Advanced Routine Method for the Analysis of Pesticide Residues by LV-PTV–GC–TOFMS and LV-PTV–GC×GC–TOFMS

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The development of advanced methods in pesticide residue screening is an enormous challenge. The scope of pesticides that require monitoring is growing parallel to the scope of matrices that are being investigated. The regulation authorities are continuously lowering the limits of detection whilst the economic pressure for commercial as well as governmental laboratories is increasing. Thus more efficient multi residue methods are essential. However, accuracy and reliability of the analytical results can not be sacrificed during this optimisation process.

Traditional instrumentation and methods are limited in fulfilling all these necessities. Modern instrumentation such as fast/low resolution GC–TOFMS, GC×GC–TOFMS, slow/high resolution GC–TOFMS and LC–MS/MS are under evaluation and there are great expectations that the combination of these technologies will result in a set of advanced methods for pesticide residue analysis in food.

Modern sample preparation methods in combination with LV-PTV injection and in combination with fast GC–TOFMS or GC×GC–TOFMS provide an ideal setup to analyse a broad range of pesticides and matrices. Using such technical and analytical methods ensures sensitive and reproducible results. In this document an in-house validated method for more than 200 pesticides will be presented as well as first validation results of a GC×GC–TOFMS validation.

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MATERIAL AND METHODS

Instrumentation

The fast/low resolution GC–TOFMS systems used for this method are a LECO (St. Joseph, MI, USA) Pegasus III GC–TOFMS (see *Figure 1*) and a LECO Pegasus 4D GC×GC–TOFMS. The Pegasus GC–TOFMS systems have a number of special technical features making them excellent tools for the described application:

Figure 1. LECO Pegasus III GC–TOFMS

- High data acquisition rate, up to 500 full spectra/sec (5–1000 amu)
- Automatic peak finding algorithm and spectral deconvolution
- No cleaning of EI ion source due to its open design
- Integrated modulator and secondary oven for comprehensive GC×GC

For sample injection the ALEX-MPS2 autosampler in connection with a CIS4 PTV cold injection system, both from Gerstel (Mülheim a.d.Ruhr, Germany) was used.

GC: Agilent (Palo Alto, CA, USA) model 6890N; LECO Quad Jet Thermal Modulator (licensed by ZOEX) and secondary oven

Injection parameters

injection volume:10 µl (1-dim.); 2 µl (2-dim)
liner: baffeld, deactivated
mode: solvent vent (1-dim.); splitless (2-dim.)

GC parameters

1-dim. separation,
Column: 30 m x 0.25 mm x 0.25 µm, VF-5ms (Varian, Middelburg, Netherlands)
Oven: 95°C(1,5 min) – 20°C/min – 190°C – 5°C/min – 230°C – 25°C/min – 300°C(20 min)
Column carrier gas: He; 1.4 ml/min constant flow
2-dimensional separation:
Primary column: 30 m x 0,25 mm x 0.2 µm Rtx-CLPesticides II (Restek, Bellefonte, PA, USA)
Secondaty column: 1.1 m x 0.1 mm x 0.1 µm Rxi 17 (Restek)
Primary Oven: 95°C(5 min) – 10°C/min – 200°C – 7°C/min – 270°C – 10°C/min – 320°C(10 min)
Secondary Oven: 105°C(6 min) – 10°C/min – 360°C(15 min)
Column carrier gas: He; 26 psi constant pressure

MS parameters

	1-dimensional	2-dimensional
Solvent delay:	180 sec	300 sec
Detector voltage:	1800 V	1800 V
Mass range:	50–450 amu	50–600 amu
Filament bias voltage:	70 eV	70 eV
Acquisition rate:	20 spectra/sec	200 spectra/sec
Ion source:	200°C	200°C
Transfer line:	250°C	250°C

SAMPLE PREPARATION

For sample preparation the QuEChERS procedure [1] was used. For this purpose 10 g homogenised sample were extracted with 10 ml acetonitrile. By addition of MgSO4 and NaCl excess water was eliminated. Cleanup of the extract was carried out using PSA sorbent. The method is presented in *Figure 2*.

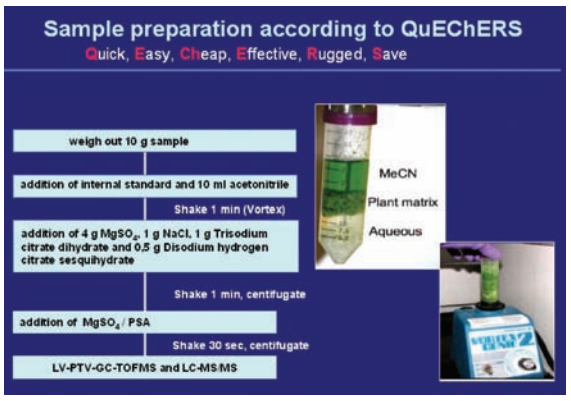

Figure 2. Presentation of QuEChERS procedure [1]

RESULTS AND DISCUSSION

Sample preparation according to QuEChERS

Mixtures of 30–40 compounds from a range of 200 (see *Table 1*) pesticides of different chemical classes like organo phosphorus, organo chlorine, strobilurines, carbamates, pyrethroides etc. were spiked into pesticide free matrices of apple, paprika, tomato, cucumber, grape and peach. The concentration range was 0.005–5 mg/kg. The matrix standards were quantitatively analysed by LV-PTV–GC–TOFMS (see Material and Methods).

In a validation process LODs lower than 0.005 mg/kg could be achieved for most of the pesticides in all matrices. This fulfils EU directives of MRLs lower than 10 ppb for baby food [2]. For 46 pesticides the LODs were between 0.005 and 0.1 mg/kg, fulfilling the regulation of the German RHmV [3]. Of these 46 pesticides, most are better analysed by LC methods, e.g. methamidophos, demethon-S-methyl.

Overall, the mean recovery values for 200 pesticides (spike level 0.05 mg/kg; n=3) in the matrices were between 85.5 % (cucumber) and 93.8 % (paprika).

In routine work up to 30 injections onto the same liner may be common practice. However, certain pesticides in some matrices show a steep decrease of response can be observed from injection to injection. In this case the use of an automatic liner exchange system could help to analyse longer sequences automatically.

During final processing a specific pesticide library and retention time database was helpful for fast automatic processing. Automatic peak find and spectral deconvolution made automatic processing possible even in cases were multiple co-elutions of target and matrix peaks occurred. Final checking of automatic processing results and report generation was around 20 minutes per analysis.

Since non-targeted automatic peak finding, spectral deconvolution and library search can be achieved in parallel with multi compound targeted quantification, it is possible to detect un-calibrated and "unexpected" residues. For example, additional pesticides, PAHs and other contaminants.

Impressively, when over 10000 injections were performed the ion source of the GC–TOFMS system was never cleaned.

Table 1. List of validated pesticides

acephate	chlorothal-dimethyl	dimethomorph
aclonifen	chlorothalonil	diniconazole
acrinathrin	chlorthion	diphenylamine
aldrin	chlorthiophos	disulfoton
azinphos-methyl	clodinafop-propargyl	endosulfan-α
azinphos-ethyl	coumaphos	endosulfan-β
azoxystrobin	cyanofenphos	endosulfan-sulfate
bifenox	cycloat	endrin
bifenthrin	cyfluthrin	EPN
bitertanol	cyhalothrin-λ	epoxiconazole
bromfenvinphos-ethyl	cypermethrin	esfenvalerate
bromfenvinphos-methyl	cyproconazole	ethion
bromophos-ethyl	cyprodynil	ethofumesate
bromophos-methyl	DDD-o,p	ethoprophos
brompropylate	DDD-p,p	ethoxyquin
bupirimate	DDE-o,p	etofenprox
buprofezin	DDE-p,p	etridiazole
captan	DDT-o,p	etrimfos
carbofuran	DDT-p,p	fenamiphos
carbophenothion	deltametrhrin	fenarimol
carbosulfan	demethon-S-methyl	fenbuconazole
chinomethionat	diazinon	fenchlorphos
chlorbensid	dichlobenil	fenhexamide
chlorfenapyr	diclofenthion	fenitrothion
chlorfenson	dichlofluanid	fenoxycarb
chlorfenvinphos	dichlorobenzophenone-4,4	fenpropathrin
chloridazon	dichlorvos	fenpropidin
chlormephos	diclobutrazole	fenson
chlorobenzilate	dicloran	fensulfothion
chloroneb	dicofol	fenthion
chloropropylate	dieldrin	fenthion-sulfone
chlorpropham	difenoconazole	fenthion-sulfoxide
chlorpyrifos-ethyl	dimethenamid	fenvalerate
chlorpyrifos-methyl	dimethoate	fipronil
fluazifop-p-butyl	metalaxyl	propyzamide
fludioxonil	metazachlor	prothiophos
flufenacet	methacriphos	pyrazophos
flumetralin	methamidophos	pyridaben
fluorodifen	methidathion	pyrifenox
fluquinconazole	methiocarb	pyrimethanil
fluroxypyr-methylester	methoxychlor	pyriproxyfen
flurtamone	metolachlor	quizalfop-ethyl
flusilazole	metribuzin	quinalphos
folpet	monocrotophos	quinoxyfen
fonofos	myclobutanil	quintozene
formothion	nitrofen	S 421
fuberidazole	nuarimol	sulprophos
HCB (hexachlorobenzene)	ofurace	tau-fluvalinate
HCH-α	oxadixyl	tebuconazole
HCH-β	paraoxon-ethyl	tebufenpyrad
HCH-δ	paraoxon-methyl	tecnazene
HCH(lindane)-γ	parathion-ethyl	terbufos
heptachlor	parathion-methyl	tetrachlorvinphos
heptachloroepoxid cis	penconazole	tetraconazole
fonofos	myclobutanil	quintozene
formothion	nitrofen	S 421
fuberidazole	nuarimol	sulprophos
HCB (hexachlorobenzene)	ofurace	tau-fluvalinate
HCH-α	oxadixyl	tebuconazole
HCH-β	paraoxon-ethyl	tebufenpyrad
HCH-δ	paraoxon-methyl	tecnazene
HCH(lindane)-γ	parathion-ethyl	terbufos
heptachlor	parathion-methyl	tetrachlorvinphos
heptachloroepoxid cis	penconazole	tetraconazole
heptachloroepoxid trans	pendimethalin	tetradifon
heptenophos	permethrin	tetramethrin
hexaconazole	phenthoate	thiabendazole
hexythiazox	phorate	thiometon
imazalil	phosphamidon	tolclofos-methyl
iprobenphos	piperonyl butoxide	tolylfluanid
iprodione	pirimicarb	triadimenol
isofenphos	pirimiphos-ethyl	triallate
iodofenphos	pirimiphos-methyl	triazophos
kresoxim-methyl	procymidone	trifloxystrobin
malaoxone	profenophos	trifluralin
malathion	propachlor	vinclozolin
mecarbam	progargite	
mepronil	propham	

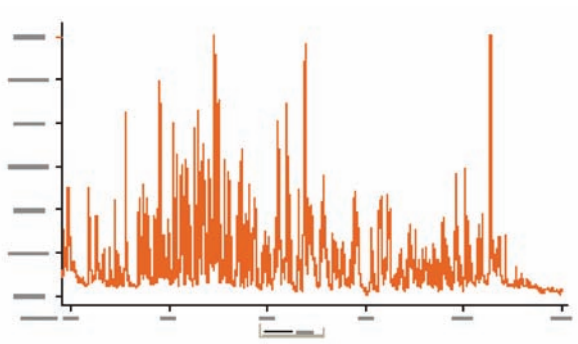

Figure 3. TIC 1-dimensional chromatogram showing all pesticides studied

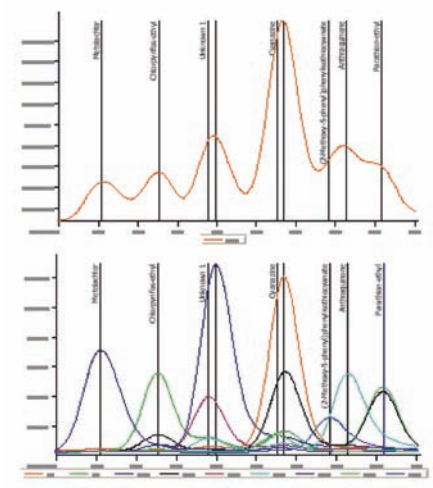

Figure 4. Zoomed TIC (top) and EIC (bottom) of coeluting compounds. Peak identifications are displayed at the peak markers

Sample preparation according to S19 method § 35 LMBG L 00.00-34

QuEChERS sample preparation results in relatively dirty sample extracts with a low extract concentration of only 1 g sample/ml and thus containing a low pesticide concentration in the extract. In contrast, the S19 method results in cleaner extracts, and higher concentrations of the pesticides in the extracts. However, the disadvantage of this sample preparation method is that it is more time and solvent consuming than QuEChERS.

The benefit of combining the S19 method with LV-PTV–GC–TOFMS is that a wider range of matrices can be investigated. Thus, it is possible to focus on matrices such as tea, spices and dried herbs like parsley.

An additionally benefit of LV-PTV–GC–TOFMS compared to quadrupole MS is the full spectra information which is gained even at low levels. This makes identification simple and more reliable. Validation data for an extended method are currently under review

Comparison of GC–TOFMS with GC×GC–TOFMS

As seen by viewing the second dimension of the GC×GC surface plot in *Figure 5*, many coelutions would occur when analysing in a single dimension mode. *Figure 6* displays a zoomed section of the contour plot indicating the coelution of four pesticides along the first dimension.

However, these four pesticides are separated due to the selectivity of the second orthogonal separation. Although the peak capacity and resolving power of GC×GC is very high, coelutions can still occur. The True Signal Deconvolution® of ChromaTOF® software can be used to identify coeluting peaks, delivering true peak spectra.

Comprehensive GC×GC–TOFMS in pesticide routine analysis allows to produce reliable quantitative results in very complex matrices like tea, spices and animal feed. Also better sensitivities can be achived for most of the tested target analytes.

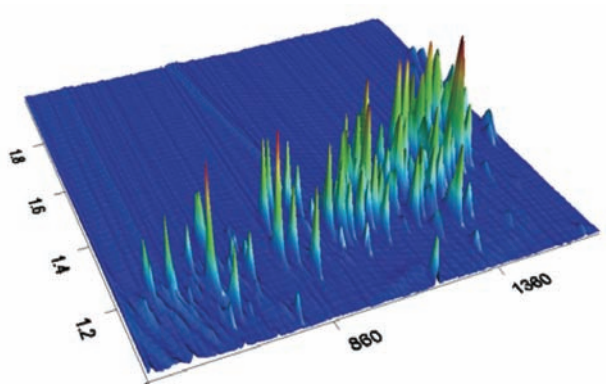

Figure 5. TIC surface plot showing all pesticides studied

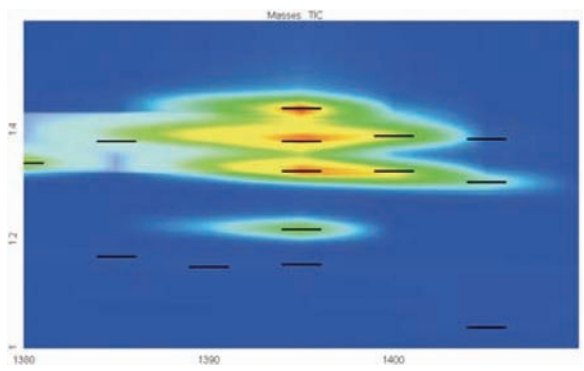

Figure 6. Zoomed contour plot indicating coelution in the first dimension, but separation in the second dimension

CONCLUSION AND OUTLOOK

It is shown that by combining modern sample preparation methods with modern analytical equipment it is possible to develop an advanced multi method for pesticide residue analysis.

LV-PTV–GC–TOFMS is one way towards creating new validated methods. Furthermore and in the future, the combination of LC- and GC-related analytical methods will cover the full scope of pesticide residues in all matrices.

For the described LV-PTV–GC–TOFMS method the recovery data, detection limits and calibration curves for a 200 pesticides multi method are now available. Data collection for a 300-pesticide method in a wider range of matrices is on the way.

The use of comprehensive GC×GC–TOFMS provides an enhanced separation that helps to eliminate coelutions. Better selectivity and sensitivity can be achieved especially when very complex samples like tea and spices is analysed.

The day-to-day work of pesticide residue analysis in routine labs will show which set of methods to be successful and find their way into European legislation.

REFERENCES

[1] M. Anastassiades, S. Lehotay, D. Stajnbaher, F.J.Schenck: "Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce", J. AOAC Int. Vol. 86, No.2, 2003

[2] Commission directive 2003/13/EC and 2003/14/EC Baby foods, infant foods and infant formulae)

[3] Verordnung über Höchstmengen an Rückständen von Pflanzenschutz- und Schädlingsbekämfungsmitteln, Düngemitteln und sonstigen Mitteln in oder auf Lebensmitteln und Tabakerzeugnissen, 21.10.1999 (BGBl. I S.2082, i.d.F. vom 07.04.2006 (BGBl. I S. 838)