Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1084 (2005) 80–89
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Computerized separation of chromatographically unresolved peaks

J. Krupčík[a,*], J. Mydlová[a], I. Špánik[a], B. Tienpont[b], P. Sandra[b]

[a] *Department of Analytical Chemistry, Chemical and Food Technology, Slovak Technical University, Radlinskeho 9, 81237 Bratislava, Slovakia*
[b] *RIC, Kortrijk, Belgium*

Available online 5 November 2004

Abstract

A computerized peak deconvolution software and mass spectra were successfully applied for the deconvolution of overlapped peak cluster in the chromatogram obtained separating the complex mixture of pesticides by retention time locking gas chromatography–mass spectroscopy. The method based on the unique fragment ions in the spectra can be used for deconvolution of peak clusters if mass spectra of overlapped peaks differ. This method allows determining actual retention times of overlapped peaks. Peak areas found by this method however, cannot be used naturally for the quantitative purposes as the abundance of fragment ions used for this deconvolution procedure can dramatically differ. Computer assisted deconvolution of peaks in the peak clusters gives more realistic peak area ratios as at this method it is supposed equal response for all peaks overlapped in a cluster.

Keywords: Pesticides; Multi-residue analysis; Capillary GC–MS; Retention time locking (RTL); Deconvolution of overlapped peaks

1. Introduction

Gas chromatography coupled to mass spectrometry (GC–MS) is the technique most commonly employed today for the analysis of volatile organic pollutants in environmental samples. The very high number of applications is the result of the efficiency of gas chromatography separation and the good qualitative information and high sensitivity provided by mass spectrometry (MS). The MS fragmentation pattern can often provide unambiguous component identification by comparison with library spectra. When gas chromatography (GC) and MS are combined, the GC separation usually provides isomer selectivity, while the MS shows compound class and homologue specificity [1].

In recent years, regulatory agencies have emphasized more and more the need for the development and use of analytical methods able to determine, in food products, as many residues as possible from the many insecticides, fungicides and other compounds applied in For multi-residue analysis by capillary GC–MS, important improvements have been made in recent years. It has been stated that a single chromatographic technique cannot monitor the currently used 800 and almost 600 superceded pesticides (herbicides, fungicides, insecticides, araricides, nematicides, growth regulators, synergists, etc.) as listed in The Pesticide Manual [3], and the application of both GC and HPLC is mandatory. Half of the currently used pesticides are, however, amenable to capillary GC analysis and by replacing the classical selective detection methods by the universal and specific mass spectrometer, many classes of pesticides can be analyzed in a single run. Moreover, the need for confirmation of positive samples by a secondary technique becomes obsolete and the MS has the sensitivity required for residue analysis [2].

In the chromatographic analyses of complex samples of pesticides complete resolution of all compounds can rarely be achieved even using an optimum selectivity and extremely high performance of the separation columns [4]. With the multidimensional separations or coupling of gas chromatography with mass spectrometry (GC–MS) several methods for resolution of overlapping chromatographic peaks have been developed. In hyphenated chromatography, overlapping chromatographic peaks can be resolved into pure spectra and pure chromatographic profiles by several multivariate decon-

* Corresponding author. Tel.: +421 7 5325314; fax: +421 7 393198.
E-mail address: jan.krupcik@stuba.sk (J. Krupčík).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.09.073

volution techniques [5]. In general, these methods require bilinearity, which implies that the spectrum of each analyte is constant. The slow scan speeds normally used in GC–MS will destroy bilinearity and introduce systematic noise in the data because the concentration in the detector changes during the scan. This effect, described as the scan effect, may hinder successful resolution by multivariate deconvolution. An important advantage of the absence of concentration biasing in time-of-flight (TOF) instruments offers the possibility of performing spectral deconvolution of partially overlapping chromatographic peaks if the fragmentation patterns for the overlapping components are significantly different. Up to now, however, the most of the papers published on the analyses of pollutants in environmental and food samples, use the single capillary gas chromatography hyphenated to linear quadrupole mass spectrometry, where the acquisition rate of the quadrupole analyser is highly significant.

For the computer assisted peak deconvolution of peak clusters in complex chromatograms, various softwares can be

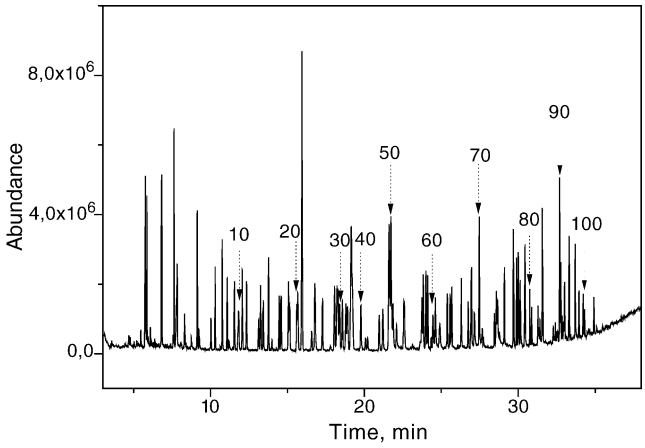

Fig. 1. Total ion chromatogram of the capillary GC–MS separation of 1 μL of a model mixture of pesticides at individual concentrations of 10 ng/μL. For separation conditions, see Section 2.

Table 1
Data obtained by RTL GQ5WS analysis

Compound No.	Compound name	TIon	Exp_RT	Q1	Q2	Q3
1	Dichlorvos (DDVP): 2,2-dichlo	109	5.87	185	79	187
2	Methanridophos (Monitor): *o*,*s*-	94	5.80	95	141	64
3	EPTC: *s*-ethyl dipropylthiocar	128	6.82	86	132	189
4	Butylate(Sutan): *S*-ethyl di-I	57	7.63	146	156	174
5	Acephate (ortran): *o*,*s*-dimeth	136	7.84	94	95	96
6	Isoprocarb (MIPC.Mipcin): *o*-c	121	9.14	136	91	122
7	Fenobucarb (BPMC.Bassa): 2-se	121	10.31	150	91	77
8	Ethoprophos (Ethoprop.Mocap)	158	10.77	97	139	126
9	Chlorpropham (IPCJ: isopropyl	127	11.08	213	153	154
10	Cadusafos: *S*,*S*-di-sec-butyl O	159	11.83	158	88	97
11	Bendiocarb: 2,2-dimethyl-1,3-	151	11.75	126	166	223
12	Thiometon (Ekatin): *S*-2-ethyl	88	12.35	125	89	93
13	a-BHC: 1a,2a,3b,4a,5b,6b-hexa	181	12.07	183	219	217
14	Terbufos: *S*-tert-butylthiomet	231	13.80	57	103	97
15	Lindane (r-BHC): la,2a,3b,4a	181	13.43	183	219	217
16	Dimethipin: 2,3-dihydro-5,6-d	54	13.14	53	118	76
17	Diazinon: *O*,*O*-diethyl *O*-2-is	179	14.47	137	152	304
18	Etrimfos: *O*-6-ethoxy-2-ethylp	292	15.16	181	153	168
19	Tefluthrin: 2,3,5,6-tetrafluo	177	15.08	197	178	199
20	Pirimicarb: 2-dimethylamino-5	166	15.69	72	238	167
21	b-BHC: 1a,2b,3a,4b,5a,6b-hexa	219	13.23	181	183	217
22	Ethiofencarb: a-ethylthio-*O*-*t*	107	15.61	168	77	108
23	Benfuresate; 2,3-dihydro-3,3-	163	15.97	256	121	91
24	ɒ-BHC: 1a,2a,3a,4b.5b,6b-hexa	181	14.55	219	183	217
25	Tolclofos-methyl (Rizolex): *O*	265	16.79	267	125	250
26	Parathion-methyl: *O*,*O*-dimethy	263	16.57	109	125	79
27	Carbaryl (NAC): 1-naphthyl me	144	16.80	115	116	145
28	Pirimiphos-methyl: *O*-2-diethy	290	18.30	276	305	233
29	Esprocarb (Fuji-grass): *S*-ben	91	18.23	222	71	162
30	Dichlofluanid (Euparen): *N*-di	123	18.39	167	224	226
31	Methiocarb: 4-methylthio-3,5-	168	18.04	153	109	94
32	Fenitrothion (MEP.Sumithion)	277	18.10	125	109	260
33	Malathion (Malathon) diethyl	173	18.81	125	127	93
34	Metolachlor: 2-chloro-6′-ethy	162	18.91	238	240	146
35	Thiobencarb (Benthiocarb, Satur	100	18.58	72	125	257
36	Chlorpyrifos (Dursban): *O*,*O*-d	197	19.24	199	314	97
37	Fenthion (MPP): *O*,*O*-dimethyl	278	19.11	125	109	169
38	Dimethylvinphos: 2-chloro-1-(	295	19.15	297	109	296
39	Diethofencarb: isopropy13,4-	267	0.00	225	124	151

Table 1 (*Continued*)

Compound No.	Compound name	TIon	Exp_RT	Q1	Q2	Q3
40	Isofenphos-oxon: *O*-ethyl *O*-2-	229	0.00	201	58	120
41	Parathion: *O*,*O*-diethyl *O*-4-ni	291	19.27	109	97	139
42	4,4′-dichlorobenzophenone (Dico	139	19.18	111	250	141
43	Fosthiazate-1: (RS)-*S*-sec-but	195	20.07	97	126	104
44	Fosthiazate-2: (RS)-*S*-sec-but	195	20.28	97	104	126
45	Isofenphos: *O*-ethyl *O*-2-isopr	213	0.00	58	121	255
46	Pendimethalin: *N*-(1-ethylprop	252	20.98	281	253	162
47	Phenthoate {PAP}: *S*-a-ethoxyc	274	21.72	121	125	93
48	Chlorfenvinphos-Z (CVP): 2-ch	267	21.59	323	269	325
49	Quinalphos: *O*,*O*-diethyl *O*-qui	146	21.63	157	156	118
50	Chinomethionat	206	21.86	234	116	148
51	Captan (Orthocide): 1,2,3,6-t	79	0.00	77	80	149
52	Triadimenol-1: 1-(4-chlorophe	112	22.08	168	128	70
53	Paclobutrazol: (2RS,3RS)-1-(4	236	22.56	125	238	167
54	Prothiofos (Tokuthion): *O*-2,4-	309	23.75	267	162	113
55	Pretilachlor: 2-chloro-2′,6′-	238	24.21	162	176	202
56	Flutolanil (Moncut): a,a,a-tr	173	23.83	145	281	323
57	*p*,*p*-DDE: 1,1′-(dichloroethyli	246	24.01	318	248	316
58	Tricyclazole (Beam): 5-methyl	189	23.57	162	161	135
59	Flusilazole: bis(4-fluorophen	233	24.61	206	234	315
60	Myclobutanil: 2-(4-chlorophen	179	24.45	150	181	82
61	Chlorobenzilate (Akar): ethyl	251	25.40	139	253	111
62	Cyproconazole-1: (2RS,3RS:2RS	222	24.90	139	224	83
63	Fensulfothion: *O*,*O*-diethyl *O*-	292	25.55	293	156	97
64	*p*,*p*-DDD: 1,1-dichloro-2,2-bis	235	25.69	237	165	236
65	Mepronil (Basitac): 3′-isopro	119	26.30	91	269	120
66	Edifenphos (EDDP): *O*-ethyl S,	109	26.75	173	310	201
67	Propiconazole-1: (RS)-l-[2-(2	259	27.14	173	261	175
68	Lenacil: 3-cyclohexyl-1,5,6,7	153	26.94	154	110	136
69	Thenylchlor: 2-chloro-*N*-(3-me	127	27.47	288	141	287
70	Tebuconazole: (RS)-1-*p*-chloro	250	27.47	125	83	70
71	Captafol (Difoltan): *N*-(1,1,2	79	0.00	80	77	151
72	EPN: *O*-ethyl *O*-4-nitrophenyl	157	28.67	169	185	141
73	Iprodione (Rovral): 3-(3,5-di	314	28.44	316	187	56
74	Tebufenpyrad: *N*-(4-tert-butyl	318	29.10	171	333	276
75	Acetamiprid: (E)-*N*-{(6-chloro	56	28.55	152	126	166
76	Pyriproxyfen: 4-phenoxyphenyl	136	29.89	78	96	77
77	Cyhalothrin-1 (Karate)	181	30.12	197	208	209
78	Phosalone (Rubitox): *S*-6-chlo	182	29.70	184	367	97
79	Mefenacet: 2-(1,3-benzothiazo	192	30.02	77	120	136
80	Acrinathrin: (*S*)-a-cyano-3-pe	181	30.75	208	93	289
81	Fenarimol (Rubigan): 2,4′-dic	139	30.43	219	107	251
82	Pyraclofos: (RS)-{*O*-1-(4-chlo	360	30.87	194	138	139
83	Permethrin-1 (Adion): 3-pheno	183	31.41	163	165	184
84	Bitertanol-1 (Baycoral): all-	170	31.27	168	171	112
85	Permethrin-2 (Adion): 3-pheno	183	31.60	163	165	184
86	Pyridaben: 2-tert-butyl-5-(4-	147	31.55	117	148	132
87	Cyfluthrin-1: (RS)-a-cyano-4-	163	32.27	206	165	226
88	Cyfluthrin-2: (RS)-a-cyano-4-	163	32.41	206	165	199
89	Cyfluthrin-3: (RS)-a-cyano-4-	163	32.58	206	165	226
90	Halfenprox: 2-(4-bromodifluor	263	32.76	265	183	184
91	Cypermethrin-1 (Agrothrin)	163	32.70	181	165	77
92	Cypermethrin-2 (Agrothrin)	163	32.76	181	165	77
93	Flucythrinate-1	199	33.01	157	181	184
94	Silafluofen: (4-ethoxyphenyl)	179	33.31	286	258	151
95	Flucythrinate-2	199	33.28	157	181	184
96	Pyrimidifen: 5-chloro-*N*-{2-{2	184	33.70	186	185	161
97	Fenvalerate-1 (Sumicidin): (RS)-	167	33.94	125	181	152
98	Fluvalinate-1 (Mavrik): (RS)-	250	34.22	252	251	181
99	Fluvalinate-2 (Mavrik): (RS)-250	250	32.68	252	181	251
100	Difenoconazole-1: *cis*,*trans*-3	323	34.47	265	325	267
101	Difenoconazole-2: *cis*,*trans*-3	323	34.56	265	325	267
102	Deltamethrin	181	34.91	253	251	255
103	Imibenconazole: 4-chlorobenzy	125	35.97	82	253	375

cal A, average; L, linear; LO, linear w/origin; Q, Quad; QO, Quad w/ori; #Qual, number of qualifiers; A/H, area or height; ID R, R.T.; B, R.T.; Q Q, Q-value; L, largest; A, all.

Table 2
Data obtained for chromatographically non-separated peaks by deconvolution methods based on unique fragment ions in the MS spectra and a chemometric computer assisted software

Peak No.	Retention time	Compound	Retention time	Compound	Ident. probab.	No. in cluster	Area (MS)	Area (Dec.)	Area ratio (MS)	Area ratio (Dec.)
36	16.79	Tolclofos-methyl	16.78	Tolclofos-methyl	91	1	13165742	252642	1.68 (1/2)	1.63 (1/2)
	16.8	Carbaryl	16.92	Carbaryl	93	2	7823303	155453		
41	18.39	Dichlofluanid	18.35	Dichlofluanid	70	1	4987611	194762	0.57 (1/2)	1.19 (1/2)
			18.39	Phtalate?		2	8696891	231853		
45 + 46	19.11	Fenthion	19.09	Fenthion	99	1	6149469	103856	0.50 (1/2)	0.49 (1/2)
	19.15	Dimethylvinphos	19.14	Dimethylvinphos(z)	95	2	12227712	213266	0.50 (1/3)	0.23 (1/3)
	19.18	4,4-dichlorobenzophenone	19.20	4,4′-dichlorbenzophenone	95	3	12300477	452285	0.88 (1/4)	0.37 (1/4)
	19.24	Chlorpyrifos	19.22	Chlorpyrifos	91	4	6974558	278788		0.63 (1/5)
	19.27	Parathion	19.27	Not identified		5		165595		
52	21.59	Chlorfenvinphos-z	21.56	Chlorfenvinphos	50	1	4175528	87778	0.88 (1/2)	0.20 (1/2)
	21.63	Quinalphos	21.60	Isofenphos	92	2	4768792	434859	0.56 (1/3)	0.41 (1/3)
			21.63	Quinalphos	95	3	7397088	215289	0.64 (2/3)	2.02 (2/3)
53	21.72	Phenthoate	21.70	Phenthoate	95	1	2940218	501694	0.21 (1/2)	0.58 (1/2)
			21.76	Alkane		2	13716514	294502		
56	22.56	Paclobutrazol	22.57	Paclobutrazol	90	1	5496689	238167	2.05 (1/2)	3.02 (1/2)
			22.63	Pyrifenox II	78	2	2678788	78760		
82	29.7	Phosalone	29.67	DEHP	86	1	10593919	368196	1.45 (1/2)	1.42 (1/2)
			29.72	Phosalone	92	2	7322972	259919		
87	30.43	Fenarimol	30.42	Flurecol-butyl	22	1	3511281	201032	0.54 (1/2)	0.66 (1/2)
			30.44	Fenarimol	95	2	6471534	303704		
103	33.01	Flucythrinate-1	33.00	Cypermethrin-2	52	1	3225836	97647	0.79 (1/2)	0.39 (1/2)
			33.02	Flucythrinate-1	86	2	4491579	250807		
104	33.28	Flucythrinate-2	33.29	Flucythrinate -2	50	1	3014701	115855	0.28 (1/2)	0.29 (1/2)
	33.31	Silafluofen	33.32	Not identified		2	10695083	404030		

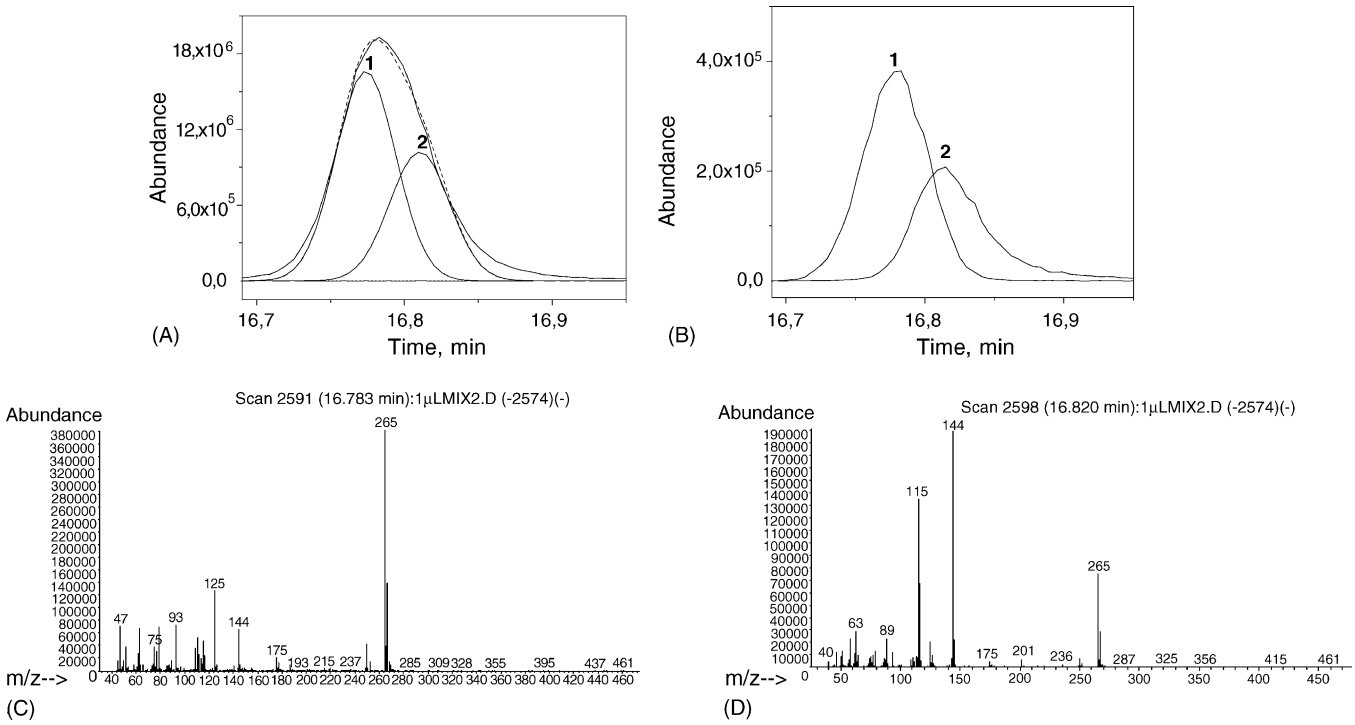

Fig. 2. Deconvolution of the peak cluster (16.7–16.9 min) by a chemometric peak fitting program (A) and by MS fragmentography (B). (C) Mass spectrum of the peak no. 1 (Tolclofos-methyl) and (D) mass spectrum of the peak no. 2 (carbaryl).

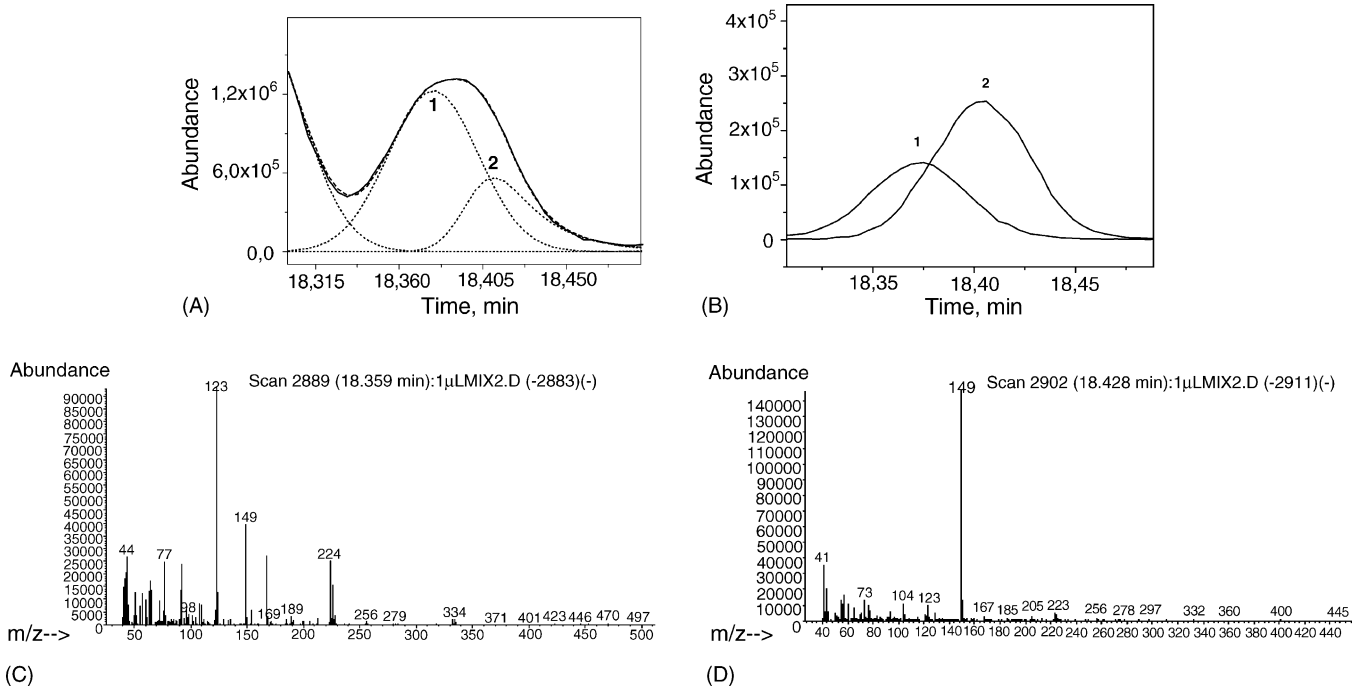

Fig. 3. Deconvolution of the peak cluster (18.31–18.50 min) by peak a chemometric fitting program (A) and by MS fragmentography (B). (C) Mass spectrum of the peak no. 1 (dichlofluanid) and (D) mass spectrum of the peak no. 2 (phtalate?).

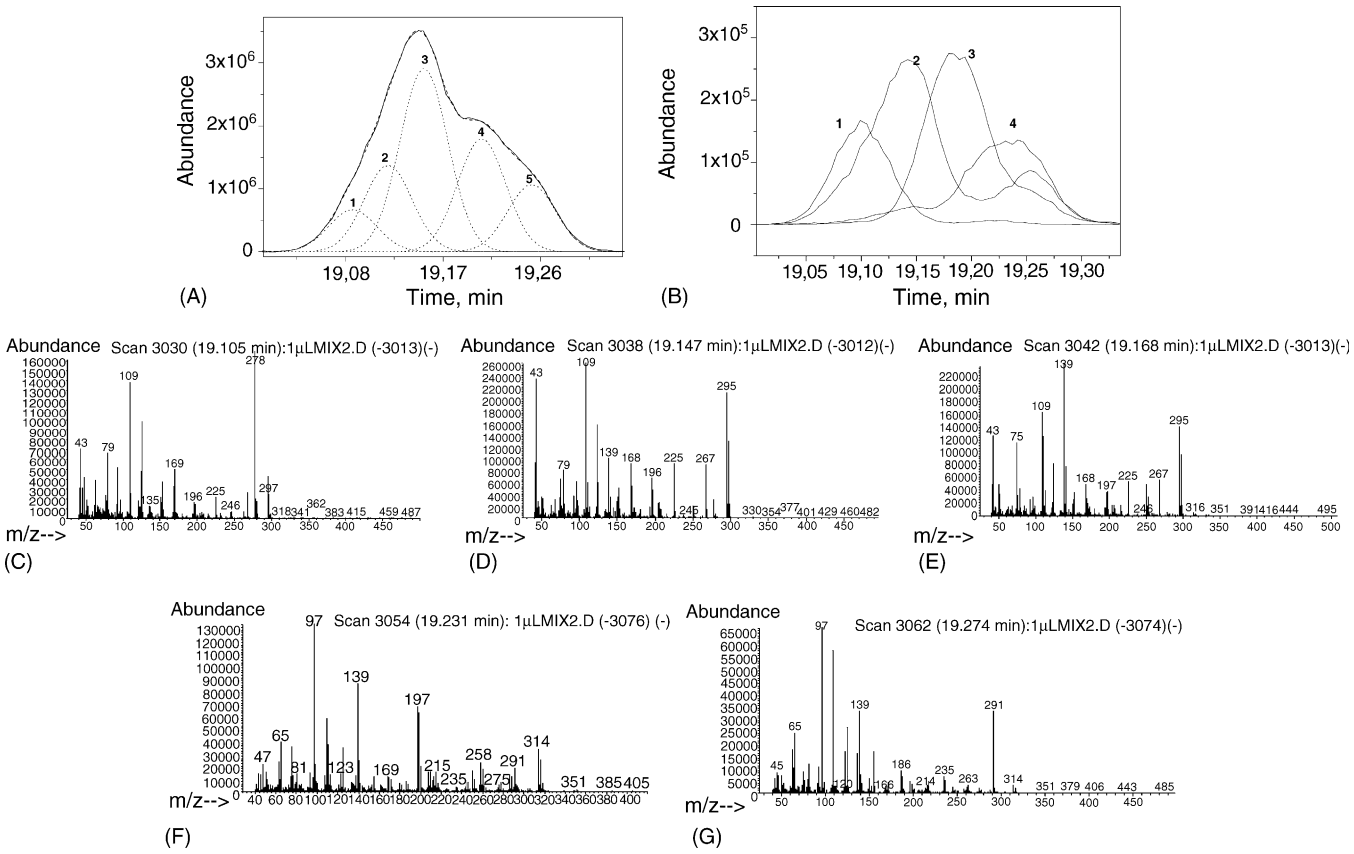

Fig. 4. Deconvolution of the peak cluster (19.02–19.34 min) by peak a chemometric fitting program (A) and by MS fragmentography (B). Mass spectra (C) peak no. 1 (fenthion), (D) peak no. 2 (dimethylvinphos), (E) peak no. 3 (4,4′-dichlorobenzophenone), (F) peak no. 4 (chlorpyrifos), (G) peak no. 5 (not identified—parathion?).

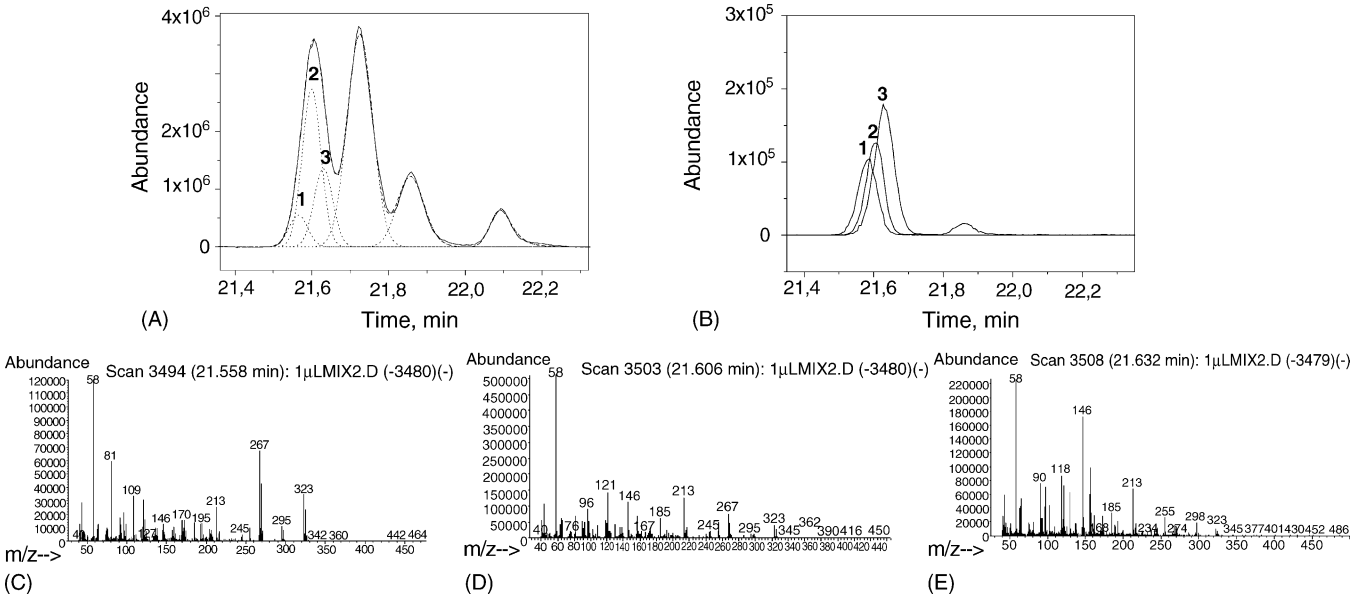

Fig. 5. Deconvolution of the peak cluster (21.4–22.30 min) by peak a chemometric fitting program (A) and by MS fragmentography (B). Mass spectra (C) peak no. 1 (chlorfenvinphos), (D) peak no. 2 (isofenphos), (E) peak no. 3 (quinalphos).

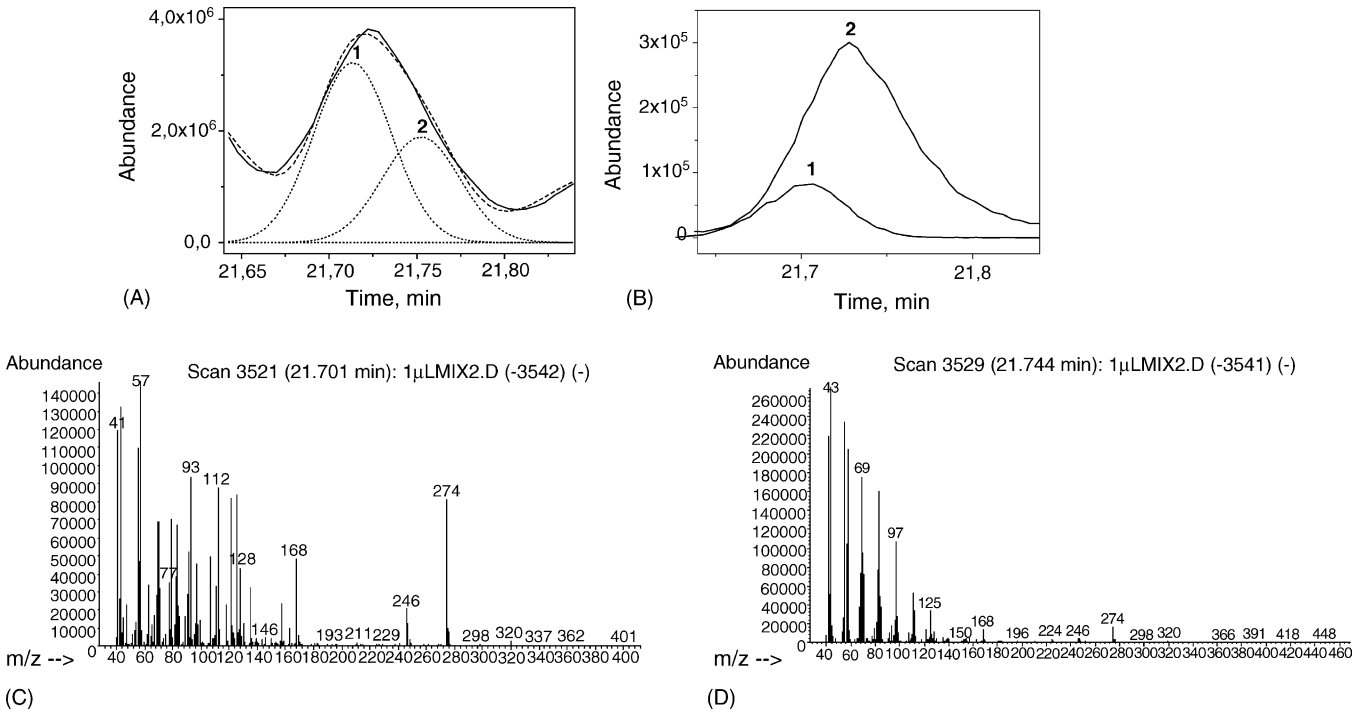

Fig. 6. Deconvolution of the peak cluster (21.64–21.82 min) by a chemometric peak fitting program (A) and by MS fragmentography (B). Mass spectra (C) peak no. 1 (phenthoate), (D) peak no. 2 (alkane?).

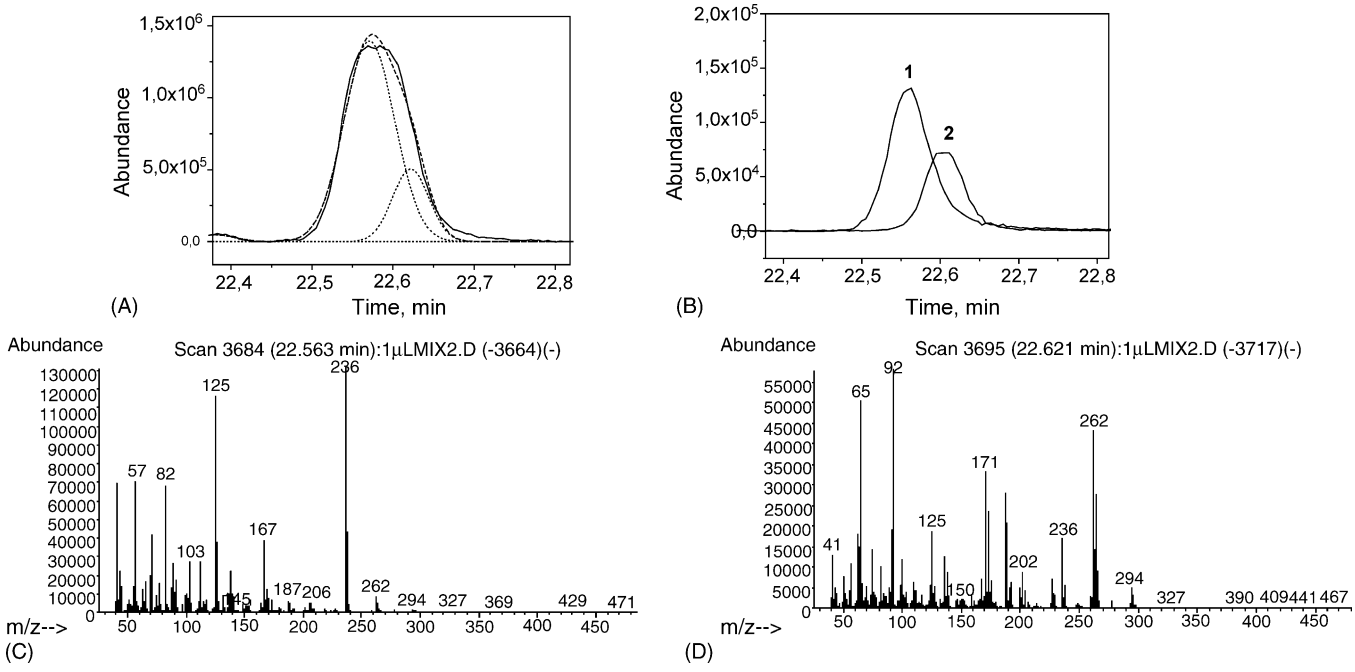

Fig. 7. Deconvolution of the peak cluster (22.4–22.8 min) by a chemometric peak fitting program (A) and by MS fragmentography (B). Mass spectra (C) peak no. 1 (paclobutrazol), (D) peak no. 2 (pyrifenox II).

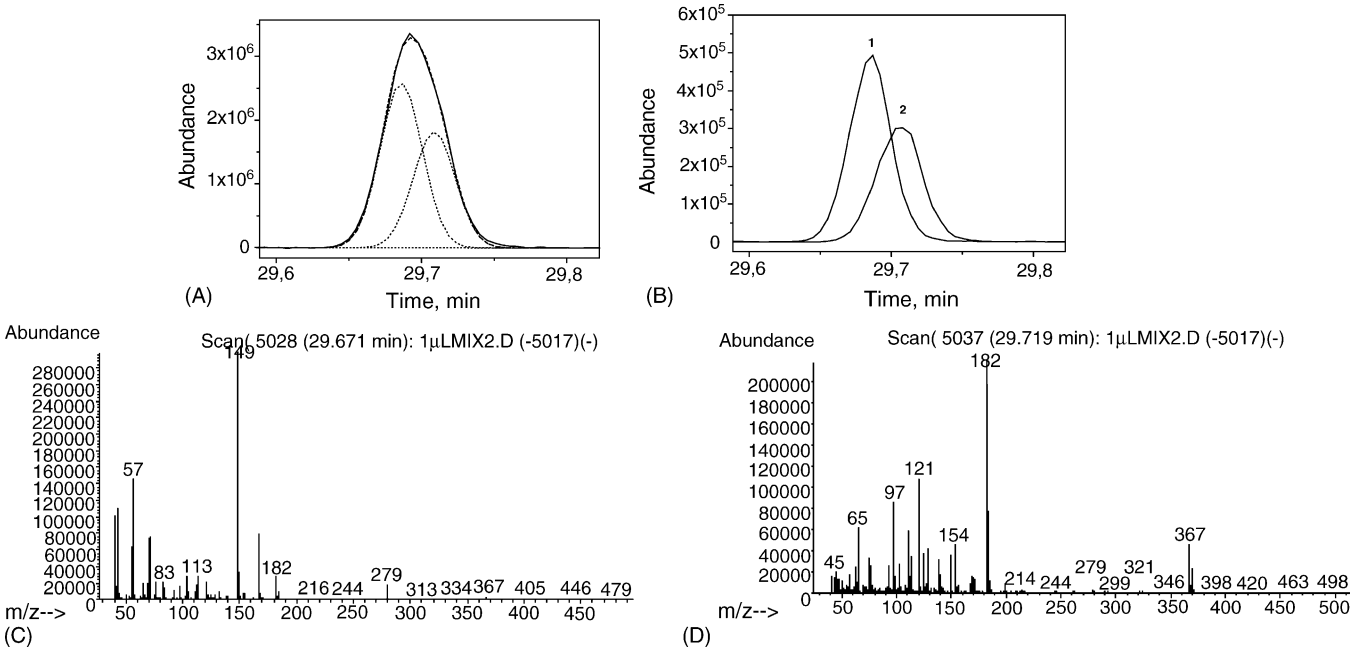

Fig. 8. Deconvolution of the peak cluster (29.6–29.8 min) by a chemometric peak fitting program (A) and by MS fragmentography (B). Mass spectra (C) peak no. 1 (DEHP), (D) peak no. 2 (phosalone).

used [6-8]. The number of peaks and design of peak shapes belong to basic input parameters in the computer assisted peak deconvolution procedures. It is a problem to determine initial peak parameters for a deconvolution procedure for a peak cluster, particularly if the number of peaks present in the selected part of the chromatogram is not known [9].

In this paper, we try to highlight the possibilities and problems of the use of both mass spectral as well as the computer assisted procedures for the deconvolution of overlapped peaks in the chromatograms obtained by the separation of complex samples of pesticides by capillary GC–quadrupole MS under the conditions of Agilent retention time locking (RTL) pesticide method [9,10].

2. Experimental

Capillary GC analysis was performed on a 30 m × 250 μm i.d., 0.25 μm d_f HP-5MS column (Agilent Technologies). The oven was programmed from 70 °C (2 min) at 25 °C/min to 150 °C, at 3 °C/min to 200 °C, and finally, at 8 °C/min to 300 °C. This is the temperature program required for the RTL

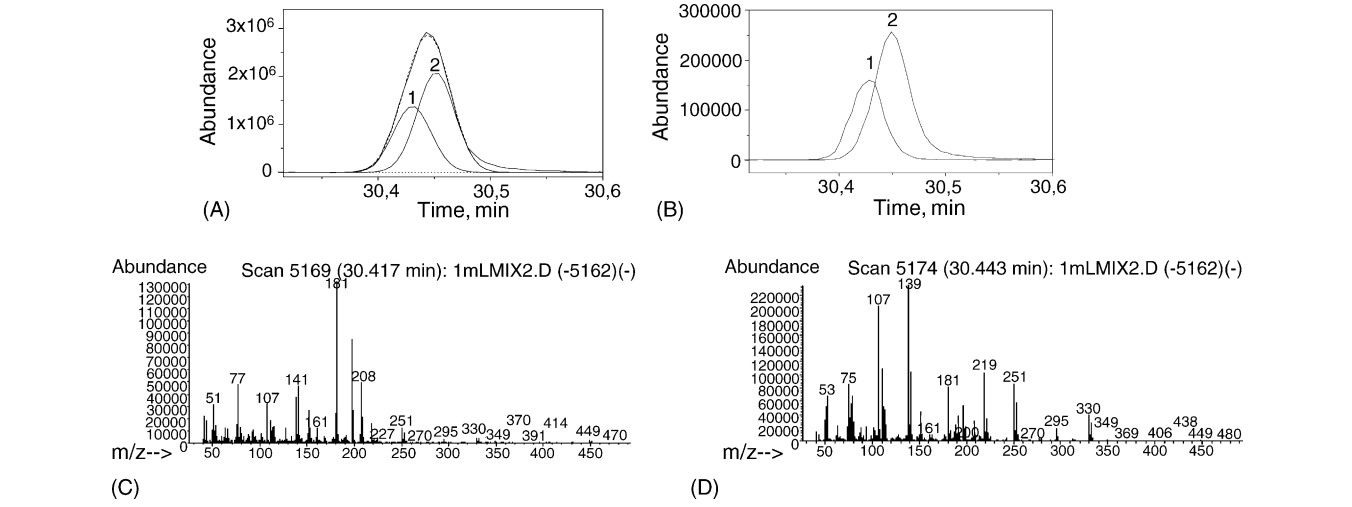

Fig. 9. Deconvolution of the peak cluster (30.31–30.60 min) by a chemometric peak fitting program (A) and by MS fragmentography (B). Mass spectra: (C) peak no. 1 (flurecol-butyl), (D) peak no. 2 (fenarimol).

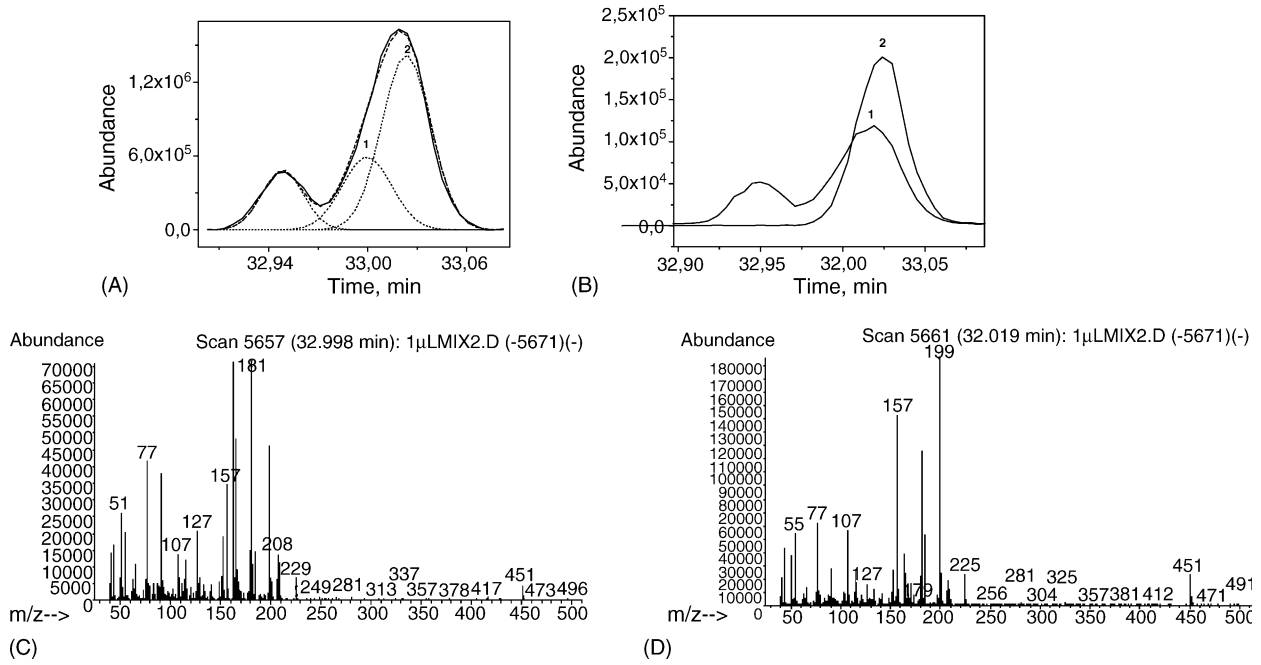

Fig. 10. Deconvolution of the peak cluster (32.88–33.08 min) by a chemometric peak fitting program (A) and by MS fragmentography (B). Mass spectra (C) peak no. 1 (cypermethrin II), (D) peak no. 2 (flucythrinate I).

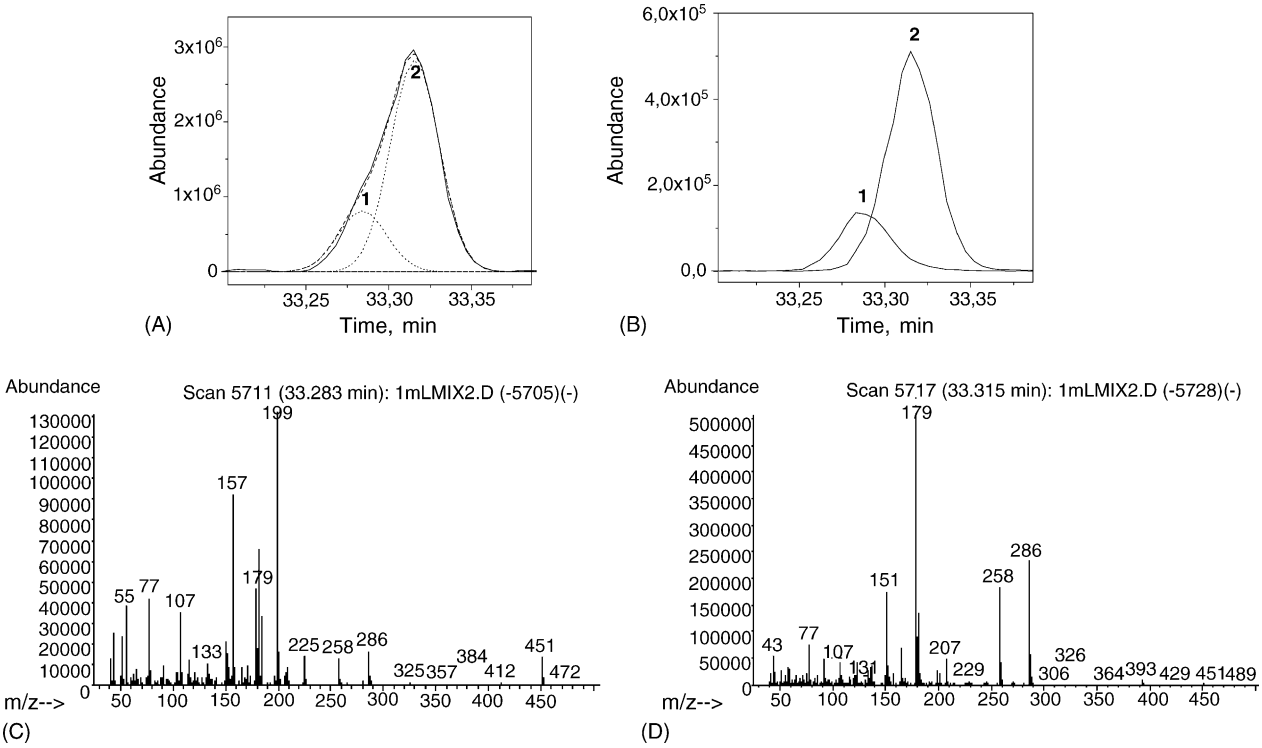

Fig. 11. Deconvolution of the peak cluster (33.21–33.37 min) by a chemometric peak fitting program (A) and by MS fragmentography (B). Mass spectra (C) peak no. 1 (flucythrinate II), (D) peak no. 2 (not identified).

screener option (Agilent Technologies). Helium was used as carrier gas. The head pressure was calculated, using the RTL software, so that *p*,*p*′-DDT was eluting at a constant retention time of 26.98 min. An Agilent 5973 MS was used in the scan mode (*m*/*z* 40–500).

The standard pesticides were obtained from Dr. Ehrenstorfer, Augsburg, Germany. Screening of pesticides was performed using the automatic RTL screener software in combination with the Agilent RTL pesticide library [2,11,12].

3. Results and discussion

Fig. 1 shows the separation of 1 μL of a model sample of pesticides at individual concentrations of 10 ng/μL. From the formal reasons there are not shown labels on all peaks. They, however, are shown in the Table 1 where the peak identification in Fig. 1 is listed as it was elucidated with the retention time locked gas chromatography–mass spectroscopy method (RTL-capillary GC–MS of Agilent).

For the identification peaks, both the retention data and MS spectra is obviously used in this method. The positive identification was, however based on the comparison of measured and reference spectra. Although, the library search is a powerful tool for the identification of unknowns, for correct identification a series of conditions must be satisfied:

(i) The compound must be included in the library.
(ii) The MS conditions at which both spectra have been obtained must be similar.
(iii) The GC separation must be sufficiently efficient to obtain a clean mass spectrum.

In order to guarantee correct identification in the Fig. 1 and prevent false positives, the purity of each peak in the Fig. 1 was verified comparing mutually MS spectra measured at three points (inflexes and peak maximum) with the library MS reference spectra. A spectral match and fit factor was used to define the peak purity.

Table 2 shows data obtained for ten peak clusters registered by the RTL capillary GC–MS of pesticides (columns 1–3) and by the comparison of mass spectra obtained for peak cluster in the above defined points with tabulated spectra (columns 4–6). Columns 8 and 9 list the individual peak areas found in the peak clusters by mass spectral deconvolution using unique ion fragments (column 8) and by a computer assisted software (column 9). The last two columns show the peak area ratios in the corresponding peak clusters calculated from the peaks areas of ion fragmentograms (column 10) and those found by a chemometric deconvolution (column 11). The ratios listed in columns 10 and 11 in brackets labels the number of peaks on Figs. 2–11 for which the peak areas are compared. There are peak clusters in Table 1 for which both deconvolution procedures shows similar peak area ratios (peak nos. 36, 82, and 104) and those with very different peak area ratios (peak nos. 41, 45, 46, 52, 53, 56, and 103). The nature of peak areas indicates that the chemometrical deconvolution procedure is more realistic as it manipulates peaks recorded as the TIC response on time, whereas spectral deconvolution procedure manipulates peaks recorded as the ion fragment (differing in *m*/*z*) response on time.

Acknowledgments

The authors acknowledge the Grant Agency of the Slovak Republic for VEGA 1/9127/02 and to the Agency for International Science and Technology Cooperation in Slovakia for Grant No. 035/2001.

References

[1] F.J. Santos, M.T. Galceran, J. Chromatogr. A 1000 (2003) 125–151.
[2] P. Sandra, B. Tienpont, F. David, J. Chromatogr. A 1000 (2003) 299.
[3] C.D.S. Tomlin, The Pesticide Manual, 12th ed., The British Crop Protection Council, Farnham, Surrey, 2000.
[4] P. van Zoonen, Analytical Methods for Pesticide Residues in Foodstuffs. Part 1. General Inspectorate for Health Protection, RIVM, Bilthoven, The Netherlands, 1996.
[5] S.A. Mjøs, Anal. Chim. Acta 488 (2003) 231.
[6] G. Vivó-Truyols, J.R. Torres-Lapasió, R.D. Caballero, M.C. García-Alvarez-Coque, J. Chromatogr. A 958 (2002) 35.
[7] J. Li, J. Chromatogr. A 952 (2002) 63.
[8] Microcal Origin, Version 7, Microcal Sofware, Northamtion, MA.
[9] J. Hrouzek, J. Krupcík, M. Ceppan, S. Hatrík, P.A. Leclercq, Chem. Pap. 54 (2001) 314.
[10] P. Wylie, B. Quimby, Agilent Application Note 5967-5860E, 2000.
[11] C. Kai Meng, Agilent Application Note 5988-4392EN, 2001.
[12] www.richrom.com/html/ric_appnotes.html.