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Retention time repeatability as a function of the injection automatism in the analysis of trace organochlorinated compounds with high-resolution gas chromatography

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Abstract

Short-term precision in retention time determination of different injection systems (three manual and three autosamplers) is assessed in the present study. This evaluation has been performed on eight different instrumental set-ups equipped with the same type of capillary column (30 m, 5% phenyl–95% methyl) which have been used for the analysis of standard mixtures of α-, γ-, and δ-hexachlorocyclohexanes. The results show that, as expected, retention time repeatability increases with the degree of automatization of the injection system. They also show that only the autosamplers and the manual injector provided with the syringe-operated start device are suitable for analysis of compounds with retention time differences in the order of 0.1 min. Conversely, none of the systems included in the study, not even the autosamplers, is adequate for the analysis of analytes having retention time differences of about 0.01 min. © 1997 Elsevier Science B.V.

Keywords: Retention times; Injection methods; Organochlorine compounds; Pesticides; Hexachlorocyclohexanes

1. Introduction

The analysis of complex mixtures of organic chemicals poses particular problems when compound assignment is based on detectors that do not provide structural information [1–5]. In such cases, identification is only based on retention time and its reliability depends critically on the precision of the retention data supplied by the chromatographic system. Poor retention time precision can lead to mistaking nearby eluting peaks or missing the analyte peak.

Misidentification is of especial concern when analyzing toxic compounds, particularly in procedures developed for the analysis of large sets of samples. In these cases, one of the main targets is the minimization of the time needed for the determinations. This goal commonly involves the reduction of the clean-up steps and the extensive exploitation of the separation power of the instrumental techniques [6–8]. In the case of compounds amenable to gas chromatographic analysis, capillary columns constitute an ideal tool for their high resolution power. The high chromatographic resolution is in fact recorded as a high time resolution (in the order of few seconds) entailing stringent requirements in terms of time precision of the injection system.

The development of analytical methods for the identification and quantification of organochlorinated pesticides in large series of human blood sera samples constitutes a representative example of this type of cases [2,9–11]. Hexachlorocyclohexanes (HCHs) are frequently encountered at low amounts (in the order of ng/ml) and their determination requires precise measurements for differentiation

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between the gas chromatographic peaks of the analytes and nearby eluting interferences.

This problem is used in the present study as a test example to evaluate the suitability of the retention time repeatability of several injection systems commonly used in gas chromatography. The performance of three manual methods of injection and three autosamplers installed in eight chromatographic setups is evaluated. The precision of each system is assessed from the repeatability of the retention times corresponding to the α-, γ- and δ-HCH isomers.

2. Experimental

2.1. Materials

Residue analysis *n*-hexane (ref. 1.04371), isooctane (ref. 1.15440), concentrated sulfuric acid 95–97% (ref. 1.00731) and acetone (ref.1.00012) were from Merck (Darmstadt, Germany). The purity of the solvents and reagents was checked by analyzing 2 ml of Milli-Q water with the same procedure and dilution factors as the samples. No peaks eluting in the HCH zone were detected.

The standard mixtures of HCH isomers and surrogate solution (1,2,4,5-tetrabromobenzene, TBB) were prepared in isooctane solution. The γ-HCH and the TBB used to prepare the solutions were from Aldrich-Chemie (Steinheim, Germany), α- and δ-HCHs were from Promochem (Wesel, Germany) and β-HCH from Dr. Ehrenstorfer (Augsburg, Germany).

2.2. Extraction and clean-up

Organochlorinated compounds in the samples (serum, in this case) were analyzed according to a previously described method [10]. Briefly, an internal standard of 1,2,4,5-tetrabromobenzene, *n*-hexane and concentrated sulfuric acid were successively added to serum samples. After reaction, the mixture was stirred and the supernatant *n*-hexane phase was separated. The remaining sulfuric acid solution was re-extracted two times with *n*-hexane. The combined *n*-hexane extracts were additionally cleaned with concentrated sulfuric acid. Then the *n*-hexane phase was separated and concentrated under a gentle nitrogen stream.

2.3. Instrumental analysis

DB-5 columns, 30 m×0.25 mm I.D. (J&W Scientific, Folsom, CA, USA) coated with 0.25 μm of 5% diphenylpolydimethylsiloxane were used for all the analyses. Fused-silica tubings, 2 m×0.32 mm I.D., were connected between the column and the injector for column preservation. The oven temperature was programmed from 90°C (holding time 2 min) to 150°C at 15°C/min, and finally to 280°C at 4°C/min, keeping the final temperature for 10 min. The injector and detector temperatures were 270°C and 310°C, respectively. Injection was performed in the splitless mode, keeping the split valve closed for 35 s. Carrier gas (helium) linear speed was 50 cm/s.

These DB-5 columns were connected to the different instrumental settings evaluated in this study. These included five gas chromatography (GC) instruments equipped with electron-capture detection (ECD) systems and two GC instruments coupled to mass spectrometry (MS) systems, one operating in the electronic impact mode (EI) and the other in the negative ion chemical ionization mode (NICI). The characteristics of these instruments are summarized in Table 1 where the injection systems used in each case are also described. The detector operating conditions of the GC–ECD instruments are described in Table 2. All these instrumental settings were connected to computerized data acquisition systems allowing the automatic recording of the chromatographic profiles.

Temperature interface in the GC–MS instruments was 280°C. Source temperatures were 200°C and 150°C for EI and NICI, respectively. The dwell time was 0.06 s and the interchannel delay 0.02 s. Methane (0.8–1 Torr; 1 Torr=133.322 Pa) was the reactive gas in the NICI mode. Quantification ions were *m/z* 71 and 181 in the NICI and EI modes, respectively. Confirmation ions were *m/z* 255 and 219 for NICI and EI, respectively.

3. Results and discussion

3.1. Injection systems

As shown in Table 1, three different manual injection systems and three autosamplers have been

Table 1
Injection techniques compared in this study

Trade company	GC-Model	Detection	Injection system	Key
Konik	HRGC 3000 C	ECD	Manual. Two start points	A
Shimadzu	GC-9A-Series	ECD	Manual. Two start points	B
Fisons	Mega 2 Series	ECD	Manual. One start point	C
Varian	STAR 3600	ECD	Manual. Injector with start device	D
Fisons	Mega 2 Series	ECD	Autosampler AS-800	E
Fisons	MD-800	NICI-MS	Autosampler AS-200	F
Fisons	MD-800	EI-MS	Autosampler AS-200	G
Hewlett-Packard	HP-5890	ECD	Autosampler 7673-A	H

Table 2
Instrumental conditions of the GC–ECD settings described in Table 1

	Fisons	HP	Konik	Shimadzu	Varian
GC-Model	MEGA 2 Series	HP-5890	HRGC 3000 C	GC-9A-Series	STAR 3600
Make-up(N_2)	40 ml/min	60 ml/min	80 ml/min	86 ml/min	28 ml/min
Radiation source	^{63}Ni 10 mCi	^{63}Ni 15 mCi	^{63}Ni 15 mCi	^{63}Ni 10 mCi	^{63}Ni 15 mCi
Reference intensity	0.8 nA	NA	14 nA	1 nA	NA
Voltage	50 V	NA	50 V	NA	NA
Pulse width	1 μs	NA	1 μs	NA	NA
Range switch	NA	NA	NA	1[a]	1[a]

NA, not applicable.
[a] Two positions of range switch: 1 and 10. The most sensitive position (1) has been chosen.

evaluated. The manual injectors studied encompass three alternative methods. In one method, oven temperature program and acquisition system are initiated independently after injection (two start points). This system is represented by the A and B settings (Table 1). In another method both temperature program and acquisition are started by pressing one single button after sample introduction (one start point; system C in Table 1). Finally, a modification of this method towards higher degrees of automatization involves the installation of a spring coupled to the outer piece of the injector that starts both temperature program and acquisition when pressed by the syringe barrel during manual injection (system D).

On the other hand, the three autosamplers considered in this study are represented by the four autosampler-equipped instruments included in Table 1 (systems E–H). One autosampler is evaluated in connection with two different GC–MS instruments operating in chemical ionization and electron impact modes (systems F and G, respectively).

3.2. Retention time dispersion

The retention time repeatability in these injection systems has been evaluated from the replicate analysis of standard mixtures of α-, γ- and δ-HCH. The standard deviations ($n=5$) resulting from the retention time determinations of these HCH isomers with Table 1 instruments are reported in Table 3.

Major differences are observed between the differ-

Table 3
Standard deviations ($n=5$) of the retention time determinations with the chromatographic settings described in Table 1

	α-HCH	γ-HCH	δ-HCH	Average
A	0.045	0.047	0.057	0.050
B	0.053	0.051	0.052	0.052
C	0.026	0.024	0.025	0.025
D	0.014	0.013	0.012	0.013
E	0.006	0.006	0.006	0.006
F	0.004	0.005	0.004	0.0043
G	0.006	0.002	0.006	0.0047
H	0.006	0.006	0.006	0.006

ent injection systems considered. These differences are systematically observed for all compounds. Conversely, no major differences can be specifically assigned to the analysis of HCH isomers. Accordingly, the average values of these standard deviations have been selected to represent the major changes between injection systems. These averaged standard deviations have been used to define normal distribution functions that illustrate the dispersion in the retention time determinations of each chromatographic setting (Fig. 1).

As expected, dispersion is considerably higher in the manual than in the automatic systems, 0.013–0.052 min vs. 0.0043–0.006 min, respectively. In the manual systems the dispersion decreases dramatically as the degree of automatization increases. Thus, in the injectors with two starting points the standard deviations are 0.050 min and 0.052 min (A and B, respectively). The injector with one manual start point (C) has standard deviations of 0.025 min, which represents an improvement of 51% in relation to the former, and the injector starting oven program and acquisition by syringe pressing (D) has a standard deviation of 0.013 min, representing an improvement of 75% with respect to A and B.

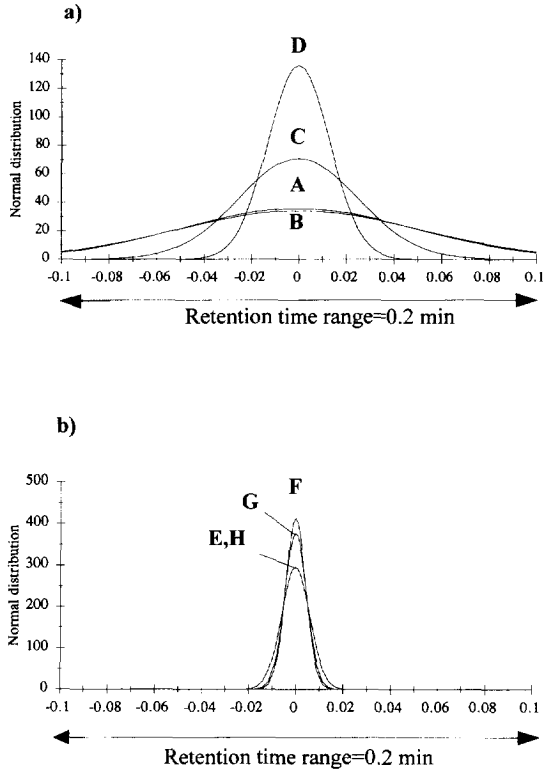

Fig. 1. Normal distributions depicting the standard deviations resulting from the retention time determination of HCHs with (a) the manual injection and (b) the automatic injection systems described in Table 1.

The differences between the automatic injectors are smaller. Autosampler AS-200 (Table 1) exhibits standard deviations of 0.0043 and 0.0047 min in the F and G instruments, respectively, and autosamplers AS-800 and 7673-A have standard deviations of 0.006 min (E and H in Table 1, respectively).

3.3. Cross-comparison

F-tests have been applied to the standard deviations reported in Table 3 to compare the differences in repeatability between injection systems (Table 4). These tests evidence that A and B are equivalent (at 95% confidence level) in terms of precision and significantly less precise than the automatic injectors (E–H) and the manual injector provided with a start device (D). However, these two manual injectors with two starting points are not significantly less precise (at 95% confidence level) than the manual injector with one start point (C). The one start point injector (C) exhibits a retention time dispersion that is significantly higher than in the automatic injectors (E–H) but not in the two start point injectors (A, B) or the injector provided with a start device (D).

In general terms no significant differences in standard deviation values are observed among the automatic injectors. These injectors exhibit significant differences from the one point and two point manual injectors but not with respect the injector provided with the start device. Therefore, this injector corresponds to an intermediate situation only showing significant standard deviation differences from the two point manual injectors.

3.4. Analysis of HCH in blood sera

Fig. 2 illustrates the ECD chromatograms that are usually encountered in the analysis of HCHs from sera samples using the oxidative clean up method described in this study. As may be observed, the analyses are complicated by three impurities showing

Table 4
F test comparison of the average standard deviations in the HCH retention time determination with the instrumental settings considered in this study ($F=s^2_{column}/s^2_{row}$, $F_{4,4,\alpha=95\%}=6.4$)

	A	B	C	D	E	F	G	H
A	1[a]	1.4						
	1[b]	1.2						
	1[c]	–[d]						
B	–	1						
	–	1						
	1.2	1						
C	3.0	4.2	1	–				
	3.8	4.5	1	–				
	5.2	4.3	1	–				
D	10	14	3.4	1				
	13	15	3.4	1				
	23	19	3.4	1				
E	56	78	19	5.4	1			
	61	72	16	4.7	1			
	90	75	17	4.0	1			
F	130	180	42	12	2.2	1	2.2	–
	88	100	23	6.8	1.4	1	–	1.4
	200	170	39	9.0	2.2	1	2.2	2.2
G	56	78	19	5.4	1	–	1	–
	550	650	140	42	9	6.2	1	9.9
	90	75	17	4.0	1	–	1	–
H	56	78	19	5.4	1	2.2	1	1
	61	72	16	4.7	1	–	–	1
	90	75	17	4.0	1	–	1	1

a, *b* and *c* correspond to the dispersion values of α-, γ- and δ-HCH, respectively.
d, only the values involving $F>1$ ($s^2_{column}>s^2_{row}$) are indicated for consistency with the F test.

significant signals in the ECD record. Impurity a, elutes near α-HCH, impurity b elutes between β- and γ-HCH and impurity c almost coelutes with δ-HCH. The analysis of this type of mixtures must face two problems: retention time precision and peak overlapping. As indicated in the introduction, the present study is only focused on the first. High precision in the injection systems is needed for the correct identification and quantitation of the species of interest in mixtures like that shown in Fig. 2. Obviously, other alternatives such as further clean-up may also be considered. However, this is not always a practical solution, namely when a large number of analyses has to be performed. In any case, this study is focused on the evaluation of the precision available with the injection systems of common use in gas chromatography.

Table 5 depicts the retention times of the analytes and interferences present in the serum samples taken as reference (Fig. 2) and the retention times of the analytes in the standard mixture. One sample t-tests have been performed to retention times of analytes and interferences to establish the suitability of every injector for the unequivocal identification of HCHs. The results from these tests are reported in Table 6. Interferences a and b elute at significantly different retention times (at 95% confidence level) than the analytes in the autosamplers and the manual injection provided with the syringe-operated start device. In contrast, interference c cannot be confidently distinguished from δ-HCH with any of the injection systems evaluated. Further clean-up, a different chromatographic column or GC–MS analysis [11] are needed for δ-HCH determination in this case.

4. Conclusions

As expected, retention time repeatability increases at a higher degree of automatization of the injection systems. Significant dispersion decreases are observed when comparing the retention time standard deviations of manual injectors requiring the independent activation of temperature program and data acquisition, their simultaneous activation, and simultaneous activation with a mechanism activated by the syringe barrel at injection (standard deviations 0.045–0.052, 0.025 and 0.013, respectively). The autosamplers provide even better repeatability (standard deviations 0.0043–0.006 min). No significant retention time dispersion differences (at the 95% significance level) have been observed among the three autosamplers tested in this study.

In terms of analytical performance, only the autosamplers and the manual injector provided with the syringe-operated start device are suitable for analysis of compounds with retention time differences of ca. 0.1 min. Conversely, none of the systems included in this study is adequate for the analysis of analytes having retention time differences in the order of 0.01 min. In this latter case, besides the problem of peak overlapping, none of the injectors has sufficiently low dispersion to differentiate (at 95% confidence level) between the retention times of such closely eluting peaks.

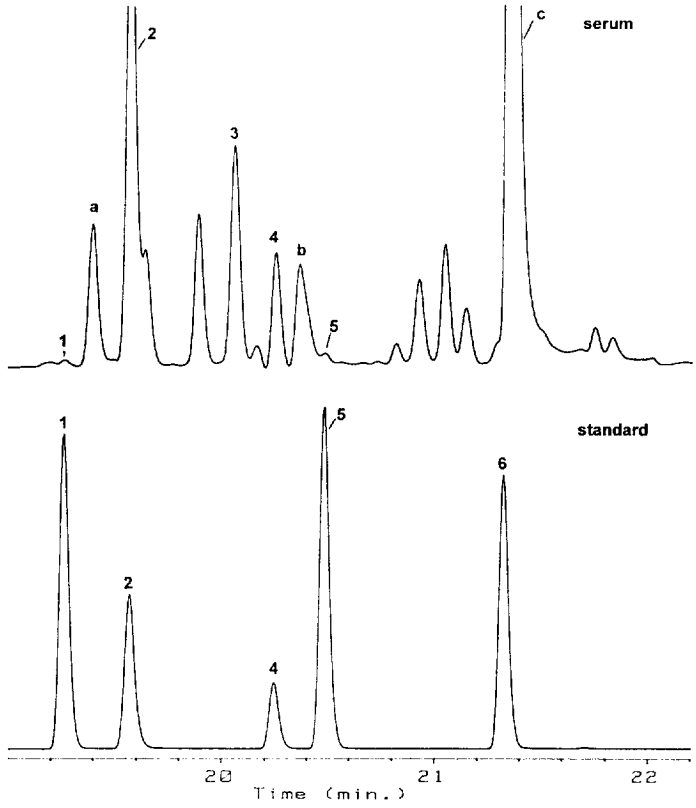

Fig. 2. ECD chromatograms of blood serum samples (acid treated) and standard mixtures. Peak identification: 1=α-HCH; 2=HCB; 3=surrogate; 4=β-HCH; 5=γ-HCH; 6=δ-HCH; *a*, *b*, and *c*=interference compounds.

Table 5
Retention times (min) of the analytes and interferences from the blood sera HCH extracts described in Fig. 2

Compound	α-HCH	*a*	β-HCH	*b*	γ-HCH	δ-HCH	*c*
Serum sample	19.266	19.396	20.248	20.362	20.483	n.d.	21.357
Standard mixture	19.258	n.d.	20.240	n.d.	20.480	21.343	n.d.

n.d., not detected.

Table 6
One-sample *t*-test comparison of the analyte and interference retention times reported in Table 5

Instrumental setting	α-HCH vs. a	γ-HCH vs. b	δ-HCH vs. c
A	1.4[a]	1.1	0.11
B	1.2	1.0	0.12
C	2.4	2.2	0.25
D	4.4	4.1	0.52
E	10	8.8	1.0
F	15	11	1.6
G	10	26	1.0
H	10	8.8	1.0

Tabulated *t* for four degrees of freedom at the 95% level of confidence=2.78.
[a] calculated with the standard deviation values of Table 3.

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