesticides at concentration 0.05 μg/mL in iso-octane:toluene (9:1). Injection volume: 20 μL, injection temperature: 60 °C and split vent time 1.1 min. Same legends as (A).

3. Results and discussion

3.1. Optimalisation of the PTV injector

In order to get the highest possible sensitivity, the PTV injection system must be optimized. In solvent mode injection two important factors must be considered; the solvent vent temperature and the solvent vent flow. The solvent vent temperature must be kept high enough to evaporate the solvent, but low enough to avoid evaporation of the lowest boiling analytes. Low boiling solvents like hexane and dichloromethane are recommended solvents for PTV injections. However, instead of changing solvent, we decided to test injecting samples dissolved in iso-octane:toluene (9:1) (Bp 99 and 110 °C), since this mixture already was adapted for our multiresidue method using selective detectors.

3.1.1. Solvent vent temperature

The lowest temperature tested (60 °C) gave the best response for most of the pesticides except prochloraz and azoxystrobin which show a little higher peak areas at the highest temperatures (Fig. 1A). Dichlorvos has the most apparent reduction (6 times) in peak area at 85 °C compared to 60 °C. This can be explained by a much higher vapour pressure of dichlorvos (2100 MPa at 25 °C) compared to the other pesticides investigated, where the vapour pressure ranged from 1.1×10^{-7} to 170 MPa at 25 °C [12].

3.1.2. Solvent vent flow

By keeping the solvent vent flow low, too much solvent may be introduced into the column. This may cause distortion of the chromatographic peak shapes resulting in bad sensitivity. On the contrary, keeping the solvent vent flow too high may result in a loss of pesticides by sweeping them out of the liner. Fig. 1B shows the optimum for most of the pesticides tested to be at 5 mL/min.

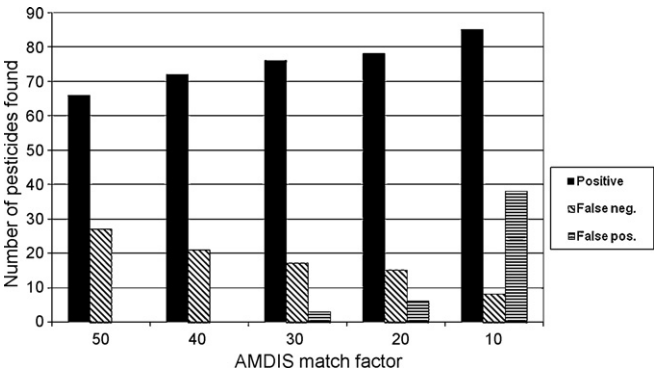

Fig. 2. Relationship between the percentage of correct positives, false negatives and false positives versus AMDIS matching factor threshold for apple extracts spiked with 93 pesticides at 0.02 mg/kg equivalent concentrations.

3.2. AMDIS match factor

The AMDIS match factor is a quality measure of the deconvoluted mass spectrum compared to the spectrum in the database. A perfect match has a value of 100. The AMDIS software allows one to preset a threshold level for the match factor, so that deconvoluted compound spectra with a quality match below the set value is not reported. If the threshold level is set too low or too high the possibility of reporting false positive or false negative results increases. An investigation on apples spiked at 0.02 mg/kg level with 93 pesticides was made. Fig. 2 shows that using an AMDIS match factor threshold of 20 resulted in 15% of the pesticides reported as false negative. An AMDIS match factor threshold of 10 gave only 8% false negatives, but then the false positives increased dramatically. As a compromise we decided to keep the AMDIS match factor at threshold 20 in our study to exclude most of the false positives, and regard this as the detection threshold. Regarding how good a quality match should be, Stein [13] has suggested that a match factor above 80 is sufficient to exclude false positive identifications but match factors 70–79 are acceptable. In the AMDIS the retention time is also an important identification criterion, and it is therefore acceptable to rely on a match factor of 70 or higher for our identification purposes. However, for a full confirmation two or more independent analyses should be in agreement [14].

3.3. Blind study

To test the ability of the DRS system to find pesticides in real samples, a blind study was performed by analysing extracts of 52 samples of different fruits and vegetables on both GC–NPD/ECD and GC–MS/DRS (Table 1). 177 pesticides (Table 2) in the monitoring programme for GC–NPD/ECD were also included in the AMDIS database. The samples represented 27 different commodities with incurred pesticides. A total of 158 pesticides were determined in the analysis and the concentration range was 0.01–2.3 mg/kg (results from GC–NPD/ECD). The GC–MS/DRS system identified 73 additional pesticides (+46%) compared to the GC–NPD/ECD system, and among these 43 different pesticides were only identified by GC–MS/DRS. The additional pesticides were identified by comparison of the deconvoluted mass spectra with the AMDIS library spectra as demonstrated in Fig. 3. However, 6 pesticides detected by GC–NPD/ECD were not identified by GC–MS/DRS. These were imazalil, omethoate, monocrotophos, chlorothalonil, cyprodinil and iprodione. The reason for this was that these pesticides

Table 1
Detection of pesticides in incurred samples from routine analysis. Comparison of GC–NPD/ECD and GC–MS/DRS in their ability to detect pesticides.

	Found by NPD/ECD	Found by DRS		Not found compared to NPD/ECD	Hits in common
		Total	Additional to NPD/ECD		
Apple (3)	6	8	2		6
Aubergine (1)	1	2	1		1
Banana (1)	2	3	1		2
Beans with pod (6)	13	24	13	2	11
Broccoli (1)	0	0			0
Carambola (1)	1	2	1		1
Carrot (2)	2	4	2		2
Cauliflower (1)	0	1	1		0
Celery (2)	3	8	6	1	2
Chilipepper (1)	1	2	1		1
Clementine (2)	7	6		1	6
Cucumber (1)	0	2	2		0
Dill (1)	2	3	1		2
Grape (2)	4	5	1		4
Grape fruit (1)	1	4	3		1
Kiwi (1)	1	2	1		1
Lettuce (5)	11	21	12	2	9
Orange (2)	4	6	2		4
Papaya (1)	2	2			2
Paprika (2)	1	5	4		1
Pear (3)	3	6	3		3
Peas with pod (1)	0	2	2		0
Pineapple (2)	4	5	1		4
Plum (1)	2	2			2
Potato (2)	2	5	3		2
Spinache (1)	1	1			1
Spring onion (1)	0	1	1		0
Strawberry (2)	7	10	3		7
Tomato (2)	4	10	6		4
Total	85	152	73	6	79

Number of samples in parenthesis.

are medium polar, and may degrade on GC or have lower sensitivity on the MS compared with NPD/ECD detection.

3.4. Screen for untargeted pesticides

EPN (o-ethyl o-(4-nitrophenyl) phenyl-phosphono-thionate, CAS RN 2104-64-5) was detected in concentrations up to 0.80 mg/kg in several samples of long beans from Thailand. EPN was at that time not included in the Norwegian monitoring programme, but appeared in the DRS report because EPN was present in the Agilent RTL pesticide library [9]. The AMDIS match factor was as high as 88, and the retention difference was only 5.2 s indicating a high degree of certainty. Fig. 3 shows the raw spectrum (A), the deconvoluted mass spectrum (B) and the AMDIS database spectrum (C) of the peak at 28.7 min in the chromatogram of a sample containing EPN. Also shown are the deconvoluted ion traces; *m*/*z*: 185, 157, 141 and 169. The raw spectrum shows a high amount of interfering ions which make the identification of EPN impossible. However, the background subtracted spectrum clearly matches the database spectrum of EPN giving a good example of how GC–MS/DRS can be a powerful technique to identify additional pesticides present in the database but not included in the monitoring programme. EPN was confirmed by re-analysis of a new sample on GC–MS, together with a reference standard of EPN and comparing the mass spectra and the retention times [15,16].

3.5. Percent correct positives of pesticides in apple, orange and lettuce

To evaluate the possibility of using the DRS in routine analysis, we investigated the ability of the DRS system as a screening tool for pesticides at low and high concentration levels in different commodities. The study was performed by spiking 177 pesticides (Table 2) at concentration levels of 0.02 and 0.1 mg/kg to blank samples of apple, orange and lettuce with three replicates for each commodity. The DRS uses three different parameters to report a positive hit: (1) A hit from ChemStation. This demands that all qualifiers are met, which in our experience seldom happens. (2) The AMDIS match. (3) The reverse match from NIST. This relies almost in all occasions on the hit from AMDIS, so the AMDIS match factor therefore becomes the most important one. For that reason we decided to focus only on the AMDIS match factor. From the DRS report every positive screening result was counted together with the corresponding AMDIS match factor (AMF). For 13 pesticides giving two or more peaks, the concentration per peak will be lower than added. However, when evaluating the percent correct positives of these pesticides, a correct positive is given even though only one of the isomers was found. The large amount of AMDIS match factor data generated (177 pesticides × 3 matrices × 3 replicates × 2 concentration levels = 3186 individual AMF's) demands for simplification without loosing important information. It was therefore decided to average the AMDIS match factors ≥20 for each pesticide at each level including the three commodities and their three replicates.

Table 2 summarises the average AMDIS match factor (mean AMF value) for the different pesticides at concentration levels 0.02 and 0.1 mg/kg. For example; acephate with a mean AMF value of 37 has a standard deviation of 6 and was found at low level in all the three replicates of apple (A = 3) and lettuce (L = 3) but not detected in orange (O = 0). 51 pesticides were found in all replicates of all matrices, for example alfa-cypermetrin and azinphos-ethyl. The percent distribution of the mean AMF values is shown in Table 3. At the 0.02 mg/kg level 23% of the pesticides were identified with a mean AMF value ≥70. 11 pesticides were missed (A = 0, O = 0, L = 0). These are captan, chlordane, cyanazine, demeton-S-methylsulfone, fenamiphos, fluazinam, folpet, imazalil, indoxacarb, metamitron and prometryn. At high level 80% of the pesticides were identified with a mean AMF value ≥70. The 6 non-detected

Table 2
Average AMDIS matching factor (mean AMF) and standard deviations for 177 pesticides spiked into apple (A), orange (O) and lettuce (L). Extracts at 2 concentration levels (triplicate injections in each matrix at each level). Number of replicates in which AMF > 20 is given in the A, O and L columns.

Compound	Low level 0.02 mg/kg						High level 0.1 mg/kg				
	Time (min)	Mean AMF	Std dev.	A	O	L	Mean AMF	Std dev.	A	O	L
Acephate	7.65	37	6	3	0	3	64	20	3	1	3
Aclonifen	25.56	60	17	2	3	3	92	7	3	3	3
Acrinatrin	30.30	77	3	3	3	2	85	2	3	3	3
Aldrin	18.30	64	13	2	0	2	86	4	2	3	3
Alfa-cypermethrin	32.90	54	17	3	3	3	80	4	3	3	3
Azinphos-ethyl	30.57	47	19	3	3	3	64	28	3	3	3
Azinphos-methyl	29.56	66	12	0	3	1	63	26	3	3	3
Azoxystrobin	36.44	61	18	1	1	3	86	9	3	1	3
Benalaxyl	26.64	81	2	3	3	3	96	2	3	3	3
Bifenthrin	28.78	74	7	3	3	3	85	3	3	3	3
Binapacryl	25.07	26	2	3	0	2	46	7	3	3	3
Biphenyl	7.11	82	10	2	0	3	85	14	3	3	3
Bitertanol	31.15	45	10	3	3	2	58	9	3	3	3
Boscalid	32.69	81	2	3	3	3	96	2	3	3	3
Bromophos	19.88	85	2	3	3	3	95	1	3	3	3
Bromophos-ethyl	22.35	63	28	3	3	3	87	14	3	3	3
Bromopropylate	28.53	63	33	3	3	3	92	3	3	3	3
Bupirimate	24.69	69	11	1	3	3	88	10	3	3	1
Cadusafos	11.69	47	17	2	2	1	73	18	3	2	3
Captafol	27.52	20	0	1	0	1	78	2	3	0	0
Captan	21.04	0		0	0	0	25	14	1	1	0
Carbaryl	16.66	76	14	3	3	3	90	1	3	3	3
Chinomethionat	21.72	67	26	3	2	1	94	4	3	3	3
Chlordane	21.83	0		0	0	0	54	27	3	3	1
Chlorfenvinphos	21.44	66	19	3	3	3	80	14	3	3	3
Chlorobenzilate	25.29	84	3	3	3	3	92	2	3	3	3
Chlorothalonil	14.69	90	2	3	3	3	98	1	3	3	3
Chlorpropham	10.95	72	14	3	3	3	89	1	3	3	3
Chlorpyriphos	19.05	69	13	3	3	3	91	1	3	3	3
Chlorpyriphos-methyl	16.42	71	19	3	3	3	81	12	3	3	3
Chlozolinate	21.22	69	19	3	1	2	95	1	3	3	3
Coumaphos	31.61	72	5	3	1	0	82	14	3	3	3
Cyanazine	19.39	0		0	0	0	0		0	0	0
Cyfluthrin[a]	32.42	36	19	2	0	2	74	6	3	3	3
Cymiazol	16.65	44	8	0	2	3	81	8	0	3	3
Cymoxanil	10.65	47		0	1	0	73	1	0	0	0
Cypermethrin[a]	32.8	37	18	2	0	1	64	9	3	2	3
Cyprodinil	20.42	82	10	3	3	3	71	33	3	3	3
Cyproconazole	24.80	58	12	1	3	2	91	4	3	3	3
DDD-p,p	25.59	69	22	3	3	3	86	10	3	3	3
DDE-p,p	23.87	84	9	3	3	3	92	13	3	3	3
DDT-o,p	25.65	57	28	2	2	1	80	16	3	3	2
DDT-p,p	26.88	70	7	3	3	0	90	6	3	3	0
Delta methrin	35.75	28	8	1	1	1	60	21	3	2	3
Demeton-S-methyl	10.40	57	14	0	2	3	84	4	3	2	3
Demeton-S-methylsulfone	17.48	0		0	0	0	83	3	0	3	3
Diazinon	14.30	74	16	3	3	3	96	2	3	3	3
Dichlofluanid	18.20	66	12	3	3	3	88	3	3	3	3
Dichlorvos	5.92	68	32	0	1	3	61	33	1	3	3
Dicloran	12.44	63	22	3	3	3	95	2	3	3	3
Dicofol	27.35	33	24	2	0	1	72	37	3	1	0
Dicrotophos	11.39	51	29	0	3	3	78	13	3	3	3
Dieldrin	23.69	26	5	1	3	3	62	11	3	3	3
Diethofencarb	19.00	28		1	0	0	0		0	0	0
Dimethoate	12.55	46	12	3	2	3	83	9	3	3	3
Diphenylamine	10.40	88	3	3	3	3	92	1	3	3	3
Disulfoton	14.37	65	1	2	1	0	88	5	3	3	3
Disulfoton-sulfone	22.80	57	26	3	2	2	93	3	3	3	3
Disulfoton-sulfoxide	22.82	20	3	0	0	3	20		0	0	1
Endosulfan alpha	22.43	36	20	3	2	3	87	9	3	3	3
Endosulfan beta	25.03	56	11	3	0	3	80	19	3	3	3
Endosulfan-sulfate	26.65	49	23	3	2	3	89	6	3	3	3
Endrin	24.60	28	10	3	1	2	72	7	3	3	1
Esfenvalerate	34.60	46	15	3	2	2	77	9	3	3	3
Ethiofencarb	15.46	68	7	2	3	0	88	4	3	3	3
Ethion	25.88	72	9	3	3	3	89	7	3	3	3
Ethoprophos	10.65	52	20	3	0	3	88	10	3	3	3
Etrimfos	14.99	69	8	3	2	2	90	7	3	3	3
Fenamiphos	23.65	0		0	0	0	0		0	0	0
Fenamiphos sulfoxide	28.38	26	3	3	1	0	66	22	3	3	3
Fenarimol	30.35	54	15	3	0	3	84	7	3	3	3
Fenazaquin	29.02	70	15	3	2	3	93	4	3	3	3
Fenchlorphos	17.15	68	17	3	3	2	84	13	3	3	3
Fenhexamid	26.81	54	25	3	3	3	81	20	3	3	3

Table 2 (*Continued*)

Compound	Low level 0.02 mg/kg						High level 0.1 mg/kg				
	Time (min)	Mean AMF	Std dev.	A	O	L	Mean AMF	Std dev.	A	O	L
Fenitrothion	17.89	75	6	3	2	1	93	3	3	3	3
Fenpropathrin	28.90	52	21	3	2	2	75	11	3	3	3
Fenpropidin	17.44	65	15	2	0	3	88	12	3	1	3
Fenpropimorph	19.07	54	8	3	3	3	83	11	3	3	1
Fenthion	18.93	75	16	3	3	3	95	1	3	3	3
Fenthion-sulfoxide	25.49	50	10	3	3	2	60	2	3	3	3
Fention-sulfone	25.70	64	20	2	2	3	90	5	3	3	3
Fenvalerate[a]	34.4	38	17	3	2	0	71	24	1	3	3
Fipronil	21.77	65	28	3	0	2	78	25	3	0	3
Fluazinam	21.51	0		0	0	0	76	17	3	3	3
Flucythrinate[a]	33.0	46	13	3	3	3	71	6	3	3	3
Fludioxonil	24.05	74	22	3	3	3	96	2	3	3	3
Flusilazole	24.54	71	11	3	3	3	86	9	2	3	3
Flutolanil	23.73	81	11	1	2	3	88	10	3	3	3
Folpet	21.42	0		0	0	0	44	27	1	2	0
HCH alpha	11.94	76	7	3	3	3	96	2	3	3	3
HCH beta	13.16	72	0	2	0	0	75	16	3	3	3
Heptachlor	16.58	54	16	2	3	0	84	16	3	3	3
Heptachlor-epoxide	20.75	39		3	3	2	70	3	0	1	3
Heptenophos	9.65	54	16	1	2	3	89	8	3	3	3
Hexachloro benzene	12.23	68	34	2	1	3	89	10	3	3	3
Imazalil	23.68	0		0	0	0	0		0	0	0
Indoxacarb	35.72	0		0	0	0	84	0	0	0	3
Iprodione	28.34	60	5	3	0	3	74	26	3	1	3
Isofenphos	21.42	63	16	2	3	3	80	14	3	3	3
Isoproturon	5.79	50	2	3	3	3	57	2	3	3	3
Kresoxim-methyl	24.77	72	16	3	3	3	91	6	3	3	3
Lambda-cyhalotrin	30.3	76	9	3	3	3	91	2	3	3	3
Lindane (HCH gamma)	13.30	54	10	2	3	1	92	2	3	3	3
Linuron	17.91	20	3	2	1	1	61	11	3	2	3
Malaoxon	16.73	69	11	0	1	2	88	3	3	0	3
Malathion	18.62	44	21	3	3	3	77	20	3	3	3
Mecarbam	21.55	69	4	3	3	0	93	3	3	3	3
Mepanipyrim	22.95	71	1	0	0	3	78	14	3	1	3
Metalaxyl	17.17	55	23	3	3	3	83	15	3	3	3
Metamitron	24.43	0		0	0	0	0		0	0	0
Methacrifos	8.49	89	1	0	0	3	89	7	3	3	3
Methamidophos	5.79	18	2	0	2	2	27	12	2	3	3
Methidathion	22.12	61	21	3	2	1	81	8	3	3	3
Methoxychlor	28.77	56	13	3	2	0	81	10	3	3	0
Metribuzin	16.12	45	16	1	0	3	73	10	3	2	3
Mevinphos	7.56	64	23	3	0	3	76	29	3	2	3
Monocrotophos	11.73	32	6	0	2	3	63	9	3	2	3
Myclobutanil	24.35	70	9	3	0	3	93	4	3	2	3
Nitrofen	24.76	63	8	3	2	2	93	3	3	3	3
Omethoate	9.94	20	3	0	0	2	41	12	3	2	3
Orto-phenylphenol	8.73	71	15	3	3	3	92	4	3	3	3
Oxadixyl	25.79	55	15	3	3	3	75	28	3	3	3
Paraokson	17.20	36	19	3	0	1	78	14	3	3	3
Paraoxon-methyl	14.43	46	18	3	0	3	82	18	3	3	3
Parathion	19.0	50	22	3	3	3	81	13	3	3	3
Parathion-methyl	16.42	71	16	1	3	3	80	22	2	3	3
Penconazole	20.86	67	16	3	3	3	91	10	3	3	3
Pencycuron	21.79	20	2	0	2	0	31	14	3	1	3
Permethrin[a]	31.3	48	17	2	3	3	75	7	3	3	3
Phenmedipham	10.86	52	45	1	0	1	80	3	2	0	0
Phorate	11.82	58	24	2	0	3	87	8	3	3	3
Phosalone	29.59	66	11	0	3	3	81	12	3	3	3
Phosmet	28.41	74	10	3	0	3	87	5	3	3	3
Phosphamidon[a]	15	52	19	3	3	1	83	6	3	3	3
Pirimicarb	15.52	68	27	2	3	3	91	8	1	3	3
Pirimiphos-methyl	18.11	77	22	3	3	3	95	3	3	3	3
Prochloraz	31.68	20		0	1	0	37	23	3	3	3
Procymidone	21.79	56	23	3	2	2	79	23	3	3	3
Profenofos	23.78	43	18	3	1	3	87	10	3	3	3
Prometryn	17.23	0		0	0	0	73	18	0	3	3
Propachlor	10.25	69	18	3	3	3	91	3	3	3	3
Propargite	27.59	24	5	3	1	1	43	12	3	1	3
Propham	7.87	58	30	3	3	3	75	28	3	2	3
Propiconazole[a]	26.9	61	12	3	3	3	92	2	3	3	3
Propoxur	10.24	74	9	3	3	3	84	1	3	3	3
Propyzamide	13.84	62	27	3	2	3	92	1	3	3	3
Prothiofos	23.61	58	17	3	3	3	86	8	3	3	3
Pyraclostrobin	34.29	28	16	2	2	1	59	21	3	3	3
Pyrazophos	30.6	68	12	3	3	3	88	4	3	3	3

Table 2 (*Continued*)

Compound	Low level 0.02 mg/kg						High level 0.1 mg/kg				
	Time (min)	Mean AMF	Std dev.	A	O	L	Mean AMF	Std dev.	A	O	L
Pyrethrins[a]	24.54	32	8	3	3	3	54	16	3	3	3
Pyridaben	31.45	62	12	3	3	3	87	4	3	3	3
Pyrifenox[a]	22.5	24	5	0	3	1	40	22	2	3	2
Pyrimethanil	14.05	86	15	1	3	3	97	1	3	3	3
Quinalphos	21.47	76	4	3	3	0	84	9	3	3	2
Quintozene	13.52	70	16	0	3	3	91	9	3	3	3
Simazine	12.87	76	1	0	0	3	92	1	3	3	3
Spiroxamine[a]	17.1	44	19	1	3	1	58	27	2	3	3
Sulfotep	11.68	80	3	3	2	3	95	3	3	3	3
Tau-fluvalinate[a]	34.6	50	9	2	3	3	73	7	3	3	3
Tebuconazole	27.35	68	16	0	1	3	83	20	3	2	3
Tecnazene	10.14	69	15	3	1	3	90	8	3	3	3
Terbufos	13.62	77	8	2	2	3	95	1	3	3	3
Terbuthylazine	13.72	69	20	3	3	3	94	1	3	3	3
Tetraconazol	19.75	80	5	3	3	2	96	1	3	3	3
Tetradifon	29.29	67	20	3	3	3	91	5	3	3	3
Thiabendazole	20.88	51		0	0	1	86	2	0	0	3
Thiometon	12.21	62	10	1	1	3	77	15	3	3	3
Tolclofos-methyl	16.63	87	3	3	3	3	96	2	3	3	3
Tolylfluanid	21.04	58	17	3	3	3	73	11	3	3	3
Triadimefon	10.24	70	7	3	3	3	89	2	3	3	3
Triadimenol[a]	21.7	47	16	2	0	0	74	11	3	3	0
Triazophos	26.37	62	25	3	3	3	92	6	3	3	3
Trichlorfon	8.54	26	8	1	3	0	55	20	3	3	2
Trichloronate	19.64	84	1	3	3	3	95	1	3	1	3
Trifloxystrobin	27.20	67	22	3	2	3	91	7	3	3	3
Vamidothion	22.43	35		1	0	0	0		0	0	0
Vinclozolin	16.42	69	21	3	3	2	91	1	3	3	3
Number of presumtive positives				376	336	381			472	454	472
Percent among 177 pesticides				71	63	72			89	85	89

[a] Compounds with two or more peaks. Retention time only for the first eluting isomer.

pesticides at the 0.1 mg/kg level were; cyanazine, diethofencarb, fenamiphos, imazalil, methamitron and vamidothion. Some of the pesticides not detected at 0.02 mg/kg were either not detected at the 0.1 mg/kg level. These are cyanazine, fenamiphos, imazalil and metamitron. Diethofencarb and vamidothion are examples of pesticides detected at low level, but not at high level. We have no explanation of this except that the mean AMF values for these pesticides are quite low (28 and 35 respectively). Table 3 shows that a five-fold increase in concentration increases the number of pesticides with mean AMF ≥70 by a factor close to 3.5.

3.6. The individual detection rate of pesticides in apple, orange and lettuce

The number of times a pesticide was detected in each matrix is given in Table 2. For each commodity and concentration level, a total number of 531 detections were performed (177 pesticides and 3 replicates). At spiking level 0.02 mg/kg in apple, pesticides were detected in 376 out of 531 detections. This means a detection rate in apple of 71%. For orange and lettuce the detection rates were 63% and 72%, respectively. The detection rates at 0.1 mg/kg spike level for apple, orange and lettuce were 89%, 85% and 89%, respectively.

Table 3
Distribution of mean AMDIS match factors for apple, orange and lettuce at two spiking levels.

Mean AMF	Percent among 177 pesticides	
	Spiking level (mg/kg)	
	0.02	0.1
<20	10	3
20–29	6	2
30–39	6	2
40–49	11	2
50–69	44	11
≥70	23	80
Sum	100	100

3.7. Detection of matrix interferences

To evaluate the performance of the DRS system to detect pesticides, a more ideal situation would preferably be if all compounds had the same extraction efficiency, the same chromatography (no tailing or discrimination in the injector) and equal response in the MS. This is, however, not the real situation and the performance of the DRS system will also be influenced by such factors. The Mini-Luke extraction method used in this study gives low recovery for polar pesticides like methamidophos, acephate, omethoate, monochrotophos, metamitron, metribuzin and thiabendazole. Some compounds like captan, folpet and dicofol are not well suited for gas chromatography and give tailing of the chromatographic peak. The electron ionization mass spectra may be unfavourable for some compounds giving few or less intense ions, which is the case for indoxacarb. Another possibility, when pesticides are not detected, is when the interference totally overlaps the target peak making spectral deconvolution impossible. Some pesticides degrade or are masked by matrix interferences in specific matrices. An example is acephate (stable at low pH) which is detected in apple and lettuce, but not in orange, probably because of matrix interferences at low m/z values early in the chromatogram. In Table 2 there are 23 situations where a pesticide is only to be found in a distinct matix. In one category, no peaks are detected in the actual matrix like acephate, DDT-p,p′, dicrotophos, iprodione, methoxychlor, myclobutanil, phosmet, quintozene, thiabendazole and triadimenol. In a second category, peaks are present, but deconvolution was not possible; cymiazol, ethoprophos, fenarimol, indoxacarb, mecarbam, methacrifos, phosalone, quinalphos and simazine. In the last category, the peaks are outside the retention time window set in the AMDIS software; fipronil, malaoxon and prometryn.

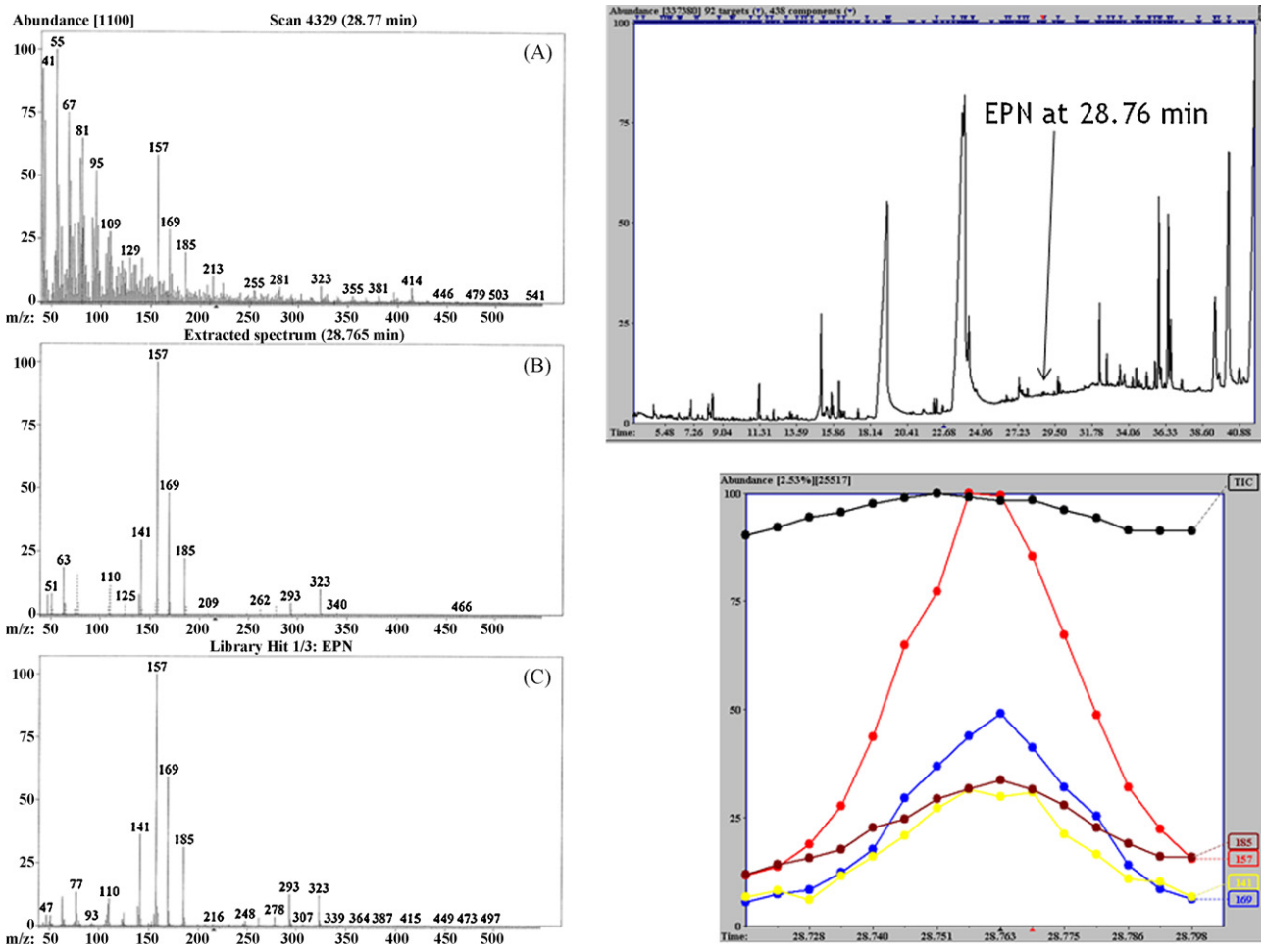

Fig. 3. Example of mass spectral deconvolution of EPN in long beans from Thailand. Chromatogram showing EPN at 28.7 min. A: Raw spectrum, B: deconvoluted spectrum and C: AMDIS library spectrum of EPN. Bottom right: deconvoluted ion traces of EPN at 28.7 min.

3.8. GC–MS/DRS in routine analysis

The results from the spiking experiments indicate that it is not possible to trust 100% on the DRS [17]. The reason is a combination of the relatively low sensitivity of the single quadrupole instrument and high matrix background which masks pesticides and make deconvolution at low concentrations difficult. The situation where spiked samples are considered is different from analysing real samples. In spiked samples an AMDIS match factor ≥20 for a certain pesticide can be considered as positive identification, because you know the pesticide was added. However, this can also be a false positive because there is a chance that DRS reported a peak where the quality of the spectrum is too low to identify the pesticide. The results in Tables 2 and 3 may therefore be some degree overestimated especially at low match factors. In real samples one must consider additional procedures for identification of pesticides which are not so easily detected. In unknown samples an AMDIS match factor at the preset threshold level of 20 is only an indication of the presence of a pesticide. With increasing match factor, the probability of a real positive sample increases, and hits with match factors ≥70 are deemed reliable. This can be regarded as the identification threshold. By comparison of samples of apple, orange and lettuce (spiked at 0.02 mg/kg) with same type of samples free of pesticides, no false positives with match factor ≥70 was found. To investigate false positives, samples of organic origin was analysed over a period of 3 months. Results from 1 sample of apple, 2 samples of orange and 3 samples of lettuce, gave a mean value of 7 false positives with AMDIS match factor over 20. This corresponds to 4% false positives among 177 pesticides. The lowest number of false positives was 5 in apple and the highest number 11 in lettuce. For hits with match factors between 20 and 69 additional identification procedures must be performed. The inspection of the deconvoluted spectrum from AMDIS can also uncover pesticides with quality match below 70. If possible the relative intensities of four diagnostic ions should be expressed as a percentage of the intensity of the most intense ion, and compared to those of a calibration standard as suggested in Ref. [15]. Monitoring of pesticides uneasily detected by AMDIS can also be performed by a macro which routinely prints diagnostic selected ion monitoring (SIM) ion traces. This can be done in combination by running synchronous SIM/SCAN [18] in parts of the chromatogram where problematic pesticides elute. Knowledge of the pesticides normally found in the matrix of concern is still very important. As an example boscalid, fenhexamid, cyprodinil and fludioxonil are likely to be found in strawberries. If these pesticides are found with a low match factor or not mentioned in the DRS report at all, it is recommended to manually look for the pesticides in MS ChemStation. Coelution of compounds with common ions can be a problem in deconvolution systems of this kind. We have uncovered one case which is important to be conscious about. Parathion-methyl and chlorpyriphos-methyl co-elute at retention time 16.42 min. Even though they have different mass spectra, they have m/z = 125, 109, 93 and 79 in common. Since some of these ions are quite intense, there is a possibility that the DRS can pick the wrong compound. Therefore, it is important

whenever these compounds are suggested, to check if $m/z = 286$ or $m/z = 263$ is present which indicate the presence of parathion-methyl or chlorpyriphos-methyl, respectively. A suspect screening result from GC–NPD/ECD has to be identified by use of GC–MS. By introducing GC–MS/DRS as a screening tool it is no longer necessary to inject the sample twice. Despite some manual interpretations of reports from the DRS system are needed, laboratory technicians claim that screening by use of GC–MS/DRS is about 5–10 times faster per sample compared to screening using GC–NPD/ECD followed by identification on GC–MS.

4. Conclusions

The GC–MS deconvolution reporting software (DRS) from Agilent Technologies has demonstrated its ability to find 46% more pesticides than manual interpretation using GC–NPD/ECD when the same number of pesticides (177) search for using GC–NPD/ECD was included in the AMDIS database. The detection of EPN in long beans from Thailand also demonstrates the great advantage of running GC–MS in full scan mode, generating universal electron ionization spectra that makes it relatively simple to detect untargeted pesticides that could not be detected in selected ion monitoring (SIM) or MS/MS transitions. GC–MS/DRS can be used as a screening tool for pesticides in fruit and vegetables but demands extra manual interpretation procedures to uncover pesticides at low concentration level. GC–MS/DRS are a faster technique than GC–NPD/ECD, because there is no longer need for identification of screening results from GC–NPD/ECD by re- injection on GC–MS.

Acknowledgement

We gratefully acknowledge the Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division for financial support.

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