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Pesticide residue analysis in foodstuffs applying capillary gas chromatography with mass spectrometric detection State-of-the-art use of modified DFG-multimethod S19 and automated data evaluation

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Abstract

This paper focuses on recent developments in the author's laboratory and reports on the ''ultimate'' analysis scheme which has evolved over the last 20 years in our laboratory. This demonstrates the feasibility of screening analyses for pesticide residue identification, mainly by full scan GC–MS, down to the 0.01 ppm concentration level in plant foodstuffs. It is based on a miniaturized DFG S19 extraction applying acetone for extraction followed by liquid–liquid extraction with ethyl acetate–cyclohexane followed by gel permeation chromatography. The final chromatographic determination is carried out with a battery of three parallel operating gas chromatographic systems using effluent splitting to electron-capture and nitrogen–phosphorus detection, one with a SE-54 the other with a OV-17 capillary column and the third one with a SE-54 capillary column and mass selective detection for identification and quantitation. The method is established for monitoring more than 400 pesticides amenable to gas chromatography. These pesticide residues are identified in screening analyses by means of the dedicated mass spectral library PEST.L containing reference mass spectra and retention times of more than 400 active ingredients and also metabolites applying the macro program AuPest (Automated residue analysis on Pesticides) for automated evaluation which runs with Windows based HP ChemStation software. The two gas chromatographic systems with effluent splitting to electron-capture and nitrogen–phosphorus detection are used to check the results obtained with the automated GC–MS screening and also to detect those few pesticides which exhibit better response to electron-capture and nitrogen–phosphorus detection than to mass spectrometry in full scan.

Keywords: Food analysis; Pesticides

1. Introduction

Fruit and vegetables are traded worldwide and which pesticides might have been applied in their agricultural production is usually not known. Consumers, however, demand produce to be ''free of harmful pesticide residues''. Therefore, food chemists are expected to control maximum residue levels for all pesticides.

Pesticide residue analysis is carried out by means of multimethods for extraction and clean up followed by a final chromatographic determination step. Extraction must achieve the transfer of all pesticide residues from each food sample matrix into the organic phase and the clean up step is designed to protect the chromatographic system from food ingredients which cause the performance of the chromatographic separation to deteriorate rapidly or impair the sensitivity of the selected detector. With respect to deterioration of the chromatographic system, each method for pesticide residue analysis may be considered to be a compromise between the

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labour intensive and time consuming daily clean up procedure with the various food samples at the laboratory bench and the maintenance intervals with lay off times of the instrument.

Further clean up steps are necessary when applying detectors of limited selectivity. In the past decades gas chromatography (GC) with electron-capture detection (ECD) and the nitrogen–phosphorus detection (NPD) have proven their potential in pesticide residue analysis. Most of the co-extracted ingredients from the food matrix show no response with these selective detection methods. This, however, is not true for ECD which also responds to matrix compounds from fruit and vegetables, a few of which such as leek and onion are therefore called ''problem foodstuffs''. The multimethods used today in the laboratory have all been developed to achieve reliable results with GC and selective detectors. A quantum leap in productivity was observed with the advent of fused-silica capillary columns with their unrivaled separation power. The next step was the introduction of mass spectrometers designed as detectors for capillary GC. The revolution of data processing allowed the development of user friendly software including advanced chromatographic peak recognition and analyte identification algorithms. The prerequisite for this dramatic development was the takeoff in desktop computer hardware. The instrument that combines these capabilities best is the modern GC–MS benchtop instrument.

Various extraction methods are currently used for non-fatty samples. The extraction with acetone followed by liquid–liquid partitioning (LLE) into dichloromethane or a mixture of dichloromethane with hexane is very popular. This method was originally presented in 1975 by Luke et al. [1]. The acetone extraction may also be followed by LLE with ethyl acetate and cyclohexane. This latter method was thoroughly investigated by Koinecke et al. [2] and validated by Specht et al. [3] in 1995 and has grown in popularity because it avoids the use of a chlorinated hydrocarbon.

Another method which was first reported by Krijgsmann and van de Kamp in 1976 [4] has been used by many groups including our own [5], namely extraction with ethyl acetate. The ethyl acetate method was tested from 1988 in the laboratories of the Swedish National Food administration and in 1989 replaced the acetone method. Andersson and Palsheden reported the comparison of the efficiency of the two methods and concluded that the two methods gave acceptable and equivalent recoveries for the pesticides tested [6].

The extraction step is mostly followed by gel permeation chromatography (GPC), first convincingly used in 1972 by Tindle and Stalling [7] to separate pesticides from fish lipids. This stage was then introduced into the universal clean up procedure DFG S19 developed by Specht and Tillkes in 1980 and extended in 1985 [8,9]. GPC turned out to be *the* universal clean up procedure to separate the pesticides from lipids, waxes and other low volatile larger non-polar co-extractives. It offers therefore the best protection for the gas chromatographic capillary column. The same procedure is used with pesticide residue analysis of fatty food samples after extracting the pesticides together with the lipids from the food matrix. The volume of organic eluents used was considerably reduced by miniaturization [10].

In an early multimethod applying the mass-selective detector HP MSD 5970, a random sample of 76 pesticides was studied [11]. Mass spectra of good quality were produced at the relevant residue concentration levels and 72 of them were identified by means of library search in the NBS mass spectral library with McLafferty's PBM search routine [12], the missing four were found to be not included in the library. The critical detection limit of 0.01 ppm was reached by all of 18 chlorinated pesticides spiked in green pepper using selected ion monitoring (SIM) with three indicative ions [11]. The positive outcome of this study encouraged us to create a dedicated pesticide mass spectral library [13]. However, in full scan mode a great number of peaks are recorded and must be evaluated. This, and the lack of detection sensitivity for a few well known active ingredients may be the reason why GC–MS is not usually applied for universal screening but more for target compound analysis with SIM. Fillion et al. [14] presented in 1995 a multiresidue determination method for fruit and vegetables with GC–MS applying SIM for the screening and quantitation of 189 pesticides. Two injections per sample were required to cover all compounds. Positive analytes were confirmed by retention time and ion ratios. The method demonstrated acceptable performance for a

variety of crops with limits of detection (LODs) ranging from 0.02 to 0.2 ppm depending on the pesticide. The method has been used for the analysis of over 1000 samples by Agriculture and Agri-Food Canada at the time of publication. In 1998 De Kok et al. [15] reported the implementation of a multimethod for the determination of 365 pesticides (including some metabolites and isomers) for fruits and vegetables in all 13 regional laboratories of the Inspectorate for Health Protection in the Netherlands. The ultimate analysis scheme has evolved through the last 15 years and is based on a miniaturized Luke extraction applying acetone for extraction followed by dichloromethane and petroleum ether in a procedure without GPC. The final chromatographic determination is carried out with a battery of four parallel operating gas chromatographic systems applying ECD, NPD and ion trap detection (ITD) (Varian Saturn II) with SE-54 capillary columns and a OV-1701 capillary column with flame photometric detection in phosphorus mode resulting in a wide pesticide coverage. Many of the pesticides can be detected and identified with LODs of 0.01 ppm with GC–ITD applying an automated identification/quantitation method. However, this low method detection limit cannot be achieved with all pesticides, since it depends on the chemical structure as well as the gas chromatographic behavior of the individual compound.

Another selective detection method well suited to pesticide residue screening analysis is element-specific atomic emission detection (AED). Its potential was demonstrated in 1996 with 385 pesticides by Stan and Linkerhägner [16]. The combination of atomic emission detection for pesticide residue screening analysis with GC–MS as a confirmatory method was elaborated for over 400 pesticides applying retention time locking by Wiley and Quimby [17] and recently reported by Cook et al. [18] as a complete system for pesticide residue screening analysis where all components are necessarily operating in an integrated network. The system appears very sophisticated but the paper does not address adequately the problems arising from the different detection sensitivities of MS and AED.

This paper reports the ''ultimate'' analysis scheme for pesticide residue screening analysis which has evolved over the last 20 years in our laboratory. It is based on a miniaturized DFG S19 extraction applying acetone for extraction followed by LLE with dichloromethane or these days with ethyl acetate–cyclohexane followed by GPC. The final chromatographic determination is carried out with a battery of three parallel operating gas chromatographic systems using effluent splitting to ECD and NPD, one with a SE-54 the other with a OV-17 capillary column and the third one with a SE-54 capillary column with mass-selective detection (HP MSD) for identification and quantitation. The method is established for monitoring more than 400 thermostable pesticides. These pesticide residues are identified in screening analyses by means of the dedicated mass spectral library PEST.L containing reference mass spectra and retention times of more than 400 active ingredients and also metabolites applying the macro program AuPest (Automated residue analysis on Pesticides) for automated evaluation which runs with Windows based HP ChemStation software.

2. Experimental

2.1. Gas chromatography with various detectors

GC analyses were performed with gas chromatographs from Hewlett-Packard (HP) Model 5890A Series, equipped with HP 7673A autosamplers, HP ECD and NPD systems and mass-selective detector HP 5970 MSD. The parameters are compiled in Table 1.

The analytical column was protected by a precolumn of 1 m length of the same type and dimension. This was installed between the injector and the analytical column by means of a glass press-fit connector. The precolumn is usually made from clean parts of used analytical columns, but retention gaps deactivated with phenylsilicone are also in use. Helium of 99.999% purity was used as carrier gas.

The hot splitless injector was equipped with a special liner (Cyclo-Uniliner for HP GCs, Restek), septum purge flow was 1 ml/min, inlet purge flow was 30 ml/min.

Interfacing the analytical columns with the two selective detectors was carried out by means of zero-volume ''Y press-fit'' connectors and fused-silica tubing of 0.2 mm inner diameter deactivated

Table 1
Parameters for operating the gas chromatographic systems

Parameter	GC-A	GC-F	GC–MSD
Column	HP-5 Crosslinked	Permabond OV-17	HP-5 Crosslinked
Distributer	Hewlett-Packard (Agilent Technologies)	Macherey–Nagel, Düren, Germany	Hewlett-Packard (Agilent Technologies)
Phase type	SE-54	OV-17	SE-54
Length	50 m	25 m	25 m
Inner diameter	0.32 mm	0.32 mm	0.2 mm
Film thickness	0.17 μm	0.25 μm	0.33 μm
Precolumn	1 m	1 m	1 m
Hot splitless injection			
Split open	1 min	1 min	1 min
Injector temperature °C	210	210	210
Oven temperature °C			
Initial temperature	100	100	100
Initial time	1	1	1
Rate	30	10	30
Final temperature	150	140	150
Final time	2	0	2
Rate A	3	3	3
Final temperature A	205	170	205
Final time A	0	0	0
Rate B	10	10	10
Final temperature B	260	280	260
Final time B	25	17	29
Detector temperature			
ECD	300	300	
NPD	280	280	
MS			260
Range ECD	2	2	
Range NPD	0	0	

with phenylsilicone parallel into ECD and NPD. A split ratio of 2:1 for the effluent from the analytical column was obtained by varying lengths of the splitting tubes, 10 cm to the NPD system and 20 cm to the ECD system to address the different response sensitivities of the two detectors. Press-fit connectors were from Chrompack, Middelburg, NL (Universal Quickseal Connector/Splitter).

2.2. Signal processing from ECD and NPD

Analogue signals obtained from ECD and NPD were recorded in parallel, digitized and transmitted to a personal computer (80486) running under MS Windows 95 and processed with PE Nelson Analytical Chromatography Software Turbochrom V.4.1 (Perkin-Elmer). The ranges and attenuations for each detector channel were adjusted to produce approximately the same peak size for the internal standard chlorthion. The signals obtained from the more sensitive ECD were usually recorded with a range value of two and the less sensitive NPD with a range value of zero.

The quality of the system was checked with standard solutions and one special mixture containing aldrin, chlorthion and captan each at 1 ng/μl in toluene. This mixture was also used to check the retention times and to adjust the carrier gas head pressure so that aldrin appears exactly at 25.00 min on the SE-54 column and 21.00 min on the OV-17 column, respectively.

Evaluation of the chromatograms was carried out by comparison of peak signals with those of reference data such as given in Table 2.

2.3. Mass spectrometry

MS measurements were performed with electron

Table 2
Retention data of over 400 pesticides and contaminants[a]

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
2-Phenylphenol	**0.458**				
3,4-Dichloroaniline	**0.378**	0.433	≪	<	0.540
3,4-Dichlorphenylisocyanate	**0.270**	0.334	*	*	0.328
4-Chloroaniline	**0.216**	0.282		*	0.294
Acephate	**0.382**	0.418		***t**	0.631
Acetochlor	**0.899**	0.906	*	<	
Aclonifen	**1.208**	1.245	<	<	
Alachlor	**0.932**	0.932	*	*	
Aldrin	**1.000**	1.000	*		1.000
Allethrin	**1.080**	1.092	*		1.057
Ametryn	**0.935**	0.934		*	
Amidithion	**0.996**	0.998	*	*	
Aminocarb	**0.761**	0.759	*	≪	
Amitraz	**1.460**	1.490		<*	1.340
Ancymidol	**1.185**	1.218	≪	*	1.220
Anilazine	**1.064**	1.084	<	<	
Aramite I	**1.175**	1.220	≪		1.151
Aramite II	**1.162**	1.189	≪		1.163
Atraton	**0.708**	0.709		*	
Azamethiphos	**1.224**	1.262	≪	<	
Azinphos-ethyl	**1.522**	1.547	<	*	1.515
Azinphos-methyl	**1.434**	1.469	<	*	1.483
Aziprotryne	**0.817**	0.818	<	<	
Azobenzene	**0.586**	0.599	<	*	0.737
Barban	**1.148**	1.173	*		
Benalaxyl	**1.256**	1.281		≪	1.252
Benazolin-ethyl	**1.074**	1.078	*	<	
Bendiocarb	**0.643**	0.642		<	
Benfluralin	**0.650**	0.654	*	*	
Benodanil	**1.226**	1.266	*	<	
Bentazone	**1.041**	1.058	<**t**	<**t**	
Benzoylprop-ethyl	**1.452**	1.504	<	<	
Bifenox	**1.390**	1.425	*	≪	
Bifenthrin	**1.348**	1.388	*		1.249
Binapacryl	**1.183**	1.210	*	<	
Biphenyl	**0.342**				
Bitertanol	**1.592**	1.599		*	1.460
Bitertanol	**1.608**	1.615		<	1.470
Bromacil	**0.989**	0.994	*	<	
Bromocyclen	**0.858**	0.850	*		
Bromophos	**1.050**	1.051	*	*	
Bromophos-ethyl	**1.099**	1.124	*	*	
Bromopropylate	**1.350**	1.390	*		1.289
Bromoxynil	**0.648**	0.676	*	<	0.887
Bromuconazole	**1.404**	1.426	*	*	
Bupirimate	**1.167**	1.196	<	*	1.182
Buprofezin	**1.160**	1.190	<	*	1.154
Butachlor	**1.128**	1.139	<	<	
Butralin	**1.029**	1.047	<	<	1.033
Butylate	**0.368**	0.407		<	0.382
Cadusaphos	**0.655**	0.662	<	*	0.775

Table 2. Continued

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
Captafol	**1.308**	1.337	*	≪	
Captan	**1.083**	1.090	*	≪	1.163
Carbaryl	**0.928**	0.932	≪	<	1.064
Carbetamide	**1.024**	1.025			
Carbofuran	**0.732**	0.728		≪	0.952
Carbophenothion	**1.291**	1.281	*	*	
Carbosulfan	**1.394**	1.378		<	
Carboxin	**1.161**	1.184	≪	<	
Chinomethionat	**1.057**	1.078	*	*	
Chlorbenside	**1.083**	1.107	*		
Chlorbufam	**0.730**	0.728	≪ **t**	<**t**	
Chlordimeform	**0.618**	0.634		*	0.777
Chlorfenprop-methyl	**0.544**	0.561	*		
Chlorfenson	**1.137**	1.154	*		
Chlorfenvinphos	**1.084**	1.092	*	*	
Chlorflurenol-methyl	**1.089**	1.114	<		
Chlormephos	**0.387**	0.397	<	*	
Chlorobenzilate	**1.193**	1.224	<		1.192
Chloroneb	**0.456**	0.481	*		
Chloropropylate	**1.192**	1.223	<		1.178
Chlorothalonil	**0.829**	0.839	*	<*	1.016
Chlorpropham	**0.605**	0.623	<	*	0.759
Chlorpyrifos	**1.014**	1.017	*	*	1.052
Chlorpyrifos-methyl	**0.912**	0.912	*	*	1.011
Chlorthal-dimethyl	**1.028**	1.026	*		
Chlorthion	**1.030**	1.037	*	*	1.095
Chlozolinate	**1.074**	1.085	*	≪	1.090
Clomazone	**0.736**	0.752	*	*	0.933
Coumaphos	**1.644**	1.650	<	*	
Crotoxyphos	**1.083**	1.107	<	***t**	
Crufomate	**1.025**	1.042	≪	***t**	
Cyanazine	**1.021**	1.024	<	*	
Cyanofenphos	**1.256**	1.286	*	*	
Cyanophos	**0.773**	0.773	*	*	
Cycloate	**0.597**	0.598		*	
Cycluron	**0.769**	0.760		<	
Cyfluthrin	**1.713**	1.714	<	<	1.476
Cyfluthrin	**1.734**	1.735	<	<	1.483
Cyfluthrin	**1.752**	1.750	<	<	
Cyfluthrin	**1.762**	1.758	<	<	1.495
Cyhalothrin	**1.446**	1.478	≪		1.307
Cyhalothrin	**1.474**	1.505	<	≪	1.320
Cyhexatin	**1.467**	1.520	*	*	
Cymoxanil	**0.560**	0.600	<	*	
Cypermethrin I	**1.784**	1.777	*	*	1.525
Cypermethrin II	**1.808**	1.798	*	*	1.537
Cypermethrin III	**1.818**	1.816	*	*	1.549
Cypermethrin IV	**1.826**	1.824	*	*	
Cyprodinil	**1.058**	1.070		*	1.098

Table 2. Continued

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
Cyprofuram	**1.193**	1.229	<	<	
D (2,4-) isobutylester	**0.844**	0.846	*		
D (2,4-) methylester	**0.603**	0.605	*		
Dazomet	**0.706**	0.705		*	
DB (2,4)-Methyl	**0.856**	0.859	<		
o,p′-DDD	**1.152**	1.186	*		1.176
p,p′-DDD	**1.207**	1.238	*		1.195
o,p′-DDD	**1.103**	1.124	*		1.120
p,p′-DDE	**1.144**	1.172	*		1.148
o,p′-DDT	**1.210**	1.244	*		1.194
p,p′-DDT	**1.255**	1.300	*		1.209
Deltamethrin	**2.340**	2.265	<	<	1.900
Demephion	**0.496**	0.518		*t	0.729
Demeton	**0.712**	0.711	<<	*	
Demeton-*S*-methyl	**0.574**	0.587	<	*	0.801
Desethylatrazine	**0.628**	0.631		<	
Desethyl-desisopropyl-atrazine	**0.525**	0.533		<	
Desisopropylatrazine	**0.614**	0.623		<	
Desmetryn	**0.888**	0.879		*	
Dialifos	**1.507**	1.560	*	<	
Diallate I	**0.668**	0.664	<	<	
Diallate II	**0.689**	0.684	<	<	
Diazinon	**0.800**	0.796	<	*	0.900
Dichlobenil	**0.311**	0.361	*	*	0.433
Dichlofenthion	**0.896**	0.886	*	*	
Dichlofluanid	**0.990**	0.991	*	<	1.062
Dichlone	**0.810**	0.812	*t		
Dichloro-4,4′-dibenzophenone	**1.022**	1.023	*		1.070
Dichlorprop-Methyl	**0.587**	0.590	*		
Dihlorvos	**0.241**	0.296	*	*	0.312
Diclobutrazole	**1.165**	1.191	*	<	
Dicloflop-methyl	**1.269**	1.312	*		
Dicloran	**0.698**	0.717	*	<	0.910
Dicofol	**1.366**	1.324	<*		1.270
Dicrotophos	**0.625**	0.645	<<	*	0.892
Dieldrin	**1.156**	1.176	*		1.157
Diethofencarb	**1.004**	1.008		<	1.044
Difenoxuron	**1.009**	1.002		<	
Dimethachlor	**0.892**	0.888	*	<	
Dimethenamid	**0.893**	0.890	<	<	0.980
Dimethipin	**0.743**	0.742	*		
Dimethoate	**0.708**	0.720	*t	*t	0.958
Dinobuton	**1.092**	1.099	*	<	
Dinocap I	**1.275**	1.318	<<	<<<	
Dinocap II	**1.296**	1.340	<<	<<<	
Dinocap III	**1.340**	1.388	<<	<<<	
Dinoseb	**0.818**	0.813	<	<	
Dinoseb acetate	**0.942**	0.952	*	<	
Diphenamid	**1.050**	1.055		*	
Diphenylamine	**0.571**	0.592		<<	0.784

Table 2. Continued

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
Dipropetryn	**0.981**	0.994	≪	*	
Disulfoton	**0.814**	0.805	<	*	
Ditalimphos	**1.118**	1.146	*	*	
DMSA: Dichlofluanid metabolite	**0.609**	0.628		*	0.888
DNOC	**0.572**	0.591	*	≪	
Dodemorph I	**1.040**	1.040		<	0.970
Dodemorph II	**1.067**	1.071		<	0.995
α-Endosulfan	**1.260**	1.302	*		1.270
β-Endosulfan	**1.120**	1.136	*		1.121
Endosulfan-beta	**1.197**	1.232	*		1.220
Endrin	**1.183**	1.210	*		1.175
EPN	**1.334**	1.392	*	*	
Epoxiconazole I	**1.248**	1.285	<	<	1.263
Epoxiconazole II	**1.316**	1.357	*	*	1.302
EPTC	**0.322**	0.359		*	
Etaconazole I	**1.206**	1.234	*	*	
Etaconazole II	**1.211**	1.240	*	*	
Ethalfluralin	**0.629**	0.630	*	<	
Ethiofencarb	**0.860**	0.864	<	*	0.752
Ethiolate	**0.216**	0.274		*	
Ethion	**1.209**	1.245	*	*	1.220
Ethofumesate	**0.982**	0.984	≪		
Ethoprophos	**0.594**	0.605	*	*	0.758
Etridiazole	**0.397**	0.437	*	*	
Etrimfos	**0.833**	0.835	<	*	
Fenamiphos	**1.136**	1.151		*	1.164
Fenarimol	**1.497**	1.538	*	*	1.442
Fenazaflor	**1.221**	1.259	<	≪	
Fenazaquin	**1.382**	1.419		*	1.334
Fenchlorphos	**0.938**	0.948	*	*	
Fenitrothion	**0.974**	0.976	*	*	1.062
Fenoxycarb	**1.338**	1.387		*	
Fenpiclonil	**1.333**	1.372			1.430
Fenpropathrin	**1.364**	1.399	<	<	1.287
Fenpropidin	**0.951**	0.952		*	
Fenpropimorph	**1.017**	1.011		<	
Fenson	**1.040**	1.040	*		
Fensulfothion	**1.204**	1.230	<	*	
Fenthion	**1.011**	1.012	≪	*	1.085
Fenvalerate I	**2.050**	2.009	*	*	1.690
Fenvalerate II	**2.124**	2.070	*	*	1.731
Flamprop-isopropyl	**1.206**	1.229	<	<	
Fluazifop	**1.036**	1.037		<	
Fluazifop-P-butyl	**1.184**	1.204		<	
Fluazinam I	**1.074**	1.267	*	*<	1.189
Fluazinam II	**1.266**	1.267	*	*<	1.189
Flubenzimine	**1.136**	1.166	*	<	
Fluchloralin	**0.810**	0.807	*	*	
Flucythrinate I	**1.846**	1.836	*	<	1.616

Table 2. Continued

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
Flucythrinate II	**1.896**	1.884	*	<	1.629
Fludioxonil	**1.152**	1.194	<	*	1.226
Flumetralin	**1.118**	1.142	*	<	1.214
Fluometuron	**0.585**	0.598			
Fluorodifen	**1.130**	1.159	*	≪	
Fluoroglycofen-ethyl	**1.516**	1.541	*	<	1.383
Fluotrimazole	**1.312**	1.340	*	<*	
Flurenol-butyl	**1.125**	1.130	*		
Flurochloridone	**1.035**	1.047	*	<	
Fluroxypyr	**0.847**	0.850	<	≪	
Flusilazole	**1.162**	1.190	≪	*	1.320
Flutriafol	**1.130**	1.141	≪	*	1.173
Fluvalinate I	**2.126**	2.076	*	*	1.610
Fluvalinate II	**2.152**	2.097	*	*	1.624
Folpet	**1.092**	1.102	*	<	1.170
Fonofos	**0.772**	0.777	*	*	
Formothion	**0.900**	0.860	*	*	
Fuberidazole	**0.925**	0.932		<	
Furathiocarb	**1.416**	1.446		<	
γ-HCH	**0.752**	0.757	*		0.914
HCH-α	**0.683**	0.683	*		
HCH-β	**0.736**	0.752	*		0.959
HCH-δ	**0.820**	0.825	*		
Heptachlor	**0.934**	0.929	*		
Heptachlorepoxide-*cis*	**1.080**	1.076	*		1.079
Heptachlorepoxide-*trans*	**1.081**	1.082	*		
Heptenophos	**0.528**	0.544	≪	*	0.733
Hexabromobenzene	**1.347**	1.395	*		
Hexachlorobenzene	**0.699**	0.700	*		0.818
Hexaconazole	**1.138**	1.166	*	*	1.117
Hexazinon	**1.284**	1.316	≪	<	
Imazalil	**1.143**	1.170	<**t**	<**t**	1.163
Ioxinyl	**0.986**	0.982	*	≪	
Iprodione I	**1.274**	1.279	<*		1.298
Iprodione II	**1.276**	1.330	*	<	1.298
Iprodione III	**1.332**	1.379	*	≪	1.298
Isazophos	**0.826**	0.828		*	
Isofenphos	**1.088**	1.092	*	*	
Isoindol-1,3-dione: Folpet metabolite	**0.400**	0.440	*	≪≪	0.448
Isomethiozin	**1.016**	1.019	<	<	
Isoprocarb	**0.487**	0.504		<	
Isopropalin	**1.056**	1.060	*	<	
Isoxaben	**1.492**	1.520	*	*	1.153
Jodfenphos	**1.074**	1.097	*	*	
Kresoxim-methyl	**1.164**	1.196	≪	*	1.207
Landrin-3,4,5	**0.624**	0.627		<*	
Lenacil	**1.268**	1.298		<	
Lindane	**0.752**	0.757	*		0.914
Linuron	**0.985**	0.988	*	<	

Table 2. Continued

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
Malaoxon	**0.916**	0.919	<	*	
Malathion	**0.996**	0.996	*	*	1.060
(2-Methyl-4- chlorophenoxy)acetic acid-Methyl	**0.470**	0.484	≪		
Mecarbam	**1.083**	1.093	*	*	1.119
Mecoprop-Methyl	**0.507**	0.517	≪		
Mefenpyr-diethyl	**1.318**	1.359	*	*	1.293
Mepanipyrim	**1.120**	1.146		*	1.180
Merphos	**1.070**	1.077	*	*	1.034
Merphos-oxon	**1.147**	1.170	*	*	1.135
Metalaxyl	**0.943**	0.940		*	1.030
Metamitron	**1.168**	1.200	*	*	
Metazachlor	**1.070**	1.073	*	*	
Methacriphos	**0.446**	0.472	*	*	0.593
Methamidophos	**0.228**	0.294		*	0.371
Methazole I	**0.605**	0.607	<	*	
Methazole II	**0.998**	0.995	<	<	
Methidathion	**1.106**	1.120	*	*	1.178
Methiocarb	**0.980**	0.974	≪	*	1.064
Methoprotryne	**1.167**	1.188		*	
Methoxychlor	**1.374**	1.401	<		
Metobromuron	**0.866**	0.864	*	<*	
Metolachlor	**1.012**	1.008	*	<	
Metribuzin	**0.903**	0.899	*	<*	
Mevinphos I	**0.371**	0.408	*	*	0.533
Mevinphos II	**0.395**	0.431	*	*	0.561
Mirex	**1.476**	1.492	*		
Molinate	**0.488**	0.507		*	
Monalide	**0.854**	0.854		≪	
Monocrotophos	**0.650**	0.668	≪	*	0.933
Monolinuron	**0.738**	0.738	<	<	
Myclobutanil	**1.165**	1.190	*	*	1.190
Naled	**0.624**	0.625	*	*	
Napropamide	**1.132**	1.151	<	*	1.161
Nitralin	**1.305**	1.351	*	<*	
Nitrapyrin	**0.415**	0.436	*	*	
Nitrofen	**1.183**	1.209	*	<	
Nitrothal-isopropyl	**1.015**	1.032	*	*	
Norflurazon	**1.254**	1.295	*	*	
Nuarimol	**1.286**	1.328	*	*<	1.286
Ofurace	**1.243**	1.285	*	*	1.309
Omethoate	**0.546**	0.569	<**t**	***t**	
Oxadiazon	**1.180**	1.180	*	<*	1.140
Oxadixyl	**1.213**	1.252	≪	<	1.288
Oxycarboxin	**1.299**	1.344	*	<	
Paclobutrazole	**1.116**	1.124	<	*	
Paraoxon	**0.946**	0.940	*	*	1.040
Paraoxon-methyl	**0.840**	0.812	*	*	
Parathion	**1.016**	1.020	*	*	1.068
Parathion-methyl	**0.912**	0.913	*	*	1.032
PCB 101	**1.114**	1.128	*		1.111
PCB 138	**1.270**	1.307	*		1.238
PCB 153	**1.227**	1.261	*		1.197

Table 2. Continued

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
PCB 180	**1.394**	1.428	*		1.302
PCB 28	**0.892**	0.892	*		0.963
PCB 52	**0.972**	0.971	*		1.012
Pebulate	**0.404**	0.432		*	
Penconazole	**1.071**	1.082	*	*	1.096
Pencycuron	**0.650**	0.650		*	
Pendimethalin	**1.072**	1.076	*	<	
Pentachlorobenzene	**0.484**	0.506	*		
Pentachlorphenol	**0.744**	0.754	*		0.893
Pentanochlor	**0.969**	0.981	≪	<	
Permethrin I	**1.590**	1.614	*		1.426
Permethrin II	**1.614**	1.636	*		1.440
Perthane	**1.178**	1.212	*		
Phenkapton	**1.356**	1.405	*	*	
Phenothrin I	**1.392**	1.429	≪		
Phenothrin II	**1.416**	1.442	<		
Phenthoate	**1.084**	1.093	*	*	
Phorate	**0.673**	0.673	≪	*	
Phosalone	**1.426**	1.470	<	<	1.387
Phosmet	**1.347**	1.387	*	*	1.400
Phosphamidon I	**0.807**	0.800	<	<	0.964
Phosphamidon II	**0.898**	0.888	*	***t**	1.019
Phoxim I	**1.112**	1.116	<	*	1.156
Phoxim II	**1.120**	1.130	<	*	1.163
PHTHAL-BUTYLBENZYL	**1.254**	1.287	*		1.256
PHTHAL-DI(2-ETHYLHEXYL)	**1.407**	1.440	*		1.250
PHTHAL-DIBUTYL	**0.985**	0.984	*		1.012
PHTHAL-DICYCLOHEXYL	**1.372**	1.422	*		
PHTHAL-DIETHYL	**0.537**	0.557	*		0.727
PHTHAL-DIISOBUTYL	**0.871**	0.875	*		0.955
PHTHAL-DIISONONYL	**1.499**	1.552	*		
PHTHAL-DIMETHYL	**0.391**	0.425	*		0.447
PHTHAL-DI-N-OCTYL	**1.628**	1.646	*		1.350
PHTHAL-DIPHENYL	**1.398**	1.449	*		
PHTHAL-DIPROPYL	**0.761**	0.760	*		
Piperonyl butoxide	**1.330**				
Pirimicarb	**0.862**	0.860		*	1.000
Pirimiphos-ethyl	**1.051**	1.056	≪	*	1.064
Pirimiphos-methyl	**0.979**	0.978	≪	*	1.030
Prochloraz	**1.654**	1.674	<	<	1.478
Procymidone	**1.094**	1.109	*	<	1.118
Profenofos	**1.139**	1.168	<	*	
Profluralin	**0.776**	0.774	*	*	
Promecarb	**0.664**	0.670		<	
Prometon	**0.726**	0.724		*	
Prometryn	**0.949**	0.939		*	
Propachlor	**0.572**	0.582	*	<	
Propamocarb	**0.340**	0.380		*	
Propanil	**0.899**	0.908	*	*	
Propargite	**1.291**	1.342	<		1.243

Table 2. Continued

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
Propazine	**0.748**	0.744	≪	*	
Propetamphos	**0.792**	0.766	<	*	
Propham	**0.392**	0.430		*	0.502
Propiconazole I	**1.260**	1.291	<	<*	
Propiconazole II	**1.270**	1.301	<	*	
Propoxur	**0.570**	0.580		<	
Propyzamide	**0.777**	0.772	*	<	
Prosulfocarb	**0.947**	0.949		*	
Prothiophos	**1.146**	1.163	*	*	
Prothoate	**0.901**	0.907	*	*	
Pyrazophos	**1.508**	1.538	<	<	1.414
Pyrethrin (Cinerin I)	**1.162**	1.184	*		
Pyrethrin (Cinerin II)	**1.420**	1.452	*		
Pyrethrin (Jasmolin I)	**1.204**	1.236	≪		
Pyrethrin (Jasmolin II)	**1.504**	1.533	*		
Pyridaben	**1.616**	1.641	*	<	1.445
Pyridaphenthion	**1.342**	1.372	*	*	1.351
Pyrifenox	**1.128**	1.128	*	<	1.140
Pyrimethanil	**0.794**	0.795		*	0.931
Quinalphos	**1.090**	1.095	<	*	1.126
Quinomethionate	**1.107**	1.118	*	<	
Quinoxyfen	**1.253**	1.298	*	*	1.250
Quintozene	**0.764**	0.770	*	≪	0.838
Quizalofop-ethyl	**1.823**	1.817	*	*	
Resmethrin	**1.288**	1.324	<		
Resmethrin	**1.296**	1.332	<		
Sebuthylazine	**0.849**	0.842	≪	*	
Secbumeton	**0.820**	0.813		*	
Simazine	**0.727**	0.726	≪	*	
Simetryn	**0.938**	0.920		*	
Spiroxamin	**0.970**	0.964		≪	
Sulfotep	**0.663**	0.663	≪	*	
Sulprofos	**1.217**	1.254		*	
Swep	**0.734**	0.734	<	*	
2,4,5-T, methyl ester	**0.783**	0.783	*		
Tebuconazole	**1.290**	1.324	≪	*	1.260
Tebufenpyrad	**1.368**	1.405	≪	*	1.290
Tebutam	**0.656**	0.659		<*	0.766
Tecnazene	**0.580**	0.584	*	≪	
TEPP	**0.518**	0.521	*		
Terbufos	**0.769**	0.767	<	*	
Terbumeton	**0.744**	0.748		*	
Terbuthylazine	**0.772**	0.769	<	*	
Terbutryn	**0.976**	0.980	*	*	1.179
Tetrachlorvinphos	**1.120**	1.135	*	*	
Tetraconazole	**1.033**	1.044	<*	*	1.053
Tetradifon	**1.406**	1.443	*		1.377
Tetramethrin I	**1.336**	1.368	<*	<	
Tetramethrin II	**1.350**	1.384	*	<	1.311
Tetrasul	**1.228**	1.257	*		
Thiabendazole	**1.076**	1.093		*t	1.186

Table 2. Continued

Pesticide	**MS RRT**	GC-A, RRT	ECD	NPD	GC-F, RRT
Thiocyclam	**0.448**	0.476	*	<	0.585
Thiometon	**0.692**	0.692	*	*	
Thionazin	**0.568**	0.577	<	*	
Tiocarbazil	**1.032**	1.033	≪	*	
Tolclofos-methyl	**0.923**	0.923	*	*	1.028
Tolylfluanid	**1.074**	1.085	*	<	1.117
Triadimefon	**1.026**	1.025	*	*	1.045
Triadimenol I	**1.099**	1.098	<	*	1.100
Triadimenol II	**1.099**	1.110	<	*	1.106
Tri-allate	**0.836**	0.826	*	*	
Triazophos	**1.227**	1.266		*	
Trichlorfon	**0.412**	0.386	*	*	
Trichloronat	**1.025**	1.043	*	*	
2,3,5-Trichlorophenol	**0.304**	0.360	*		
Tridiphane	**0.949**	0.943	*		
Trietazine	**0.776**	0.770	≪	*	
Trifluralin	**0.640**	0.644	*	*	
Vamidothion	**1.116**	1.124	*	*	1.201
Vernolate	**0.387**	0.420		*	
Vinclozolin	**0.911**	0.912	*	<	0.978

[a] RRT: relative retention time with respect to aldrin which elutes after 25.00 min on MS and GC-A and after 21.00 min on GC-F with the carrier gas pressure adequately adjusted as described in Section 3.4. Response to ECD and NPD: *good, < weak, ≪ very weak, t=peak shows tailing. No RRT in column GC-F indicates no data available as described in Section 3.4.

impact ionization (EI) at 70 eV. In the full-scan mode, the scanned mass range was 50–550 u with a scan rate of 1.61 scans/s. Solvent delay was set to 5 min. Using SIM, three characteristic ions were selected for each compound and scanned using corresponding time windows with dwell times of 100–200 ms per ion. MS tuning was performed weekly by using the autotuning macro. Precolumn and insert liner were exchanged at least after every 50 injections, but the quality of the system was checked daily with standard solutions and the same special mixture used for the ECD/NPD check containing aldrin, chlorthion and captan each at 1 ng/μl in toluene. This mixture was also used to check the retention times and to adjust the carrier gas head pressure such that aldrin appears exactly at 25.00 min.

Evaluation of all chromatograms obtained in pesticide residue screening analysis in the full-scan mode were automatically evaluated immediately after each run with the macro program AuPest. The proposals of potential positive pesticide results made by AuPest were checked manually using the interactive capabilities of the program.

2.4. AuPest

AuPest (Automated residue analysis on Pesticides) is a macro program developed in our group for automated evaluation which runs with HP ChemStation software together with the library PEST.L containing more than 400 active ingredients and also metabolites, environmental contaminants and derivatives of pesticides otherwise not amenable to GC. All target compounds are linked to their retention times measured under fixed conditions (25 m×0.2 mm SE-54 column with standard temperature program, given in Table 1). AuPest runs automatically after each full-scan GC–MS run and creates a number of reports. This needs much less time than the instrument requires for cooling and equilibration before running the next analysis.

The analyst receives all the results in tables together with the raw data which may be simultaneously accessed in the Standalone Data Analysis of the HP ChemStation after a sample sequence has finished.

The special feature of AuPest is that the analyst is aided by helpful tools when checking the results

reported. The display is divided into two parts: on the left, the AuPest results tables and simultaneously on the right the Standalone Data Analysis chromatography software with access to the raw data.

A mouse click in the report tables activates the corresponding chromatographic data such as peaks, mass spectra of the analyte in the samples together with that of the reference pesticide. In the right window, the analyst may work manually with all the commands provided by the HP ChemStation.

The evaluation of the full scan analysis is successively carried out at two levels. Firstly, all integrated peaks in the TIC chromatogram are checked with a special background subtraction procedure (Level 1). A special search is then performed for target pesticides overlapped by matrix compounds (Level 2).

2.4.1. Short description of the macro program

AuPest is written in HP ChemStation macro language and fully integrated into this software package. It consists of several parts.

AuPest Level 1 performs an automated library search of every automatically integrated peak against PEST.L or sequentially other user-defined libraries.

AuPest Level 2 uses reconstructed ion chromatograms (RICs) to search for compounds at very low concentrations. It is in particular useful to detect peaks that are overlapped by matrix compounds and therefore not recognized by the automated integration routine.

AuPest Setup is used to set user-defined parameters, e.g. the names of the additional mass spectral libraries for automatic searching, thresholds, time windows and so on.

AuPest Control-file editor is a program to build up the so-called control-files needed for the AuPest Level 2 search.

AuPest Results is a program for viewing, editing and printing the results obtained from Level 1 and Level 2 searches.

2.4.2. Description of AuPest Level 1

A click on menu point Level 1 starts the integration of the TIC according to user-defined integration parameters. The user can choose between autointegration or a special events file. After integration, every integrated peak is background subtracted by subtracting successively the mass spectra at peak start and peak end and then searched against PEST.L or sequentially other target compound libraries. It is also possible to specify a hit quality threshold, so that Level 1 only switches into the second library if this threshold is not met. Level 1 also performs a library search without background subtraction for each peak and checks whether the hit quality is better with or without background subtraction. The best result is stored. The results are compiled in different result tables which can easily be accessed by choosing *Results* from the AuPest menu.

The first results table, called AuPest *Results Level 1*, contains the complete analysis report of AuPest Level 1 including the sample description (sample name, corresponding data file, acquisition date, method file, operator) as well as the names of the target compound libraries. All integrated peaks are listed with their retention times and search results. If library search is successful, the three best matches of each library search are reported with their substance name, hit quality and reference number in the corresponding library and the retention time together with the expected retention time for each pesticide found by library search. Peaks with no search results are reported as such, with only their retention times.

In the second results table called *Important Peak List Level 1*, only those results of the library search are compiled which have met user defined thresholds for hit quality and retention time windows! For example, this list might contain only search results with a hit quality better than 80 and also all peaks with a hit quality less than 80, if exhibiting a difference in retention time smaller than 1 min from that laid down in the library for the suggested pesticide.

This also enables the recognition of pesticides (or other target compounds) overlapped by matrix components which produce poor library search results and emphasizes the retention time as a very important independent piece of information. On the other hand, any similarity between the mass spectrum of the sample peak and the reference spectrum must be considered as purely coincidental if the retention times of the reference target compound and sample peak are significantly different.

The user can switch between the tables by a mouse click or can display them together on the screen in parallel windows. Scrolling through a result

table, the user can immediately jump to the selected peak in the TIC chromatogram of the HP ChemStation software by mouse click in order to manipulate the data manually.

AuPest Level 1 prints all results to files, which speeds up the data analysis process considerably. The analyst can check the results first on the screen and can decide later what to print out. This saves printing costs and reduces paper consumption enormously.

2.4.3. Description of AuPest Level 2

If the pesticide (or another target compound) is overlapped by a co-eluate from the matrix, a manual evaluation must usually be performed. Such a manual verification procedure to confirm the identity of a compound generating a small peak overlapped by matrix or another substance takes advantage of reconstructed ion chromatograms. The analyst inspects ion traces indicative of the target compound and attempts to single out a characteristic mass spectrum by suitable background subtraction. A disadvantage of this technique is that it is very time consuming when applied to a great number of peaks.

AuPest Level 2 was developed to execute all these manual steps automatically. The only thing the user has to do is to create control-files for AuPest Level 2. This can easily be done with the control-file editor. Along with the name of the pesticide (target compound), three indicative ions and the retention time must be entered. One control-file can hold an almost unlimited number of pesticides (target compounds).

When starting the Level 2 search from the AuPest menu, Level 2 asks for the number of control-files that should be used for the search. Having started by simply marking the control-file names on the corresponding file list, Level 2 begins to check for the first target compound in the first control-file. This is done by redrawing the specified ion traces in a user-defined time window, usually 1 min, around the calibrated retention time of the pesticide. Only if Level 2 has found peaks in the traces of the indicative ions will it proceed to check whether peaks appear at the same retention time in the other two ion traces. If their difference in retention time is less than 0.03 min (default, but user-defined), Level 2 assumes that the three (or two) ions originate from the same target compound user-defined and continues by taking a scan at the apex of the peak followed by background subtraction. This is performed by subtracting the average of scans taken at the start and at the end of the peak. The resulting spectra are searched successively against PEST.L or another specified target compound library.

All positive search results are compiled in the AuPest *Results Level 2 Table* with a complete sample description (sample name, corresponding data file, acquisition date, method file, operator), the names of the control-files and the library used. Each peak with a positive search result is described by the scan number and the retention time where the spectrum has been taken and the three best hits.

If required, a visual comparison of the two mass spectra can confirm the presence of the indicative ions of the reference in the analyte or, in the negative case, their absence.

AuPest enables the analyst to decide on average within 5 min whether a pesticide is present and if so which specific one in the corresponding sample. AuPest also supports the estimation of the concentration level. A tutorial, demonstrating the various stages of the interactive checking of results with real samples, is available on the website: http://www.aupest.de.

2.5. Material

All solvents were Suprasolve products from Merck, Darmstadt, Germany and all analytical standards were supplied by Dr. Ehrenstorfer, Augsburg, Germany, Promochem, Wesel, Germany, Riedel-De Haën, Seelze, Germany.

2.6. Sample preparation – clean up with modified multimethod S19

2.6.1. Method A

A 30 g amount of homogenized plant material was extracted with 60 ml acetone. Before the extraction began 3 μg aldrin dissolved in 1.5 ml toluene was added as surrogate standard (SSTD) representing a pesticide concentration of 0.1 ppm. The filtered extract was saturated with sodium chloride and diluted with 40 ml dichloromethane, in order to separate excess water. After drying the organic phase

with sodium sulfate and filtration, the solvent was evaporated under vacuum by means of a rotary evaporator and the residue successively dissolved in 0.75 ml ethyl acetate and 0.75 ml cyclohexane. A 1 ml volume of this solution was cleaned up by gel permeation chromatography (see Section 2.6.3).

The eluate was evaporated to dryness, dissolved in 2 ml toluene and 1 ml each were transferred to two autosampler vials and injected into two of the gas chromatographic systems. A 2 μl volume of the extract was equivalent to 20 mg plant material. After a gas chromatographic run the vials were recapped and analyzed in the third instrument or stored in the refrigerator.

2.6.2. Method B

A 40 g amount of homogenized plant material was extracted with 78 ml acetone. Before the extraction began 4 μg aldrin dissolved in 2 ml toluene was added as surrogate standard (SSTD) representing a pesticide concentration of 0.1 ppm. A 16 g amount of sodium chloride and 40 ml ethyl acetate–cyclohexane (1:1) was added and again blended. After settling, 90 ml of the supernatant organic phase was decanted, dried with sodium sulfate and the solvent evaporated to dryness. The residue was successively dissolved in 0.75 ml ethyl acetate and 0.75 ml cyclohexane and 1 ml cleaned up by GPC (see Section 2.6.3). The eluate was evaporated to dryness and redissolved in 2 ml toluene. This was divided equally between two autosampler vials and injected into two of the gas chromatographic systems. A 2 μl volume of the extract was equivalent to 20 mg plant material. After the gas chromatographic run, the vials were recapped and analyzed in the third instrument or stored in the refrigerator.

Variation: if all the three GC instruments are simultaneously available, the final extract is equally divided into three portions.

Technical hint: we have found that equal division of the final extract is easily carried out by means of adjustable micropipettes with one-way pipette tips.

2.6.3. Gel permeation chromatography

A GPC system consisting of an isocratic HPLC pump, a sample loop of 1 ml, a 370×12.5 mm I.D. GPC glass column packed with Bio-Beads SX-3 and provided with a water jacket to maintain constant temperature, a three-way valve with time or sensor switch and two volumetric funnels for fraction collecting was used. The system is a homemade semiautomatic device. The injection is made manually by filling the extract into the sample loop of 1 ml with a syringe. The GPC is started after resetting all control parameters manually. The effluent is directed into the first volumetric funnel until the liquid surface reaches the sensor, the three-way valve is then automatically switched to transfer the effluent to the sample funnel. The run is finished when the liquid surface reaches the sensor in the sample funnel and the GPC system is ready for the next run.

Eluent: ethyl acetate–cyclohexane (1:1, v/v) at a flow-rate of 1 ml/min.

The first eluate is discarded (in our laboratory the eluent solvent is recycled by fractional distillation), the second eluate collected containing the pesticides is evaporated to dryness and dissolved in 2 ml toluene.

The exact elution volumes must be determined in each laboratory, the elution volumes with our GPC system are approximately 20 ml for the first fraction (to waste) and 25 ml for the second one which contains the pesticides.

Note that some separation between low-molecular weight compounds has been observed, therefore, the corresponding publications should be consulted and critical target pesticides checked.

2.6.4. Supplementary clean up

A supplementary clean up and fractionation after GPC was carried out on silica minicolumns for the few samples where matrix interference impairs peak identification or quantitation. The final extracts used for the GC analyses were collected and subjected to the minicolumn. Modifying the original method, the sample extract was separated into three fractions, namely fraction 1 with toluene, fraction 2 with toluene–acetone (80:20, v/v) and fraction 3 with acetone. Chlorthion was added to fractions 2 and 3 as internal standard (I.S.) to give a concentration corresponding to 0.2 ppm in the sample.

Variation: fractions 2 and 3 were combined before evaporation to dryness and handled as one fraction.

2.6.5. Preparation and handling of pesticide reference standards

Preparation of pesticide reference standards was carried out on a microbalance (1872 MP 8, Sartorius, Göttingen, Germany) which is connected to a personal computer running the dedicated windows based software ''BALANCE'', first described by Lipinski and Stan in 1987 as a DOS program [19]. The program is in permanent use in our laboratory (with adaptation to new Windows versions and a few upgrades).

Pesticide stock solutions were prepared by dissolving pesticide standards in toluene at 1 mg/ml. They are stored in glass bottles with PTFE-faced screw caps. Working solutions were prepared by mixing and dissolving stock solutions by means of special displacement micropipettes (transferpettors, Brand, Wertheim, Germany). All solutions are registered in the files of the BALANCE program allowing a permanent check of the age and quality of working standards and stock solutions.

2.7. Quantitative determination of pesticide residues

Quantitative determination of identified pesticide residues was carried out by adding the standard at the estimated concentration to a blank matrix extract. Blank matrix extracts are those from other crop samples analyzed recently using exactly the same extraction and clean up procedure. Following GC analysis and recapping, the sampler vials are stored in the refrigerator. This is the reason why portions of the final extract of a crop sample are transferred with a micropipette of fixed volume to the sample vials. This procedure guarantees a realistic matrix concentration in the spiked sample. The estimate of the spike concentration of the pesticide standard is made by experienced staff comparing the responses of the standard mixtures, run daily in the same sequence as the sample analyses, in the various detector traces with those found for the pesticide identified. These standard mixtures contain about 70 frequently found pesticides and are extended in numbers when new pesticides are found in the samples. Single-point calibration is usually carried out by injecting both the sample and the spiked sample one after the other into the GC instrument. The preferred quantitation method is GC–MS in SIM with three indicative ions and quantitative determination using the peak areas of all three ions obtained by manual evaluation. The calculation of the ion peak ratios is an additional confirmatory test which is obligatory for all pesticide residues found at low concentration levels where the match of full-scan spectra with the reference spectra is of lower quality. Quantitation can also be performed with GC–ECD or GC–NPD if the pesticide is already unequivocally identified by GC–MS. The column which showed the best peak resolution from the matrix is chosen for quantitative determination. For the most accurate quantitation of positive results, it is best to calibrate with two blank matrix samples spiked at concentration levels above and below the amount present in that particular sample based on initial estimation.

3. Results and discussion

3.1. Extraction and clean up

A number of extraction and clean up procedures are widely used in pesticide residue analysis, a university laboratory with its limited resources cannot presume to give a judgement based on own comparative experience. Therefore, only a few comments should be made on the multiresidue methods starting with acetone as extraction solvent. This method is known in Germany and other European countries as DFG S19 and in North America as the Luke method. For more than a decade, the DFG S19 method has been used in our laboratory with dichloromethane as solvent in the partitioning step, it has been used unchanged for the last 7 years in the version described as method A. The method works fine and is easy to handle, a major advantage being that the dried extract after the liquid–liquid partitioning is free of residual water. A solution of the residue in ethyl acetate and cyclohexane gives a clear extract. The drawback is the time consuming filtration step before the partitioning and the decreasing acceptance of dichloromethane in the laboratory due to health and safety concerns and environmental pollution risks.

We have worked for about 2 years with the ''online'' extraction method B and have found it less

time-consuming because the filtration step is omitted. However, the ''dry'' extract always contains a considerable quantity of residual water which makes it more difficult to handle. The use of a miniaturized GPC column requires the residue to be dissolved in only 1.5 ml of eluent. The problem is to transfer the organic phase from a small two phase volume contained in a relatively large round bottom flask. This small volume does not lend itself to binding of the residual aqueous phase with sodium sulfate since this would unavoidably lead to substance losses.

Applying the DFG S19 method, the GPC clean up is followed by a silica minicolumn fractionation resulting in up to seven fractions. The procedure was developed not only to separate pesticides only detectable with ECD from matrix interference, but also to avoid polychlorinated biphenyl (PCB) congeners overlapping the group of halogenated pesticides. This was of particular importance when using packed columns with their low resolution. With capillary columns available, fractionation into up to seven individual gas chromatographic samples was no longer necessary, resulting in a dramatic increase in productivity. In our laboratory, a stepwise reduction of silica minicolumn fractions occurred. For a long time we collected three, then later two fractions and now in general we do completely without this clean up step.

The reason for this change in our attitude is easy to explain: traditionally we carried out pesticide residue screening analysis on a 50 m×0.32 mm SE-54 capillary column with splitting of the effluent to the two parallel operating detection (ECD and NPD) [5]. The simultaneous signals from two selective detectors with different responses supported the reliability of the recognition of possible residues. First attempts were undertaken to automate the data evaluation with a program written in BASIC running on a programmable integrator by comparison of retention times on two columns and the signals in the two parallel detectors [20]. GC–MS at those times was only used for confirmatory analysis of critical samples. With the advent of the lower cost mass selective detectors of quadrupole or ion trap technology, mass spectrometric analyses became more easily available. Since the minicolumn clean up is only necessary to compensate for the insufficient selectivity of ECD, its application is not necessary in a screening procedure where mass spectral detectors with their higher selectivity serve as first target compounds identifier.

3.2. Pesticide residue analysis of a typical food sample from the market: strawberries

In Figs. 1 and 2, a first example is given to demonstrate the current status of our daily work in the laboratory. They show a typical set of chromatograms which we produce for each food sample: one TIC chromatogram on HP-5 with MS, and two parallel chromatograms on HP-5 and OV-17 showing the detector responses of ECD and NPD. We evaluate the samples in the chronological order MS chromatograms first with AuPest evaluation and then check

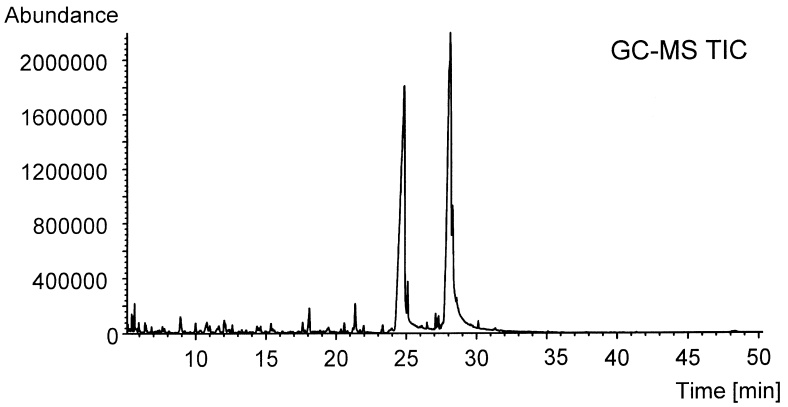

Fig. 1. TIC chromatogram on HP-5 of a strawberry sample.

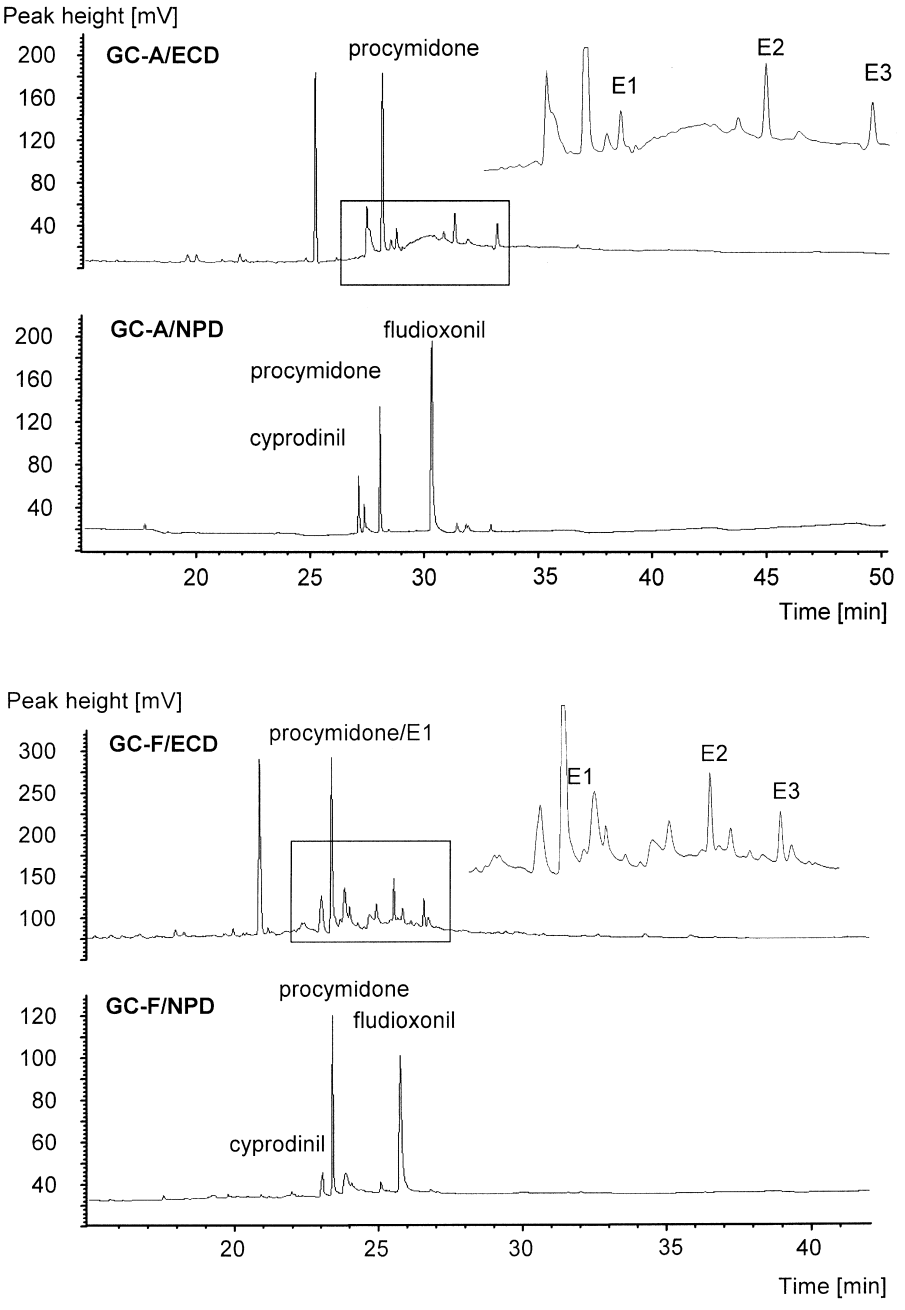

Fig. 2. Two chromatograms of a strawberry sample on HP-5 and OV-17 columns with parallel detection by ECD and NPD (experimental conditions see Section 2.1, identified residues as indicated, E1, E2=endosulfane isomers, E3=endosulfane sulfate).

the other chromatograms to see if the overall picture matches. As shown, we had to deal with a sample of strawberries grown in early summer, these fruits are vulnerable to insects and pathogenic fungi. The TIC chromatogram is dominated by two large peaks and shows a number of peaks which are relatively small

compared to the two dominating ones. Most of them are from the matrix because the TIC chromatogram shows all compounds amenable to GC without discrimination, the two large peaks are known to be free fatty acids. Automated data evaluation with AuPest allows us to extract the target compound information. Fig. 3 shows the monitor display after calling up the results of the strawberry sample.

This program is designed to automatically find out if any pesticide is in the sample. The results in the important peak list of AuPest Level 1 shown in Fig. 4 indicate the fungicides captan and procymidone. While procymidone was found with the highest match quality of 99 at the expected retention time, the other possibility, captan, with the low match quality of nine could easily be ruled out. In Fig. 5, interactive working with AuPest is demonstrated with the confirmation of procymidone in strawberries. The program allows to see the formula on mouse-click. The results in the important peak list of AuPest Level 2 shown in Fig. 4 also find the surrogate standard ALDRIN and another fungicide, namely cyprodinil. Both pesticide residues can be cross checked by inspecting the ECD and NPD chromatograms, the pesticides found are indicated in the graphical presentations in Fig. 6. The chromatograms in the ECD traces show a number of peaks but the surrogate standard ALDRIN representing a concentration level of 0.1 ppm produces together with procymidone the two most intense peaks. Since the response of the ECD to procymidone is six-fold less than that of ALDRIN, a concentration level of 0.6

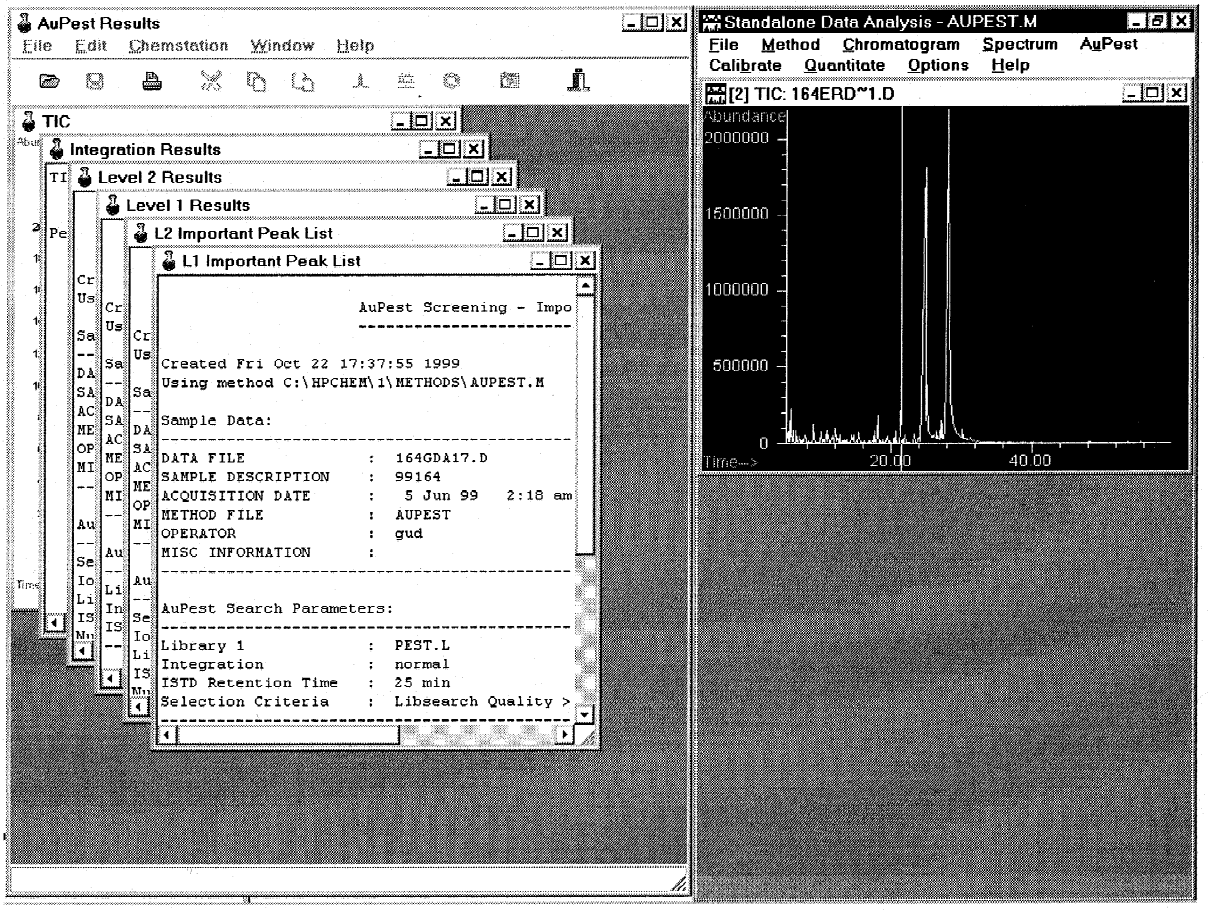

Fig. 3. Calling the results of the strawberry sample with AuPest.

AuPest Screening - Important Hits

Create Fri Jun 5 3:08:01 1999
Using method C:\HPCHEM\1\METHODS\AUPEST.M
Sample Data:

DATA FILE :	164GDA17.D
SAMPLE DESCRIPTION :	99164
ACQUISITION DATE :	5 Jun 99 2:18 am
METHOD FILE :	AUPEST
OPERATOR :	gud
MISC INFORMATION :	strawberries

AuPest Search Parameters:

Library 1 :	PEST.L
Integration :	normal
ISTD Retention Time :	25 min
Selection Criteria :	Libsearch Quality > 15 OR RT Difference < 1 min

Search Results:

Peak	Name	Ret. Time	exp.at	Q	Ref	Lib	Peak Area
20	CAPTAN	27.195	27.050	9	85	1	3049984
21	PROCYMIDONE	27.305	27.350	99	232	1	3412007

Fig. 4. AuPest Level 1 Important Peak List. Ret. Time=retention time in min, exp. at: Ret. Time of calibrated reference pesticides in min, Q: quality in %, Ref: entry number in library PEST.L, Lib: number of selected library, peak area in counts.

ppm was estimated, the final residue concentration level of procymidone was determined at 0.65 ppm. In the ECD chromatogram of GC-A, a pattern of three peaks was observed at 28.25, 30.72 and 32.45 min which is characteristic for α- and β-endosulfan and endosulfan sulfate. These cyclodiene insecticide molecules contain six chlorine atoms and are very sensitive indicated in the ECD trace, in mass spectral detection they show a less sensitive response due to their fragmentation into a large number of ions without any indicative intense main fragments. Thus, endosulfan residues at low concentration levels are always easier to spot in an ECD chromatogram than in a TIC chromatogram. In the TIC chromatogram shown in Fig. 1, no peaks can be seen at the corresponding retention times although it must be admitted that the scale is not in favor of small peaks. Nor did zooming bring any peak of the endosulfane pattern to light. The pattern was finally confirmed with the ECD chromatogram of GC-F where α-endosulfan, however, was overlapped by procymidone but the other two components were clearly observed. The residue concentration was determined to be 0.02 ppm. In the NPD chromatograms of GC-A and GC-F, a further intense peak was observed which did not have corresponding peaks in the ECD traces. From the retention data compilation given in Table 2, fludioxonil, a new fungicide, matches retention times on both columns as well as the detector response data. A check of the TIC chromatogram showed that the potential pesticide residue would be buried under the huge fatty acid peak. A manual search by extracting indicative ion chromatograms from the TIC brought the presence of fludioxonil to light. For the same reason ALDRIN was not found with AuPest Level 1 but only with AuPest Level 2. The difference between the two overlapped target compounds was the location under the overlapping peak, ALDRIN eluted with the end of the overlapping peak so that the

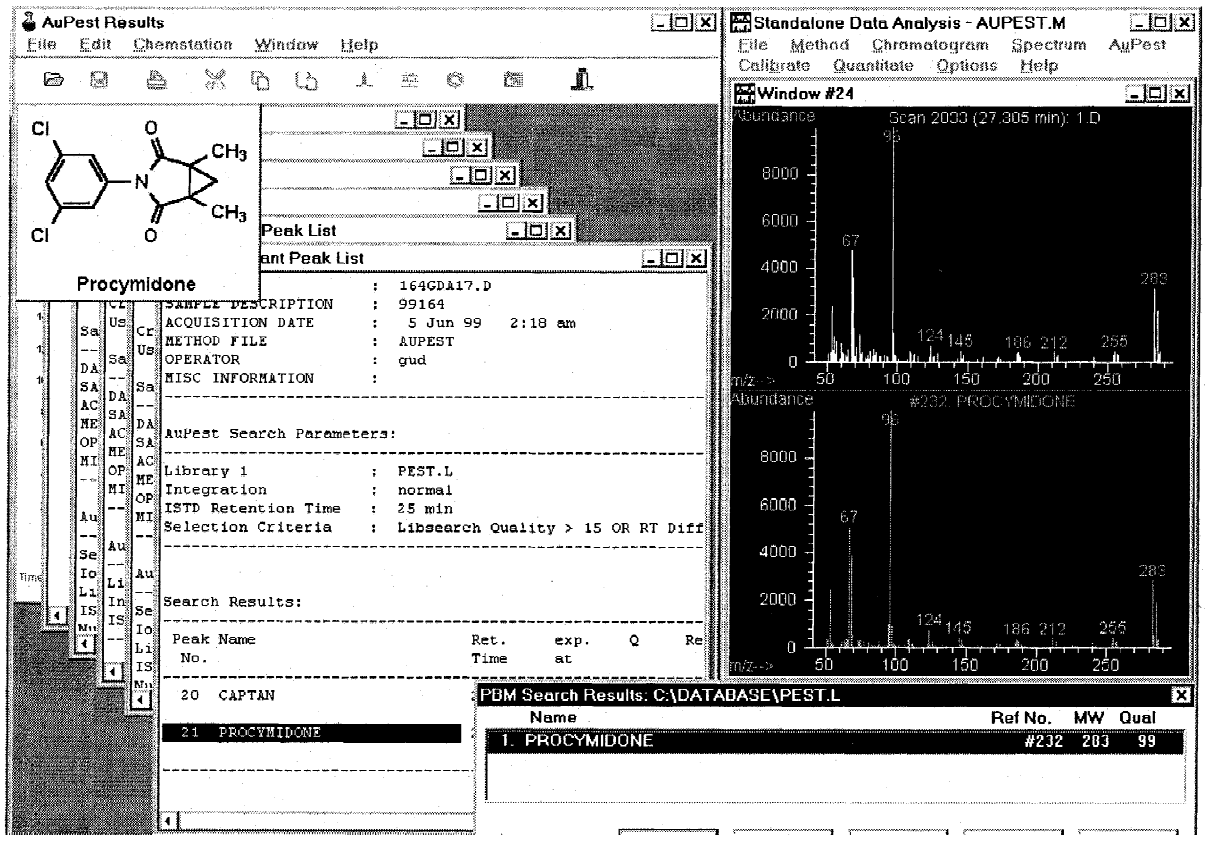

Fig. 5. Interactive working with AuPest.

dedicated background subtraction could be successful while on the other hand fludioxonil eluted more towards the center of the matrix peak.

3.3. Reliability of automated pesticide residue analysis using AuPest

It seems obvious that the application of an automated evaluation program such as AuPest increases the productivity of a laboratory doing daily routine pesticide residue analysis in a remarkable manner. The example selected demonstrated an extremely rare case that one pesticide residue could not be traced by AuPest due to its overlapping by a huge matrix peak. The situation, however, can be easily recognized by an observant analyst with the TIC chromatogram which shows the parts where trace compounds can be buried by matrix peaks. These parts need further manual inspection. Additionally, the two GC instruments with parallel ECD and NPD detectors produce chromatograms which are complementary in their data as demonstrated. Finally, all data have to fit unequivocally together although an explanation for every single peak seen in the ECD and also the NPD traces cannot be expected.

The following example deals only with the GC–MS system and should demonstrate the merits and the limits of AuPest and all automated evaluation programs. For the fast check of the performance of the GC–MS and AuPest, a calibration mixture of 61 pesticides was created, a TIC chromatogram is shown in Fig. 7. The mixture was injected at various concentration levels which easily can be made by dilution of a standard mixture. This approach allows

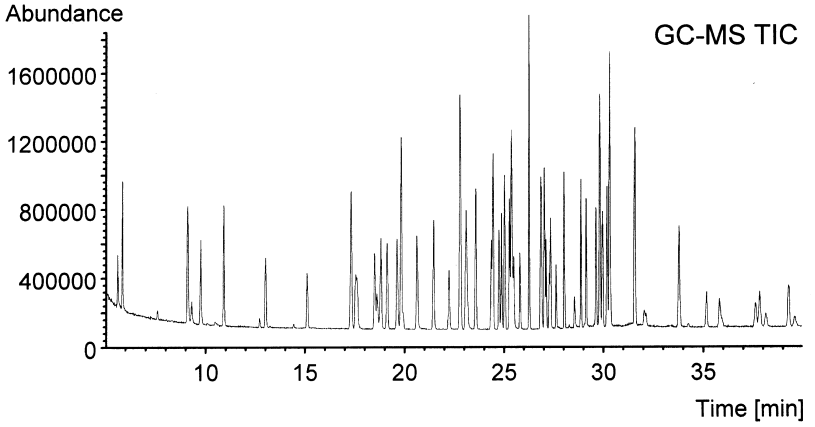

Fig. 7. TIC chromatogram on HP-5 of a calibration mixture of 61 pesticides 10 ng each.

the limits of automated data evaluation by means of AuPest has been determined. As can be drawn from Fig. 8, 57 pesticides have correctly been identified from a mixture of 61 pesticides with AuPest Level 1 at a concentration level of 10 ng for each pesticide injected. Two pesticides were not found with AuPest Level 1 because they were overlapped in critical pairs appearing in the TIC chromatogram as unresolved peaks. These two pesticides, however, were identified with AuPest Level 2 because their retention times exhibit a small difference allowing their resolution with the automated background subtraction procedure described. One critical triplet, however, was not resolved and therefore not recognized, namely chlorpyrifos-methyl, parathion-methyl and vinclozolin. These three pesticides elute with their peak maxima within 0.03 min. It turned out that with the PBM search algorithm only chlorpyrifos-methyl was identified because the isotope cluster of the molecular ion of chlorpyrifos-methyl is so dominating that other ions which are obviously there in a mixed mass spectrum are not considered by the library search algorithm. The situation is illustrated in Fig. 9 with parathion-methyl which can be easily found by means of the three indicative ions m/z 263, 125 and 109 as shown in window 3, but the PBM search algorithm does not identify the relevance of these ions in the mixed spectrum as shown for scan 1622. This means that if chlorpyrifos-methyl is found in a sample, a further search for the other two possibly overlapped pesticides has to be carried out.

3.4. Pesticide retention data

Over the years, a retention time list has been created and maintained. Our current list is given in Table 2.

As described in the Experimental section, we apply SE-54 silicone phase as the main screening column and have used, over the years, the same temperature program which gave us optimum resolution. A column length of 50 m with an inner diameter of 0.32 mm was found to give best results with respect to necessary resolution and analysis time. The surrogate standard ALDRIN was used from the beginning because this pesticide was rarely used in Europe and banned many years ago, ALDRIN was never found in vegetables or fruit. This compound served as well as an internal standard, for both adjustment and check of the retention time which was always set to 25.00 min by regulating the carrier gas pressure, as well as to check the ECD response. In it's function as a surrogate standard it indicated problems during extraction and clean up. Retention times were also collected later on the 25 m×0.2 mm HP-5 column used with GC–MS applying the same temperature program, the retention times turned out to be very well in concordance and are given here as relative retention times such as entered in our mass spectral library PEST.L. In the last 3 years, an OV-17 column was introduced replacing another of the same type but of different size and operated with a different temperature pro-

AuPest Screening - Important Hits

Created Wed Dec 09 09:39:42 1998
Using method C:\HPCHEM\1\METHODS\AUPEST.M
Sample Data:

DATA FILE :	61 CALI01.D
SAMPLE DESCRIPTION :	Calibration Mix A – 61 pesticides
ACQUISITION DATE :	10 Sep 98 1:04 pm
METHOD FILE :	GC-A
OPERATOR :	Joachim
MISC INFORMATION :	10 ng each pesticide

AuPest Search Parameters:

Library 1 :	PEST.L
Integration :	AUPEST.M
ISTD Retention Time :	25 min
Selection Criteria :	Libsearch Quality > 20 or RT Difference < 1 min

Search Results:

Peak	Name	RT	exp. at	Q	Ref	Lib	Peak Area
45	MEVINPHOS	9.097	9.275	94	81	1	27263894
46	ACEPHATE	9.305	9.800	87	168	1	6730988
49	PROPHAM	9.755	9.925	91	82	1	19198362
57	METHACRIFOS	10.906	11.100	91	89	1	26857259
79	TEPP	12.703	12.975	64	24	1	2021759
82	HEPTENOPHOS	13.010	13.200	96	90	1	17348288
106	CHLORPROPHAM	15.104	15.250	93	83	1	14812859
129	HEXACHLOROBENZEN	17.307	17.425	99	62	1	41053366
130	DICLORAN	17.538	17.475	99	175	1	16262157
131	DIMETHOATE	17.603	17.775	98	176	1	12350679
140	GAMMA-HCH	18.491	18.950	96	63	1	21974016
141	PENTACHLOROPHENOL	18.601	18.600	97	180	1	10710660
142	BETA-HCH	18.798	19.750	99	479	1	27860285
143	QUINTOZENE	19.116	19.250	97	72	1	24887143
146	PYRIMETHANIL	19.609	19.750	86	322	1	26109292
147	DIAZINON	19.829	19.950	83	91	1	52742921
152	CHLOROTHALONIL	20.618	20.725	98	288	1	28495321
159	PIRIMICARB	21.451	21.550	97	92	1	29304531
165	PHOSPHAMIDON	22.229	22.300	91	182	1	16162430
169	CHLORPYRIFOS-METHYL	22.788	23.000	86	268	1	68903721
170	TOLCLOFOS-METHYL	23.095	23.175	83	66	1	44578654
172	METALAXYL	23.578	23.450	96	242	1	36356566
180	FENITROTHION	24.356	24.375	99	243	1	18244978
181	PIRIMIPHOS-METHYL	24.444	24.475	94	93	1	40065042
183	DICHLOFLUANID	24.740	24.875	96	7	1	22285407
184	MALATHION	24.871	24.900	58	84	1	23480856
185	ALDRIN	25.025	25.000	90	230	1	34535840
186	FENTHION	25.277	25.300	97	77	1	26105967
187	CHLORPYRIFOS	25.364	25.400	99	74	1	58930554
188	4,4'-DICHLOROBENZOPHENONE	25.485	25.500	98	172	1	17062984

Fig. 8. AuPest Summary Report of the calibration mixture of 61 pesticides. Comments are entered by the analyst when checking the results of the automated evaluation. RT=retention time in min, exp.at: Ret. Time of calibrated reference pesticides in min, Q: quality in %, Ref: entry number in library PEST.L, Lib: number of selected library, peak area in counts.

Peak	Name	RT	exp. at	Q	Ref	Lib	Peak Area
90	CHLORTHION	25.803	25.750	64	171	1	15574568
194	PIRIMIPHOS-ETHYL	26.263	26.300	96	94	1	56413617
199	CHLOZOLINATE	26.855	26.850	93	75	1	45971080
200	MECARBAM	27.042	27.050	99	95	1	31522714
201	QUINALPHOS	27.118	27.100	99	185	1	16308887
202	FOLPET	27.283	27.300	99	86	1	8827841
203	PROCYMIDONE	27.349	27.350	94	96	1	22788443
205	METHIDATHION	27.623	27.350	91	97	1	13360104
208	ALPHA-ENDOSULFAN	28.028	28.000	91	68	1	29589405
213	IMAZALIL	28.543	28.475	97	186	1	8486241
214	DIELDRIN	28.872	28.875	95	61	1	28426091
215	O,P-DDD	29.048	30.475	40	360	1	1344373
216	BUPIRIMATE	29.146	29.025	98	195	1	25092725
222	CHLOROBENZYLATE	29.826	29.775	49	87	1	51813514
223	BETA-ENDOSULFAN	29.957	29.925	93	69	1	26224595
224	P,P-DDD	30.188	30.175	50	76	1	31690418
225	O,P-DDT	30.319	30.225	97	173	1	69554866
239	P,P'-DDT	31.580	31.500	83	174	1	55667450
244	DICOFOL	32.062	34.000	59	207	1	6789155
289	PHOSALONE	35.822	35.725	99	321	1	12159879
290	AZINPHOS-METHYL	35.943	34.725	50	245	1	2677225
310	FENARIMOL	37.609	37.325	87	204	1	11163782
311	PYRAZOPHOS	37.817	37.550	97	209	1	14909156
312	AZINPHOS-ETHYL	38.124	37.500	86	246	1	7209159
322	BITERTANOL	39.560	39.800	56	265	1	3549235

Comment: 57 pesticides including isomers found in Level 1 Important Peak List

239	TOLYLFLUANID	26.888	26.900	99	79	1	4843933

Comment: Tolylfluanid forms a critical pair with chlozolinate, peaks are not separated – therefore only found in Level 2

227	ETHION	30.303	30.275	88	80	1	13560604

Comment: Ethion forms a critical pair with o,p-DDT, peaks are not separated – therefore only found in Level 2

VINCLOZOLIN and PARATHION-METHYL not found with Level 1 and Level 2

Comment: Vinclozolin and parathion-methyl form a critical triplet with chlorpyrifos-methyl, peaks show absolutely no resolution –
therefore, vinclozolin and parathion-methyl could only be identified manually by their indicative ion traces!

Fig. 8. (*continued*).

gram. ALDRIN was always set to 21.00 min on the OV-17 column (GC-F) by regulating the carrier gas pressure. With this column, only the pesticides have been calibrated which are at present considered as relevant for fruit and vegetables. This is the reason for the lower amount of retention data reported for this column. On the other hand, the combination of GC-A and GC–MS will be reliable to spot the presence of those pesticide residues considered in many countries as outdated.

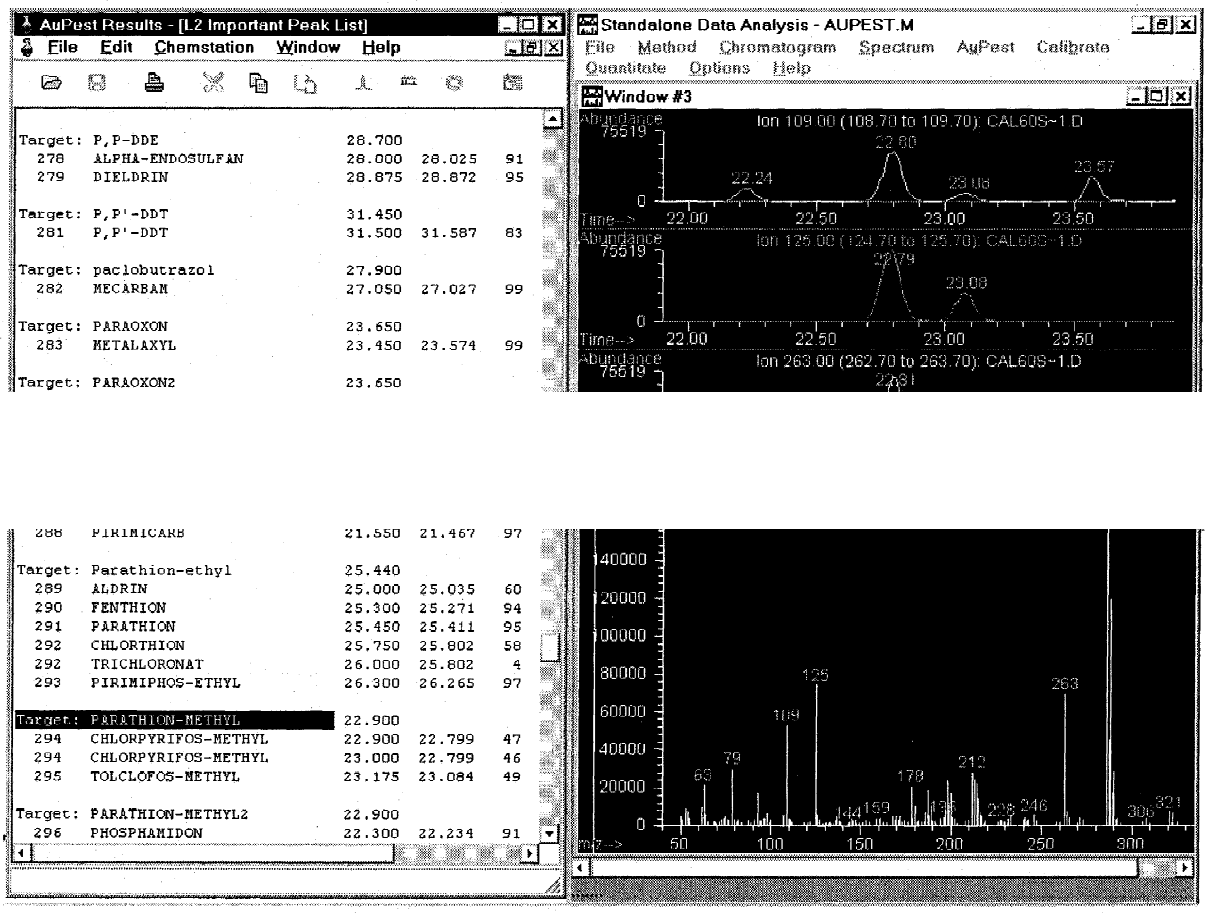

Fig. 9. Search for parathion-methyl in a critical pair with chlorpyrifos-methyl background subtraction produces no clean spectrum but always mixed spectra where chlorpyrifos-methyl is dominating.

3.5. Compatibility of AuPest with other mass spectral detectors

AuPest contains a mass spectral library of pesticides with their corresponding retention times under standard conditions and the automated evaluation procedure written in the HP ChemStation macro language. We recently received a GC–MS instrument Finnigan Magnum of 1992 as a gift from a German company. This mass spectral detector is based on ion trap technology and has a reputation for high detection sensitivity. In our hands, the instrument really does a good job exhibiting much higher detection sensitivity in pesticide residue analysis than our other instruments. TIC chromatograms obtained with this instrument are now converted into HP ChemStation format by means of MASSTransit (Palisade, Newfield, NY, USA) software developed for data interchange under Windows. AuPest has now been run for a few months with this procedure and has produced the same reliable results as with the HP GC–MS system. This means our experience may be considered as a contribution to the long lasting discussion about the applicability of mass spectral libraries created with quadrupole instruments for ion trap instruments.

3.6. Analytical setup and its proper application

Variation in retention times is less of a problem for

mass spectral detection as for selective detection such as ECD and also NPD. Therefore, the long column was used over the years to obtain resolution in the region around ALDRIN at 25.00 min where many relevant pesticides elute. Together with the two parallel detector responses we were able to identify many pesticides without always the mass spectral confirmation available. A very good complementary system is the OV-17 column also with two detectors in parallel which is the prerequisite for the reduction of the clean up. It is our experience that on one of the two columns a pesticide identified by GC–MS can be indicated. The most important use of these two columns with ECD and NPD is to recognize a few pesticides poorly identified with our GC–MS system which has already been in daily use for 14 years. The poor efficiency with a few pesticides is, however, not only a result of the age of the MS instrument but also inherent in the methods used. To give only a few examples: endosulfane appears in chromatograms almost always as three peaks, the α- and β-isomers and the oxidation product endosulfane sulfate exhibiting high responses in ECD but relatively poor responses in a mass spectral detector. They are bicyclic alkanes with six chlorine atoms causing the high sensitivity in the ECD response but they are fragmented into a great number of ions in EI with no really intense indicative ions. This results in problems with the mass spectral recognition procedures at low concentration levels where background ions cannot be completely subtracted. This is a common feature of all cyclodiene insecticides. Another situation is found with PCBs and toxaphene which are easily and very sensitively seen in ECD chromatograms but are less noticeable with mass spectral detection. Another example to mention is the widely used fungicide iprodione: this compound shows thermal degradation on HP-5 and all other SE-54 phases resulting in a typical chromatogram in ECD and also NPD which makes its recognition easy to an experienced analyst, with our OV-17 column however, no degradation is observed and the peak exhibits excellent shape. The degradation on HP-5 in our analytical set up has the consequence that iprodione is identified by mass selective detection only at higher residue concentration levels but on the other hand it certainly never has been overlooked.

3.7. Maintenance of the chromatographic systems

It is common experience that the efficiency of the separation deteriorates with use. However, a proper sample clean up and careful handling of the entire gas chromatographic system helps to reach impressive standing times. In our laboratory, on the two GC instruments with ECD and NPD, we run food samples in routine analyses as described in the Experimental section and also samples from students doing their experimental work in the university courses as well as developing new analytical methods including new derivatization procedures. On the GC–MS system, in parallel to our food samples, we run a series of water and also other environmental samples, all after derivatization. Although the instruments are busy all over the year, it is fascinating how robust the methods are at the present stage. With no obvious mistake by the users, the capillary columns can reliably be used for 600 to 900 samples and the ion source may keep its sensitivity for 3 months before cleaning is necessary. The prerequisite for such a long lasting performance, is according to our experience, the regular run of mixtures containing a number of compounds sensitive to the deterioration of the chromatographic conditions together with one less vulnerable compound. In addition to calibration samples, in our laboratory a mixture of aldrin, chlorthion and captan has proven to work very well. Aldrin is the most stable compound and serves also for retention time checking and adjustment, respectively. It exhibits medium mass spectral sensitivity. Chlorthion serves for checking the NPD response and captan is most vulnerable to degradation due to matrix deposits in the injector or at the beginning of the column. Observed degradation of captan triggers immediate exchange of the insert liner and if this does not restore the chromatographic quality, the pre-column must also be replaced. Following this regime rigorously is the prerequisite not only for reliable results but also pays with longer lifetime of costly separation columns.

The lifetime of the column is then only limited by its average thermal burden due to the decreasing film thickness as a result of column bleed as well as the ongoing cross-linking and polymerization. These latter phenomena are considered to be the cause of

retention time shifting which can be observed by running daily calibration mixtures containing the most affected analytes and doing retention time corrections in the daily evaluation procedures but not in the retention time data bank!

3.8. Combining mass spectral and atomic emission detectors

As already mentioned, the potential of the element-specific AED as another selective detection method well suited for pesticide residue screening analysis was demonstrated with 385 pesticides by Stan and Linkerhägner in 1996 [16]. After the determination of the detection limits of each pesticide in the corresponding element traces under routine laboratory conditions it turned out that extracts obtained with the clean up according to the principle of multimethod DFG S19 needed to be more concentrated than usual. After elimination of lipids and waxes by gel permeation chromatography, extracts from 10 g of the food samples had to be concentrated to 200 μl, of which 10 μl were introduced into the GC–AED with automated large-volume injection with programmed-temperature vaporization and solvent venting. The LODs achieved under these experimental conditions demonstrated the feasibility of screening analyses with the identification of pesticide residues down to the 0.01 ppm concentration level in plant foodstuffs. However, two analyses had to be usually performed for all samples with the element traces of sulfur, phosphorus, nitrogen and carbon in the first and chlorine and bromine in the second run. Fluorine was not sought in any screening analysis. The method has proven to be of great value especially with ''problem foodstuffs'' but seems costly for the average sample.

The combination of atomic emission detection for pesticide residue screening analysis with GC–MS as a confirmatory method was elaborated for over 400 pesticides and recently reported by Cook et al. as a complete system for pesticide residue screening analysis where all components are necessarily operating in an integrated network [18]. The system appears very sophisticated but the paper does not address adequately the problems arising from the different detection sensitivities of MS and AED. Most of the 44 pesticides analyzed in spiked samples could be detected at 0.1 to 0.5 ppm in these extracts. As in our earlier work demonstrated, the important element trace of nitrogen appeared as the Achilles heel of the method. Only large-volume injection enables GC–AED to achieve the detection sensitivity necessary to control maximum residue limits (MRLs) in all food samples [16]. Further studies in our laboratory showed that the introduction of the co-extractives of a 500 mg crop sample with each injection in routine analysis created a high burden for the GC–AED system which does not cause the performance of the chromatographic separation to deteriorate rapidly but impairs the sensitivity of the AED by carbonizing the discharge tube [21]. This is the reason why in our laboratory GC–AED has only been applied to several ''problem foodstuffs'' or environmental samples. The GC–AED is an analytical tool well suited for answering questions such as whether, for instance, sulfur or chlorine containing compounds are present in a sample. In our opinion, it is not the first choice for screening for specified target compounds such as pesticide residues in foodstuffs or environmental samples. Therefore, GC–AED in our laboratory is no longer applied in pesticide residue analysis. Due to its molar response to elements, however, it has been found to be unrivaled in quantitative analysis of tensides such as alkyl ethoxylates, for instance, consisting of hundreds of components where only a few compounds are available as reference standards [22].

4. General considerations and final conclusions

In a recent review of trace analysis of pesticides by gas chromatography, Van der Hoff and Van Zoonen [23] devoted a section to multiresidue methods applied to foodstuffs, in particular to fruits and vegetables. They stated that some consensus exists on the general approach in sample treatment with two accepted extraction procedures, namely with acetone followed by partitioning with dichloromethane (the Specht method) or dichloromethane and light petroleum (the Luke method) or extraction with

ethyl acetate in the presence of sodium sulfate. These procedures have been standardized at the national levels [24–28]. As described in this paper, we have long-term experience with the Specht method and more than 2 years experience with the modified Specht method in which dichloromethane is replaced by ethyl acetate–cyclohexane (1:1, v/v) [3]. The latter is less time consuming but needs more skill because of the residual water in the raw extract which is not so simple to remove with an excess of sodium sulfate in a miniaturized procedure such as ours. Although in many instances further clean up steps can be omitted to address the MRLs in the particular crop, we generally apply gel permeation GPC to clean up the raw extract with a small size column according to Tuinstra et al. [29]. This is a reasonable precautionary measure for the protection of the chromatographic system because we inject finally the equivalent of 20 mg of food sample onto the gas chromatographic column. This has proven to be necessary to reach the detection sensitivity for almost all pesticides in full scan mode which is dependent on the mass spectral fragmentation pattern of the individual target compound and also the performance of the GC–MS system. Recently, we acquired a Finnigan GC–MS Magnum from 1992 with ion trap technology which is reputed to exhibit excellent detection sensitivity in full scan mode. When analyzing our samples in parallel with the HP MSD 5970A from 1986, we found many additional pesticide residues by routinely running AuPest Levels 1 and 2 with the mass spectral data files converted into the HP ChemStation format. Quantitative estimations often resulted in pesticide residue levels of 1 ppb and even less. This is in full agreement with results recently reported by De Kok et al. [15]. In their routine pesticide residue analysis with a Varian Saturn II instrument based on the same ion trap technology as our Finnigan Magnum, they inject the equivalent of only 0.7 mg of food sample onto the gas chromatographic column. The much higher detection sensitivity of the gas chromatographic mass spectral detector allows reliable checking of the MRLs with a lower burden on the gas chromatographic system by food matrix. In De Kok's group, the throughput of samples is considerably increased by omitting the clean up step of GPC which obviously is possible without an impact on the life-time of their gas chromatographic systems.

Another approach for multiresidue analysis was reported by Fillion et al. [14]. They developed and evaluated a routine residue analysis for monitoring 189 pesticides in fruit and vegetables by GC–MS-SIM applying an HP MSD 5972A. Residues were extracted from food samples with acetonitrile and co-extractives were removed by a clean up step on a charcoal–Celite minicolumn. SIM analysis was performed time-programmed with retention time windows containing one target ion and two qualifiers for each target pesticide. Two injections were required per sample to cover all compounds. The method demonstrated acceptable performance for the analysis of a number of crops investigated, exhibiting limits of detection from 0.02 to 0.2 ppm depending on the compound. An equivalent of 4 mg of food sample was injected onto the gas chromatographic column. The drawback of this method, however, is the well-known fact that a few, but relevant, pesticides cannot be monitored because they are completely retained by the charcoal treatment. Such pesticides are chlorothalonil, dicloran, diphenylamine, hexachlorobenzene (HCB) and propanil. The method was later miniaturized and modified by substituting the charcoal–Celite minicolumn by a an activated carbon membrane, but the problem of retaining pesticides extracted from the food matrix and amenable to gas chromatography in the charcoal clean up could not be convincingly solved [30].

Therefore, it is our opinion that all clean up steps for the retention of matrix compounds by applying selective adsorption should be omitted because they are no longer absolutely necessary. The available gas chromatographic separation efficiency of capillary columns combined with the unrivaled detection selectivity of mass spectrometry and the new dimension of detection sensitivity of modern mass spectral detectors makes them superfluous. The most important aspect of the combination of gas chromatography and mass spectrometry applying electron ionization is that this is the only analytical system which allows automatic screening of hundreds of target compounds in routine monitoring such as with pesticide residue analysis.

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