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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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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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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.