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Multiple-detector responses or multiple-retention times: what is more informative for gas chromatography peak identification?☆

G. Stoev*, A. Mihailova

Central Laboratory of Veterinary Control and Ecology, Iskarsko Shosse Str. 5, 1528 Sofia, Bulgaria

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Abstract

Multiple-separation and -detection are two approaches applied at the identification of analytes in chromatography. Using them depends on the physico-chemical properties and elemental content of the analytes. When physico-chemical properties are similar multiple-separation gives better opportunities for the identification. In this case, the efficiency of the columns is very important. When analytes contain some characteristic groups as $-NO_2$, halogen, or nitrogen atoms then multiple-detection will be more useful. The sensitivity and/or selectivity of the detectors increase reliability of identification significantly.

Keywords: Detection, GC; Retention times; Efficiency; Selectivity; Sensitivity

1. Introduction

The retention time of the chromatographic peaks is a valuable characteristic for identification of analytes. Although the precision of the relative retention times measured with the modern instruments is very high (0.01–0.03%) a suspicion for overlapped peaks exists especially in complex environmental and biological samples.

As it is well known that more than one compound can have the same or similar retention time, a confirmation by additional characterization is necessary. Identification of GC peaks is carried out mainly by the most selective mass spectrometers, coupled to the chromatographic column. Multi-dimensional chromatography is a powerful technique for separation of multi-compound samples. It increases the reliability of identification several orders compared to this one using only one column [1]. However, complicated instruments are necessary for this technique.

Multiple-detector responses have been applied for peak identification when two compounds with the same retention time possess different responses to detectors, based upon different physical properties. Combination of detection method as the universal flame ionization detection (FID) and the selective electron-capture detection (ECD) and nitrogen–phosphorus detection (NPD), were often applied for distinguishing GC peaks. Multiple (FID/ECD/NPD) responses were used at the identification of polymers by pyrolysis gas chromatography [2]. It was realized by splitting of the effluent of the capillary column to every one detector in a ratio of 1:1:1 [3]. Applying relative responses of the pyrolysis products to methyl methacrylate (MMA) as an internal standard, the variation of the split ratio between the detectors and the differences in power supplied to the detector were cancelled. Multiple-detection rendered a more reliable identification of the polymers. However, it was not possible a quantitative assessment to be done.

Wentworth et al. [4] have determined the relative retention times and relative detector responses of many compounds containing numerous functional groups, varying structures, and degrees of unsaturation. They used detectors based upon different principles: FID and argon and krypton pulsed dis-

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* Corresponding author. Tel.: +359 2 9791034; fax: +359 2 9790742.
E-mail address: clvce@netissat.bg (G. Stoev).

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charge photo-ionization detectors and found out that their responses appeared to be independent. For distinguishing of GC peaks these authors used four relative detector responses in conjunction with the retention time, determined by benzene as an internal standard. The authors supposed that this approach can be used in place of mass spectrometry and possibly in some cases it may be superior to mass spectrometry in peak identification.

Intuitively, we have agreed that identification of peaks separated simultaneously by columns with different stationary phases is more reliable. We also accepted intuitively ECD and NPD as "selective" detection methods because there was no expression for quantitative estimation of the acquired information. Chromatographers are sure that the combinations of two or three detectors provide an opportunity for more reliable identification. However, we did not know quantitatively what we could gain using a combination of different detectors or when the most selective mass spectrometer was applied. That is why an expression for quantitative assessment of the reliability of identification by multiple-detection or -separation and this one of mass spectrometry is necessary. Recently, the influence of the selectivity of some HPLC detectors [5] on the reliability of the identification has been determined.

The aim of this work is a quantitative assessment of the contribution of the separation by column with different polarities and the selectivity and sensitivity of different GC detectors to the reliability of the identification.

2. Theory

The identification in chromatography at first sight is realized by comparison of retention time and peak area of the analyte with a standard substance. If we present the chromatograms as maps with r and s strips along the abscissa (retention time) and the ordinate (signal intensity), the probability, $P_{r,s}$, two peaks to be fully overlapped and the compounds, which they present, to be accepted as identical is:

$$P_{r,s} = \frac{1}{r,\, s} \tag{1}$$

The probability $(1 - P_{r,s})$ is a measure for distinguishing of the compounds. The probability, $P_{r,s}$, means also that two different compound when their peaks are fully overlapped will be accepted as identical, although in fact they are not.

When only the retention time is used the probability will be $P_r = 1/r$. The value of strips r presents the number of statistically separated peaks. Taking into account the efficiency of the column it is possible to predict the probability all analytes of a mixture with a fixed number of compounds to be separated as peaks [6–9]. In real samples, where the peaks are of an unequal size, the situation is worse. The situation is more complicated when the number of compounds is undefined. The probability that all compounds will be separated is markedly low [10]. For example, for 10 analytes and a value of peak capacity, n_c of 234, the probability is only of 0.73. Approximately, 10^6 theoretical plates, determined isocratic, are necessary for reaching a value of a peak capacity of 234. This tremendous value of efficiency is difficult to achieve in practice and, in many cases, for environmental or biological samples, a probability for co-incidental overlapping of compounds exists. The sum of separation number of the column, ΣSN, represents statistically the number of fully separated peaks. This value can be used as a measure of the strips r in Eq. (1). The separation number (SN) is determined by:

$$\mathrm{SN} = \frac{t_{R(Cn+1)} - t_{R(Cn)}}{w_{0.5(Cn+1)} - w_{0.5(Cn)}} - 1 \tag{2}$$

where $t_{R(Cn)}$ and $t_{R(Cn+1)}$ are the retention times of two homologues of hydrocarbons or fatty methyl esters with n and $n + 1$ number of carbon atoms and $w_{0.5(Cn)}$ and $w_{0.5(Cn+1)}$ are their widths at the half peak height. The sum of the separation numbers (ΣSN), usually determined at a programmed temperature, depends on the working temperature range and the selectivity of the liquid phase and the efficiency of the separation system. At a programmed temperature of the column it is impossible to predict a probability of every analyte be separated as a single compound peak and an uncertainty of overlapping exists. But, the temperature programming gives larger possibility for separation of a complex sample than isocratic separation and is mainly applied in practice.

The retention time is a measure of the absorption of the compounds being separated on the stationary phase. The last, which can range from a non-polar poly(methyl silicon), to a highly polar poly(cyanopropylsilicon). Right chosen phase is this one which best suits for most of the compounds in the mixture. Once a phase has been selected, the compounds in a homologous series for the most part will elute according to the chain length. This is caused by the additional Van der Waals attractive forces resulting from the additional carbon chain length. Consequently, compounds with same functional group will be separated to a greater or lesser degree by the attached carbon chains. Generally, for an efficient capillary column, these isomers can be separated and in general overlapping peaks are not expected. However, it is possible coincidently several compounds with different functional groups and chain lengths interact equally with the stationary phase and they could overlap. These compounds interact with different polarity stationary phases by different manner and they will be separated by this way.

Multiple-detection offers another opportunity for distinguishing of overlapped analytes, possessing different functional groups and, respectively, different signals. Applying the response of the detector, which determines the number of strips s, the probability $P_{r,s}$ (Eq. (1)) will be lower than this one when only the retention of the analyte is used. When an analyte is registered simultaneously with q-numbers of detectors based upon different physical properties the probabilities will be orthogonal because they are independent [2,4] and the

combined probability for overlapping can be expressed as:

$$^{q}P_{r,s} = P_{r,s(1)} \times P_{r,s(2)} \times \cdots \times P_{r,s(q)} |\bar{X}_A - \bar{X}_B| \quad (3)$$

95% confidential level, and if $|\bar{X}_A - \bar{X}_B|$ is less than u, then X_A and X_B are not distinguishable.

3. Experimental

Chemicals: 4,4′-Polychlorinated biphenyl (4,4′-PCB) used as an internal standard and the pesticides α-benzene hexachloride (α-HCH), diazinon, lindane, malathion, aldrin, endrine, DDE and DDT were purchased from Sigma.

Gas chromatography: Varian 3400 GC with Star data system was used for analyses. The instrument was equipped with a fused silica capillary column (25 m × 0.25 mm i.d. with 0.2 μm DB-5 stationary phase, column I and/or OV-1701, column II, OV-275, column III and a splitless injector (30 s splitless injection). The temperatures of the injector and detectors (FID and ECD systems) were 300 and 320 °C, respectively. The oven temperature program was: 60 °C for 1 min, increasing with 30 °C/min to 180 °C, after that by 4 °C/min to 290 °C, and the final temperature was hold for 15 min. ΣSN of the columns was determined by injection of 1 μl mixture of C_8–C_{25} *n*-hydrocarbons with a concentration 0.5 ppm.

The eluent of the capillary column was split in ratios 1:1:1 or 1:1 by a laboratory made splitter with pieces of quartz capillaries in equal length (25 cm × 0.20 mm i.d.). The capillaries were tightened with particles of destroyed graphitized ferules and home made former.

Polymers: Poly(ethylene) (PE), poly(isoprene) (PIP), poly(chloroprene) (PCP), and poly(cyanacrylate) (PCA), were pyrolyzed by a Currie-point pyrolyzer (Pye Unicam) at 610 °C/5 s. Pyrolysis products were separated with capillary column I at programmed temperature from 40 to 200 °C with 4 °C/min. The column was connected directly to the pyrolyzer, i.e. on-column injection [2], and high efficiency and sensitivity were achieved. Poly(methyl methacrylate) (10 μg) was laid on the pyrolysis wire as a solution in chloroform by a microsyringe; polymers (100 μg) were pyrolyzed after evaporation of the solvent.

4. Results and discussion

4.1. Multiple-detection

The value of *r* in formula (1) at simultaneous detection is constant and only the intensity of the signal of the detector *s*-values, influences on the reliability of the identification. The number of strips, *s*, can be determined by the relative ratios of the detector's signals and their standard deviations by Eq. (1).

At the beginning, we applied the approach for quantitative estimation of the reliability of the identification with multiple-detection to the results published by Wentworth et al. [4] (Table 1). FID-RePIX represents the relative FID signal of the analyte ***X*** towards the signal of the internal standard (benzene) obtained by photo-ionization detection. Using an internal standard we were able to take into account variations in sensitivity of the detectors over a long period of time. Variation in the values of FID-RePIX for alcohols ranges from 0.944 for 2-methyl-2-propanol to 0.359 for 1-butanol and depends on the attached carbon chain to the OH group. FID-RePIX magnitudes of the other compounds as hydrocarbons, halogenated hydrocarbons and nitrogen- and oxygen-containing substances are in the interval 0.100 to 1.300 and represent the contribution of the moieties and the structure on the signals. This wide range of distribution reduces the probability for coincidental overlapping of the compounds by their detector's signals, $^{q}P_s$, and gives a chance for more reliable distinguishing. FID-RePIX value for 2-methyl-2-propanol is larger than 1-butanol and its contribution to the identification should be bigger. However, taking into account the values of the standard deviation of FID-RePIX magnitudes the probability of a coincidental overlapping for 2-methyl-2-propanol is eight time higher than 1-butanol [$P_{s(2-\mathrm{Me}-2-\mathrm{propanol})}/P_{s(1-\mathrm{butanol})}$] ($2.4 \times 10^{-2}/2.8 \times 10^{-3} = 8.57$) and the identification of the last one is more reliable.

Every one of the RePIX values contributes to the identification of the GC peaks. However, the FID and Ar-RePIX magnitudes are more valuable compared to the Kr-RePIX values because the last ones of some compounds are very small and the standard deviations are higher than these of FID-RePIX values. Concerning methanol, 2-methyl-1-propanol, 1-butanol and 2-nirtopropane Kr-RePIX do not contribute to the identification. The low sensitivity of Kr-RePIX results in a low selectivity of this detector towards aliphatic alcohols (Kr-P_s values in Table 1).

We applied this approach to the reliability of the identification of polymers as PE, PIP, PCP, and PCA, by pyrolysis–GC. The main pyrolysis products of these polymers: dodecene, isoprene and dipentene, chloroprene and dichloroprene, and cyanacrylate were detected with FID, ECD and NPD. Only dodecene from the alkene's (**C_2–C_{25}–en**) homologous series, generated from PE was used for determination of contribution to multiple-detection because every alkene consists the same element's ratio (**C_nH_{2n}**). MMA was used as an internal standard. It was generated simultaneously with the pyrolysis products of the analyzed polymers from poly(methyl methacrylate), which degrades mainly to a monomer (>98%). The values of the FID-MMAReFID ratios for all pyrolysis products were in the range from 0.316 (dimer of chloroprene) to 1.352 (isoprene) (Table 2). Obviously, the chlorine atom sufficiently decreases the response factors of FID. The contribution of the relative FID-MMAReFID response factors to distinguish these compounds is $^{\mathrm{FID}}P_s \approx 2 \times 10^{-5}$. The difference between ECD-MMAReFID and NPD-MMAReFID values was quite expressive. The reason is the large responses of ECD for chloroprene and its dimer and this one of NPD for cyanacrylate. That is why the contribution of ECD to

Table 1
Contribution of the multiple-detection to the reliability of the identification of some compounds

Detection	FID-RePIX				Ar-RePIX				Kr-RePIX				^{q}P
	$\overline{x}$	σ	s	FID, $P_s = 1/s$	$\overline{x}$	σ	s	Ar, $P_s = 1/s$	$\overline{x}$	σ	s	Kr, $P_s = 1/s$	
MeOH	0.494	0.018	19	5.3×10^{-2}	0.724	0.028	17	5.9×10^{-2}	0.008	0.003	1	1.0	3.1×10^{-3}
EtOH	0.734	0.007	74	1.4×10^{-2}	0.485	0.007	48	2.1×10^{-2}	0.041	0.007	4	2.5×10^{-1}	7.3×10^{-5}
2-Pr-ol	0.712	0.003	178	5.6×10^{-3}	0.456	0.005	65	1.5×10^{-2}	0.045	0.004	6	1.7×10^{-1}	1.4×10^{-5}
2-Me-2Pr-ol	0.944	0.015	42	2.4×10^{-2}	0.411	0.006	45	$2.2 \times .10^{-2}$	0.080	0.015	3	3.3×10^{-1}	1.7×10^{-4}
1-Pr-ol	0.614	0.008	55	1.8×10^{-2}	0.509	0.004	101	9.9×10^{-3}	0.040	0.002	13	7.7×10^{-2}	1.4×10^{-5}
2-But-ol	0.545	0.013	28	3.6×10^{-2}	0.471	0.002	157	6.4×10^{-3}	0.044	0.007	4	2.5×10^{-1}	5.8×10^{-5}
2-Me-1Pr-ol	0.475	0.003	118	8.5×10^{-2}	0.542	0.002	180	5.6×10^{-3}	0.045	0.022	1	1.0	4.8×10^{-5}
2-Me-2-But-ol	0.572	0.013	30	3.3×10^{-2}	0.472	0.005	94	1.1×10^{-2}	0.139	0.020	4	2.5×10^{-1}	9.1×10^{-5}
1-But-ol	0.359	0.001	359	2.8×10^{-3}	0.567	0.002	189	5.3×10^{-3}	0.041	0.020	1	1.0	1.5×10^{-5}
Hexane	0.769	0.003	187	5.4×10^{-3}	0.703	0.002	226	4.4×10^{-3}	0.045	0.004	7	1.4×10^{-1}	3.4×10^{-6}
1-Heptene	0.601	0.005	77	1.3×10^{-2}	0.767	0.024	22	4.5×10^{-2}	0.284	0.082	10	1.0×10^{-1}	5.9×10^{-6}
Heptane	0.511	0.004	81	1.2×10^{-2}	0.692	0.011	44	2.3×10^{-2}	0.820	0.066	8	1.3×10^{-1}	3.5×10^{-5}
Cycloheptane	0.438	0.006	46	2.2×10^{-2}	0.972	0.012	56	1.8×10^{-2}	0.139	0.016	5	2.0×10^{-1}	7.8×10^{-5}
1-Heptyne	0.570	0.006	61	1.6×10^{-2}	0.910	0.036	17	5.9×10^{-2}	0.274	0.007	28	3.6×10^{-2}	3.4×10^{-5}
Ethylacetate	0.518	0.005	77	1.3×10^{-2}	0.600	0.007	61	1.6×10^{-2}	0.064	0.014	3	3.3×10^{-1}	7.1×10^{-5}
Allylacetate	0.508	0.002	221	4.5×10^{-3}	0.810	0.006	97	1.0×10^{-2}	0.159	0.027	4	2.5×10^{-1}	1.2×10^{-5}
Acetaldehyde	0.548	0.004	107	9.4×10^{-3}	0.572	0.003	114	8.8×10^{-3}	0.153	0.011	9	1.1×10^{-1}	9.1×10^{-6}
Dichlormethane	0.278	0.005	37	2.7×10^{-2}	0.830	0.006	89	1.1×10^{-2}	0.026	0.004	4	2.5×10^{-1}	7.6×10^{-5}
Dichlorethane	0.360	0.003	94	1.1×10^{-2}	1.075	0.010	74	1.4×10^{-2}	0.013	0.003	2	5.0×10^{-1}	7.2×10^{-5}
1-Bromopropane	0.503	0.010	34	3.0×10^{-2}	0.815	0.016	35	2.9×10^{-2}	0.159	0.010	11	9.1×10^{-2}	7.8×10^{-5}
1-Bromobutane	0.365	0.004	66	1.5×10^{-2}	0.798	0.017	32	3.3×10^{-2}	0.129	0.008	10	1.0×10^{-1}	4.7×10^{-5}
2-Nitropropane	0.414	0.002	165	6.1×10^{-3}	0.736	0.010	50	2.0×10^{-2}	0.031	0.013	1	1.00	1.1×10^{-5}
Acetonitrile	0.947	0.008	78	1.3×10^{-2}	0.035	0.026	1	1.00	0.045	0.014	2	5.0×10^{-1}	6.4×10^{-3}

$u = 1.03\sqrt{2\sigma^2}$; $s = \overline{x}/u$; $^{q}P = {}^{\mathrm{FID}}P_s \times {}^{\mathrm{Ar}}P_s \times {}^{\mathrm{Kr}}P_s$.

distinguishing of chloroprene, $^{ECD}P_s = 2.6 \times 10^{-5}$, and this one of NPD for cyanacrylate, $^{NPD}P_s = 2.1 \times 10^{-5}$, are three order higher than contribution $^{FID}P_s$ of FID. ECD did not contribute to the identification of isoprene, dipentene, and dodecene because it is not sensitive to hydrocarbons. The value of the combined probabilities, $^{q}P_s$, for distinguishing of these compounds with multiple-detection was not low and its contribution to their identification was small. The contribution of the sensitivity of the selective detectors is significant.

The contribution of the sensitivity of the detectors to the identification was estimated by simultaneous detection with FID and ECD of a mixture of pesticides diazinon, α-HCH, γ-HCH, malathion, aldrin, endrin, 4,4′-DDE, 4,4′-DDT, and 4,4′-PCB as an internal standard (Table 3). The contributions $^{FID}P_s = 2.5 \times 10^{-1}$ to 2.0×10^{-2} and $^{ECD}P_s = 2.3 \times 10^{-1}$ to 1.5×10^{-2} of FID and ECD, respectively, were not significant. The combined probabilities, $^{FID,ECD}P_s$ for distinguishing by simultaneous detection with FID and ECD were only one or two orders and were not significant. By decreasing of the concentration we determined the quantitative detection limits of FID for every one of the pesticides at a signal/noise ratio (S/N) of 10. The quantitative assessment at a fixed sensitivity, for example at the limit of detection (LOD), is able to be a measure for the selectivity at validation of analytical methods. We accepted the *s*-value in formula (1) to be one, because it was impossible for lower concentrations quantitatively to be determined by FID. Here, the contribution of FID to distinguishing is zero because $^{FID}P = 1.00$. In practice, ECD detects much lower concentrations than these ones of FID and demonstrates its selectivity. The peak's areas of every one of the pesticides at S/N = 10 of FID was accepted as a unit at the comparison of the signal of ECD. The ECD peak areas of the analyzed pesticides were higher by several orders than these ones at FID. For aldrine, the ECD/FID peak area ratio was 8365. It means that ECD is able to distinguish 8365 substances with signals which are 1, 2, . . ., 8865 times larger than this one of FID for aldrin at S/N = 10. That is why the *s*-value in formula (1) is 8865 and $^{ECD}P_{8865} = 1.19 \times 10^{-4}$ (Table 4). This value means that the high sensitivity of ECD increases the reliability of identification 8865 times towards this one with FID.

4.2. Multiple-separation

Despite of the high reliability of identification with multiple-detection a probability exists for overlapping analytes and they will not be distinguished. In this case, the only way for their distinguishing is chromatographic separation or mass spectrometry with a selected-ion monitoring mode. For example, the relative retention times (Table 3), of diazinone, lindane, and malathione are very similar and at the beginning of the experiments two of them were overlapped. They were separated by an adjustment of the temperature program and the separation system revealed a sum of separation numbers

Table 2
Contributions of FID, ECD and NPD to the identification of the pyrolysis products[a]

Pyrolysis product detection	FID-ReMMA (FID)				ECD-ReMMA (FID)				NPD-ReMMA (FID)				$^{q}P = P_{FID} \times P_{ECD} \times P_{NPD}$
	$\bar{x}$	σ	s	$P_s = 1/s$	$\bar{x}$	σ	s	$P_s = 1/s$	$\bar{x}$	σ	s	$P_s = 1/s$	
Dodecane	1.344	0.009	102	9.8×10^{-3}	–	–	–	–	0.164	0.045	2	5×10^{-2}	4.9×10^{-3}
Isoprene (PIP)	1.352	0.012	75	1.3×10^{-2}	–	–	–	–	0.212	0.037	4	2.5×30^{-1}	3.2×10^{-3}
Dipentene (PIP)	0.432	0.006	48	21×10^{-2}	–	–	–	–	0.184	0.034	3	3.3×10^{-1}	6.9×10^{-3}
Chloroprene (PCP)	0.746	0.008	67	1.5×10^{-2}	720	0.013	37894	2.6×40^{-5}	0.852	0.019	30	3.3×10^{-2}	1.3×10^{-8}
Dichloroprene (PCP)	0.316	0.007	31	3.2×10^{-2}	0.308	0.020	10621	9.4×10^{-5}	0.104	0.046	1	1	3.0×10^{-7}
Cyanacrylate (PCA)	0.882	0.010	58	1.7×10^{-2}	1.650	0.012	97	1.0×10^{-2}	614	0.009	47231	2.1×10^{-5}	3.6×10^{-9}

[a] 100 μg polymer + 10 μg poly(methyl methacrylate) dissolved in $CHCl_3$; t_{pyr} = 610 °C/5 s; five numbers of measurements.

Table 3
Contribution of multiple-detection $^{q}P_s$ with ECD and FID and the efficiency of the column P_s to distinguish some of the pesticides

Compounds	R_x/R_M (4,4′-PCB) mean	ECD-Re (4,4′-PCB) ECD				FID-Re (4,4′-PCB) FID				$^{q}P_s$	$P_{r,s} = {}^{q}P_s \times P_r$[a]
		$\bar{x}$	σ	s	$P_s = 1/s$	$\bar{x}$	σ	s	$P_s = 1/s$		
α-HCH, t_R = 10.920	0.959 (σ = 0.0060)	5.865	0.0964	44	2.27×10^{-2}	0.468	0.0501	6	1.66×10^{-1}	3.77×10^{-3}	2.02×10^{-5}
Diazinone, t_R = 11.886	0.961 (σ = 0.0047)	0.175	0.0285	4	2.38×10^{-1}	0.710	0.0389	13	8.0×10^{-2}	1.90×10^{-2}	1.02×10^{-4}
Lindane, t_R = 11.945	0.966 (σ = 0.0001)	6.736	0.2406	19	5.20×10^{-2}	0.488	0.0064	51	1.96×10^{-2}	1.02×10^{-3}	5.45×10^{-6}
Malatione, t_R = 14.9	1.205 (σ = 0.0018)	2.027	0.0447	31	3.22×10^{-2}	0.325	0.0355	6	1.59×10^{-1}	5.12×10^{-3}	2.74×10^{-5}
Aldrin, t_R = 15.549	1.258 (σ = 0.0009)	8.389	0.6119	9	1.06×10^{-1}	0.896	0.0214	28	3.57×10^{-2}	3.79×10^{-3}	2.03×10^{-5}
Endrin, t_R = 20.63	1.672 (σ = 0.0009)	8.907	0.0931	65	1.50×10^{-2}	0.531	0.0771	4	2.50×10^{-1}	3.75×10^{-3}	2.01×10^{-5}
DDE, t_R = 21.36	1.728 (σ = 0.0036)	5.707	0.3285	11	9.09×10^{-2}	0.994	0.0459	14	7.14×10^{-2}	6.49×10^{-3}	3.47×10^{-5}
DDT, t_R = 23.143	1.848 (σ = 0.0046)	4.906	0.0792	42	2.38×10^{-2}	0.546	0.0302	12	8.33×10^{-2}	1.98×10^{-3}	1.06×10^{-6}

[a] $P_r = 5.3 \times 10^{-3}$ ($\Sigma SN = 187 \rightarrow P_r = 1/187 = 5.3 \times 10^{-3}$).

Table 4
Influence of the sensitivity of ECD to the reliability of identification at multiple-detection

Substance	Concentration (μg/l) at S/N = 5; 1 μl	Sig^{ECD}/Sig^{FID}[a] (m)	$P = 1/m$
α-HCH	0.6	8865	1.13×10^{-4}
Diazinone	16	14300	6.97×10^{-5}
γ-HCH	0.8	9184	1.09×10^{-4}
Malation	30	5820	1.88×10^{-4}
Aldrin	0.6	8365	1.19×10^{-4}
Endrin	1.2	11365	8.79×10^{-4}
4,4′-DDE	3.0	4066	2.46×10^{-4}
4,4′-DDT	2.0	4906	2.04×10^{-4}

[a] Area FID at S/N = 5.

187. This value means that the system was able to separate 187 compounds, statistically distributed over the applied temperature range and the number of strips r along the abscissa in Eq. (1) is 187. Now, the probability for coincidental overlapping of the analyzed pesticides detected by all detectors is 5.4×10^{-3} ($1/187 = 5.4 \times 10^{-3}$) times lower and this one of the multiple-detection (Eq. (3)) will be $5.4 \times 10^{-3}\ {}^{q}P_s$ (Table 2).

Splitting the eluent immediately after an injector gives opportunity analytes to be separated simultaneously by two columns with different polarity. In this case, overlapping will be $P_{col1} \times P_{col2}$ are the separations are orthogonal, where P_{col1} and P_{col2} are the probability of distinguishing with column I and column II, respectively. We used a second column with OV-1701, column II, with ΣSN = 202. Small differences between the flows through columns were equilibrated by length of the capillary piece of the spliter. Contribution of OV-1701 column to distinguish analytes was $1/202 = 5.0 \times 10^{-3}$ and the combined probability at the multiple-separation in this case was $^{\text{DB-5/OV-1701}}P_r = 2.7 \times 10^{-5}$. OV-1701 column was replaced by another one with the most polar liquid phase Silar OV-275, column III. By programming the temperature of the column and at 240 °C final temperature we obtained ΣSN = 236 and $^{\text{OV-275}}P_r = 4.2 \times 10^{-3}$. The combined probability from the multiple-separation in this case was $^{\text{DB-5/OV-275}}P_r = 2.2 \times 10^{-5}$. However, separation with two columns was not truly orthogonal because liquid phases did not reveal only one type of interactions, i.e. the value of orthogonality (O_r) was below 1 ($O_r < 1.0$). The true O_r-value is difficult to be determined because this procedure requires a lot of experiments (1, 11). The value of orthogonality depends on the difference between the polarities of the liquid phases, the bigger difference, the higher value of the orthogonality. Taking into account that the value of orthogonality between C_{18} and the other stationary phases for RP-HPLC is above 0.6, at multiple-separation with DB-5/OV-1701and DB-5/Silar OV-275 liquid phases we accepted this parameter to be 0.6 and 0.8, respectively. Of course, this acceptance is not precise and further investigations are necessary. Now the combined probability at the multiple-separation with DB-5/OV-701and DB-5/Silar OV-275 liquid phases for

Table 5

Mass range (u)		FWHM resolution (u)		Scan speed (u/s)	n^a	$P^k_{6,n}$ [b] ($m = 6$)			
						$k = 3$	$k = 4$	$k = 5$	$k = 8$
Low	15–200	Standard	0.5	5500	370	1.99×10^{-8}	5.40×10^{-11}	2.96×10^{-12}	5.65×10^{-18}
Standard	50–2000	TurboScan	2.2	66000	886	1.44×10^{-8}	6.54×10^{-12}	3.70×10^{-14}	1.83×10^{-20}
		Standard	0.5	5500	3900	1.70×10^{-11}	1.76×10^{-14}	1.67×10^{-17}	4.28×10^{-23}
		ZoomScan	0.15	275	13000	4.55×10^{-13}	1.40×10^{-16}	5.39×10^{-20}	3.26×10^{-30}
		Ultra Zoom	0.08	27.5	24375	6.91×10^{-14}	1.13×10^{-17}	2.33×10^{-17}	4.06×10^{-32}
High	100–4000	Standard	0.8	5500	4875	8.65×10^{-12}	7.09×10^{-15}	7.28×10^{-18}	2.12×10^{-26}
		Unit	0.5	1830	7800	2.09×10^{-12}	1.76×10^{-15}	1.13×10^{-18}	5.64×10^{-25}
		ZoomScan	0.3	275	12666	4.93×10^{-13}	1.56×10^{-15}	6.17×10^{-20}	1.03×10^{-29}
UltraHigh	500–20000	Standard	2.5	5500	7800	2.09×10^{-12}	1.76×10^{-15}	1.13×10^{-18}	5.64×10^{-25}

[a] n: mass range/resolution.
[b] $m = 6$ (at ±10% reproducibility of abundance).

distinguishing of every one of the investigated pesticides is $(0.6^{\text{DB-5/OV-1701}} P_r) = 0.6/2.7 \times 10^{-5} = 4.4 \times 10^{-5}$ and $(0.8/^{\text{DB-5/OV-275}} P_r) = 2.2 \times 10^{-5}/0.8 = 2.8 \times 10^{-5}$.

These values are approximate to the probability of distinguishing with multiple-detection and column I, $P_{r,s}$ (Table 3). Increasing efficiency of column at multiple-separation or sensitivity and/or selectivity of the detectors at multiple-detection render an increased reliability of identification of analytes. The approach, which will be applied, depends on the analytes. When the analytes do not posses specific moeities and differ only by their volatility, multiple-separation will be preferred because difference in the polarity of the column will be useful for distinguish analytes with near physico-chemical properties. Multiple-detection will be preferred for identification of analytes with specific groups such as $-NO_2$, halogen atoms (ECD), or N-compounds (NPD). In this case, their selectivity and/or sensitivity are very useful for discrimination of the analytes on the base of difference in the elemental content (Table 5).

5. Conclusion

Multiple-detection and -separation give approximately equal possibility for distinguishing of analytes. When physico-chemical properties of the analytes are similar multiple-separation gives better opportunities for their identification. In this case, the efficiency of the columns is very important. When analytes contain some characteristic groups as $-NO_2$, halogen, or nitrogen atoms multiple-detection will be more useful. The sensitivity or selectivity significant increases the reliability of identification.

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