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Short communication

Trace-level determination of pesticides in food using difficult matrix introduction–gas chromatography–time-of-flight mass spectrometry

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Abstract

A procedure for the fully automated analysis of food samples by means of difficult matrix introduction–gas chromatography–time-of-flight mass spectrometry (DMI–GC–ToF MS) is discussed. After extraction, samples require very little clean-up and are injected in a micro- or μ-vial which is held in a liner. Next, the liner is placed in the injector and the contents of the vial are thermally desorbed and led directly to the capillary GC column. After GC–ToF MS analysis, the data are processed automatically using a peak deconvolution algorithm. The practicability of the procedure was demonstrated by analysing spiked grape and pineapple samples down to the 1–10 ng/g concentration level.

Keywords: Food analysis; Difficult matrix introduction; Direct thermal desorption; Automation; Gas chromatography–time-of-flight mass spectrometry; Pesticides

1. Introduction

The routine analysis of food samples for residues of pesticides by means of gas chromatography–mass spectrometry (GC–MS) requires high-quality analytical techniques and poses high demands on contract laboratories. Next to proper analytical performance, cost efficiency is becoming increasingly important today. Because of the frequently complex matrix of food samples, a wide variety of interfering compounds has to be eliminated by means of, often, time-consuming clean-up procedures. Studies carried out by Amirav [1] and Lehotay [2] showed that the use of a μ-vial placed in a GC injector is an interesting option to handle complex samples. With this approach, sample clean-up can be virtually eliminated. However, the set-up used by these authors cannot easily be automated. Recently, de Koning et al. [3] introduced an automated liner-exchange unit. The liner is wide enough to hold a μ-vial and is sealed with a standard 11-mm magnetic crimp cap. With this set-up it is, in principle, possible to perform the complete sample preparation procedure in an automated fashion, whilst using a fresh liner-plus-vial for each analysis.

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Another important aspect is the time required for data analysis, i.e. the data processing time. Since selected ion monitoring cannot be used for the present purpose because, next to target analytes, also unknowns have to be detected, the real problem is the abundance of peaks in a full-scan GC–MS trace. In this study, the problem is solved by combining direct thermal desorption (DTD) on-line with gas chromatography–time-of-flight mass spectrometry to enable so-called difficult matrix introduction, i.e. DMI–GC–ToF MS. After ToF MS data acquisition, the data are processed by a peak deconvolution algorithm which extracts ''pure'' analyte peaks and spectra, even when there is multiple overlap of analyte and matrix-compound peaks.

2. Experimental

2.1. Chemicals and samples

Pesticides-mix standards containing either 4 μg/ml or 400 ng/ml of each compound, and pesticide-free extracts of grapes and pineapples dissolved in ethyl acetate were obtained from a commercial contract laboratory. The 400-ng/ml mix was used for standard injections to set up the application. The 4-μg/ml mix was used to prepare spikes of the extracts in the range of 10–200 ng/ml. The matrix content of the extracts was kept constant at 4.7 g/ml.

2.2. Instrumentation

2.2.1. GC–MS analysis

GC analysis was carried out on a Model 6890 GC (Agilent Technologies, Palo Alto, CA, USA) instrument which was equipped with an OPTIC 3 injector (ATAS GL International, Veldhoven, The Netherlands), a DTD direct thermal desorption unit and a FOCUS XYZ sample preparation robot, both from ATAS GL. The capillary GC column was a CP-SIL 8 CB Low/Bleed 25 m×0.25 mm I.D. capillary GC column with a film thickness of 0.25 μm (Varian, Middelburg, The Netherlands). The liner was a DTD liner (ATAS GL) which holds an MPI-μ-vial (ATAS Deutschland, Magdala, Germany). This combination comprising a liner with a μ-vial is the DMI set-up.

Detection was carried out on a PEGASUS III time-of-flight mass spectrometer (Leco, St. Joseph, MI, USA) controlled by a ChromaTOF software package (Leco) for data acquisition, data processing and peak deconvolution.

2.2.2. Injection

The OPTIC 3 is a stand-alone injector consisting of an electronic controller (injector temperature, carrier gas flow-rate) and an injector body mounted in the GC oven wall. The injector body consists of a holder with a very low thermal mass into which an 80 mm×3.4 mm I.D. liner can be inserted. The liner can be packed with a solid sample for thermal desorption analysis, or a μ-vial for DMI analysis; the latter option was used in this study. Heating can be applied at a rate of up to 16 °C/s. The liners can be exchanged using a sample preparation robot equipped with a DTD direct thermal desorption unit, as discussed below.

2.2.3. Sample preparation robot

The Focus XYZ sample preparation robot, which is located on top of the GC system, has a robotic XYZ arm with a motorised syringe, holds separate vial trays for samples, solvents and reagents, a syringe wash station and a heated sample agitator for mixing. Control is via a local module or a PC with software running under Windows. When using the PC software control, the system can be user-programmed to emulate procedures commonly used in sample preparation for GC analysis such as liquid–liquid extraction and derivatisation. The procedures can be selected, programmed and carried out automatically to meet specific analytical requirements. Changes to the programmes can be readily made. Due to the flexible programming of the robot it is also possible to add auxiliary equipment such as a direct thermal desorption unit and a 98-position (7 columns×14 rows) liner tray, which were needed in the present study.

2.2.4. Direct thermal desorption unit

The DTD desorption unit is a mechanical injector head that is controlled by the sample preparation robot. It can be opened and closed automatically by means of a pneumatic cylinder. Prior to installation of the DTD unit, the standard injector head of the OPTIC injection interface was removed and the DTD

unit was installed instead. When the head is open, the XYZ robotic arm picks a liner, which is sealed with a magnetic septum cap, from the liner tray and inserts it into the injector. Next the head is automatically closed. The DTD injector head contains a needle which is connected to the electronic gas regulator of the OPTIC via a flexible gas line. When the head is closed, the needle penetrates the liner septum and the carrier gas stream, which was disconnected by opening the head, is reconnected.

3. Results and discussion

The main goal of this preliminary study is to show the potential of the proposed system for the automated screening of pesticides in food samples after rapid sample extraction and very limited sample clean-up. The set of pesticides (Table 1) was selected on the basis of the application of interest, the analysis of grapes and pineapple.

The injection parameters were optimised in two steps. First, the minimum transfer temperature was optimised by a sequence of splitless injections where the transfer temperature of every next injection was increased by 10 °C in the range of 250–350 °C. It was found that, as a minimum, a temperature of 280 °C is needed. Higher temperatures will only result in the transfer of more and higher boiling matrix compounds. Next, the solvent evaporation time for 5-μl injections was optimised at an injector temperature of 50 °C and a carrier gas flow of 150 ml/min—values selected on the basis of earlier experience—by decreasing the evaporation time from 180 to 100 s in 10-s steps. It was found that, for the set of pesticides being studied, the evaporation time is not a very critical parameter: a time window of 110–130 s could be used. Shorter evaporation times caused flooding of the column and, consequently, poor peak shapes while longer evaporation times led to evaporation of the sample to dryness and loss of analytes. An evaporation time of 120 s was used in further work.

Because one can expect that the chromatograms will contain a large number of co-eluting matrix compounds next to the analytes of interest, and because a peak deconvolution algorithm (see below) will have to be used anyway for data processing, it was decided not to perform an extensive optimisation of the GC oven programme, but to use a rather simple one. Because the boiling point of ethyl acetate is 77 °C, the initial oven temperature was set at 60 °C to create some solvent effect in the capillary column. After a 2-min hold, the temperature was linearly increased to 280 °C at 20 °C/min, with a final hold of 12 min. MS data acquisition was carried out over the mass range of 50–450 a.m.u., with an acquisition rate of 20 spectra/s.

Table 1
Pesticides studied and analytical data

Compound	Quant. mass	Correlation coefficient[a]		RSD (%)[b]	
		Grapes	Pineapple	Grapes	Pineapple
Biphenyl	154	0.994	0.993	2.5	6.0
Dichlofluanid	123	0.992	0.998	16	20
Diflubenzuron	153	0.999	0.996	6.0	16
Flusilazol	233	0.999	0.999	4.0	3.0
Iprodion	314	0.993	0.990	18	45
Myclobutanil	179	0.999	0.999	3.5	6.0
Oxadiazon	175	0.999	0.995	3.0	5.5
Pyrimethanil	198	0.998	0.997	5.5	3.0
Teflubenzuron	223	0.998	0.999	3.7	18
Tetradifon	159	0.997	0.996	5.5	4.5
Tolylfluanid	137	0.999	Deconv. problem	13	Deconv. problem
Trifluralin	264	0.999	0.998	2.7	3.3
Vinclozoline	212	0.999	0.997	1.5	3.0

[a] Range 10–200 ng/ml, five data points in duplicate.
[b] Spike, 50 ng/ml; $n = 10$.

Finally, after the detailed description of the procedure given in the Experimental section, the analytical procedure can be summarized in a few lines of text. Vials containing fruit extracts were placed in the sample tray of the FOCUS XYZ sample preparation robot and a DTD liner containing a μ-vial was placed in the liner tray. Next, the robot takes an aliquot of an extract from the sample vial and introduces it into the μ-vial. Then, the injector is opened automatically and the DTD liner is put into place. After closure of the injector head, the sample solvent is vented. Next, the compounds are thermally desorbed from the μ-vial and transferred to the capillary GC column. After GC–ToF MS analysis, the data are processed by the ChromaTOF software.

3.1. Analytical performance

The information provided with the sample extracts (cf. above) was that the extraction of a fruit or vegetable sample contaminated with pesticides at a level of 10 ng/g (the baby-food level) will lead to a pesticide concentration of about 50 ng/ml in the extract. In order to be able to obtain reliable data at the 10 ng/g level, the detection limit of the analytes in the extracts should, preferably, be 10-fold lower, i.e. 5 ng/ml. Since the specified detection limit of the Leco mass spectrometer is 2 pg injected for hexachlorobenzene, one can assume that a 5-μl injection will certainly create the possibility to record clean mass spectra. In order to check whether the injection of such a fairly large mass of analytes will not cause overload conditions, the injection linearity was studied in the range of 1–5 μl injected (five data points) for a pesticide standard in ethyl acetate at the level of 400 ng/ml. For all eight analytes tested, peak area measurements gave R^2-values in the 0.992–0.999 range.

As a next step, the system was matrix-calibrated for grapes. Pesticide-free extracts were spiked with the pesticides-mix at five concentration levels in the range of 10–200 ng/ml; all measurements were carried out in duplicate. With a matrix content in the extracts of 4.7 g/ml (cf. above), one can calculate that the pesticide levels then are in the range of 2–40 ng/g, which nicely brackets the baby-food level of 10 ng/g (e.g. spike, 50 ng/ml; matrix, 4.7 g/ml; amount of pesticide/amount of sample, 50:4.7=10.7 ng/g). After the successful completion of this work, an identical study was carried out for pineapple extracts. As is shown below, comparable results were obtained in both instances.

Processing of the data by the software package and the construction of the calibration plots were carried out automatically. The results, which are included in Table 1, are fully satisfactory. In order to study the repeatability of the total procedure, 10 subsequent injections were made of extracts spiked at the 50 ng/ml level; quantification was fully automated. Most of the results shown in Table 1 are according to expectations, with RSDs of about 6% or below. However, there are a few exceptions, notably dichlofluanid, iprodion and tolylfluanid. As regards iprodion, this analyte is known [4,5] to be sensitive to thermal degradation, which can occur in both the injector and the capillary column. Moreover, its detectability was poorer than that of the other pesticides studied. With dichlofluanid and tolylfluanid, there were serious deconvolution problems due to matrix interferences, which are discussed in the next section. For the analyses of both grape and pineapple extracts, the correlation coefficients obtained for the calibration plots were in the range of 0.990–0.999. On the basis of the several sets of analytical performance data which were obtained, the limits of detection for all analytes could be calculated to be 1–10 ng/g, i.e. in the expected range. Because the software builds the MS spectra which are compared with reference spectra before quantitation is carried out, it will be clear that the limits of detection and quantification are actually the limits of identification.

3.2. Peak deconvolution

The main problem of data processing of complex samples is the overload of peaks in the GC chromatogram. In such a case, data processing of full-scan chromatograms without loss of mass spectrometric information can only be achieved using a peak deconvolution algorithm. The data analysis software searches the whole chromatogram for the presence of peaks in every mass trace. With this procedure, peaks with different m/z values found at the same retention time and having the same shape, are combined to build the compound spectrum. Co-

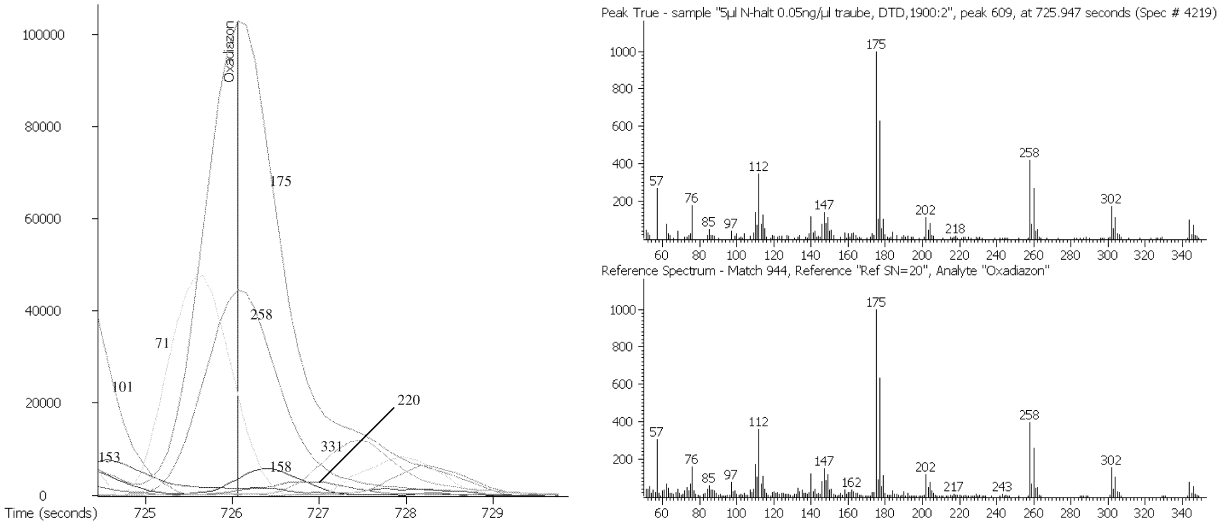

Fig. 1. Left: Extracted ion chromatograms of grape extract spiked at the 50 ng/ml level (equiv. to 10 ng/g). Mass traces of oxadiazon (quantification mass, m/z 175; unique mass, m/z 258) and co-eluting compounds (unique masses, m/z 71, 101, 153, 158, 220 and 331). Right: experimental (top) and reference spectrum (bottom).

eluting peaks with slightly different retention times, i.e. with one or more data points between their peak apices, and/or peak shapes are attributed to other, i.e. additional, compounds. For this research, an acquisition rate of 20 Hz was used, which means that ''pure'' deconvoluted spectra can be obtained from peaks which are only 0.1 s apart. Two examples of successful deconvolution are displayed in Figs. 1 and 2. They both show a multiple co-elution problem in grape extracts, viz for oxadiazon and trifluralin,

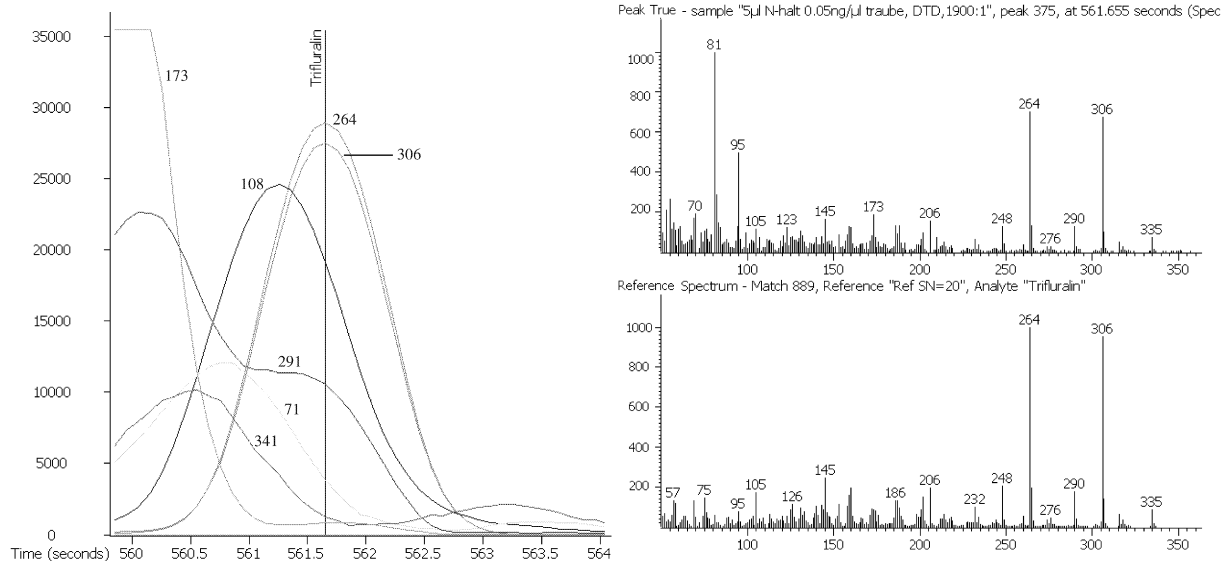

Fig. 2. Left: Extracted ion chromatograms of grape extract spiked at the 50 ng/ml level (equiv. to 10 ng/g). Mass traces of trifluralin (quantification mass, m/z 264; unique mass, m/z 306) and co-eluting compounds (unique masses, m/z 71, 108, 152, 173, 291 and 341). Right: experimental (top) and reference spectrum (bottom).

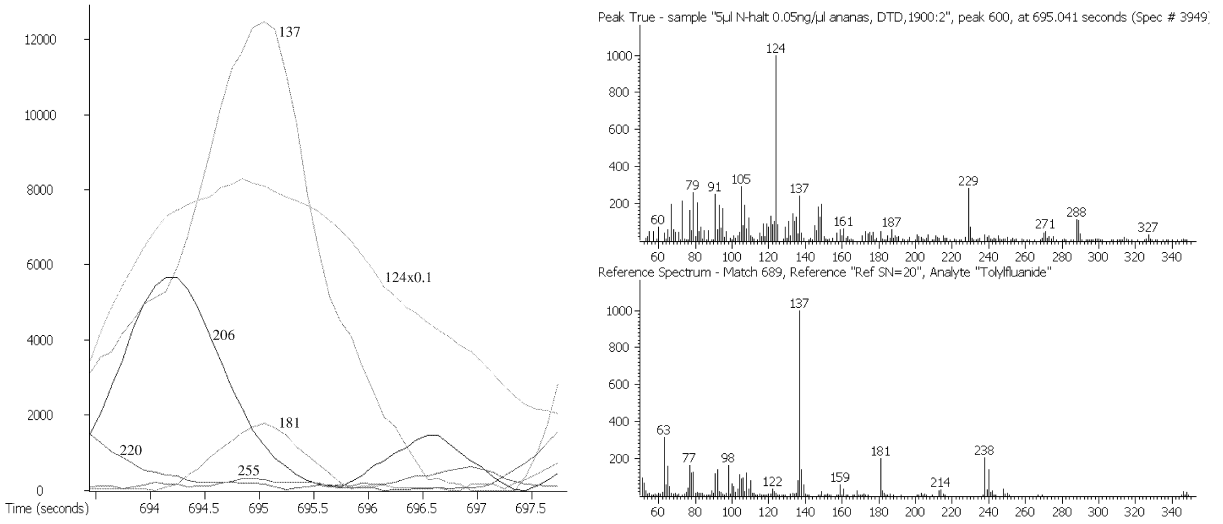

Fig. 3. Left: Extracted ion chromatograms of pineapple extract spiked at the 50 ng/ml level (equiv. to 10 ng/g). Mass traces of tolylfluanid (quantification mass, m/z 137; unique mass, m/z 181) and co-eluting compounds (unique masses, m/z 124 (10 times reduced), 206, 220 and 255). Right: experimental (top) and reference spectrum (bottom).

respectively. The merits of the deconvolution approach are illustrated best by a comparison of the experimental and the reference spectra included in the figures. In the case of oxadiazon, the mass spectra are virtually identical. In the case of trifluralin, the similarity (see m/z 248, 264, 290, 306 and 335) is also fully satisfactory, but the high peaks at the low m/z 81 and 96 values appearing in the spectrum of the extract, indicate a somewhat less than ideal outcome.

The practicability of DMI–GC–ToF MS is demonstrated by the fact that recognisable mass spectra were obtained in 25 of the 26 cases studied. The only exception was with the determination of tolylfluanid in pineapple extract. As Fig. 3A shows, there was a huge interfering peak at m/z 124. As a consequence, the resulting experimental mass spectrum was much to impure to even tentatively recognise the presence of tolylfluanid. But again, this was the exception to the rule.

4. Conclusions

The use of the fully automated DMI–GC–ToF MS system for the screening of pesticides in fruits shows a promising analytical performance. Due to the flexibility and the automation of the DMI/DTD introduction approach and the powerful deconvolution algorithm of the MS data handling, sample clean-up can be reduced to a minimum without loss of performance. The possibility of loading 98 samples in the sampling tray and automated liner exchange makes the system very user-friendly. In all probability, the system can create a breakthrough in the field of pesticide screening in food samples. At the time of writing this publication, the system is being tested for routine application in a commercial contract laboratory. The general experience is that the proposed procedure is robust. The analysis of a wider range of pesticides, fruits and vegetables over a prolonged period of time is part of the on-going research.

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