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Journal of Chromatography A, 811 (1998) 241–245

JOURNAL OF CHROMATOGRAPHY A

Short communication

Use of persistent trace gas chromatography artifacts for the calculation of pseudo-Sadtler retention indices

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Received 8 December 1997; received in revised form 6 April 1998; accepted 6 April 1998

Abstract

Pseudo-Sadtler retention indices were calculated using persistent trace level capillary column artifacts, evident in the gas chromatography–mass spectrometry analysis of a natural product extract. The artifacts consisted mainly of a series of polymethylcyclosiloxanes eluting during a linear temperature program. These were used to determine retention indices of structures in the extract, and these indices were then compared with those derived from the more traditional *n*-alkanes calibration method.

Keywords: Retention indices; *Curcuma longa*; Polymethylcyclosiloxanes

1. Introduction

Gas chromatography–mass spectrometry (GC–MS) was used to analyse a cyclohexane extract of the *Curcuma longa* L. with the following theme; that the combination of specially calibrated retention indices (I_{exp}) with GC–MS for the identification of organic structures could have a much higher analytical performance than GC–MS alone [1,2].

Capillary column GC can generate retention indices very precisely, assuming standardisation of stationary film polarity, carrier gas flow-rate, stationary phase film thickness and temperature programming rate. However, in practice there is little inter-laboratory standardisation. Initial development work [3,4] to circumvent this problem, involved the successful discovery of ''pseudo-Sadtler'' GC conditions. The strategy used was the following: one dataset was adopted as a founding core, that is the 8°C on OV-1 stationary phase subset of the excellent Sadtler compilation. Then experimental conditions were sought after that generated I_{exp} values which closely approximated the analytically well defined I_{Sad} values and so allowed a comparison of unknown structures with those in the Sadtler library [5]. This method is robust because of the existence of a plateau of these ''pseudo-Sadtler'' calibration conditions (Ref. [3]; Table 1).

Normally I_{exp} values are measured using co-injected *n*-alkane standards. However we observed in the course of our experiments a systematic series of artifacts persistently seen at trace levels, even with thoroughly conditioned capillary columns and low bleed GC injector septa, which we assign to siloxane impurities. Their reproducibility suggested to us that these artifacts could be exploited for calibration purposes. Once their I_{exp} values are established, these can be used in turn to calculate retention

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0021-9673/98/$19.00
PII: S0021-9673(98)00285-4

Table 1
Analytical artifacts found during GC–MS analysis

I_{exp}	M_r	Library fit	$I_{Lee\ Smith}$
1003.88±0.02	296	Octamethyltetrasiloxane	1000
1165.99±0.54	370	Decamethylcyclopentasiloxane	1161
1342.27±0.50	444	Dodecamethylcyclohexasiloxane	1339
1518.59±0.79	518	Tetradecamethylcycloheptasiloxane	1518
1693.43±0.43	592	Hexadecamethylcyclooctasiloxane	1691
1761.80±0.55	346	Diphenyltetramethylcyclotrisiloxane	–
1858.43±0.68	666	Octadecamethylcyclononasiloxane	1855
1873.66±1.43	406	3,5-Bis(4-*tert*.-butylphenyl)-2,3,4,5-tetramethylhexane	–
2818.37±0.84	410	Squalene	–
2853.84±1.03	618	Tetraphenylhexamethylpentasiloxane isomer 1	–
2955.26±0.96	618	Tetraphenylhexamethylpentasiloxane isomer 2	–

The ± values in the I_{exp} column refer to the standard deviation from six measurements. The literature $I_{Lee\ Smith}$ values [8] are reported here to add weight to the identification of the various siloxane species.

indices (I_{bleed}) which should be close to the I_{Sad} values.

2. Experimental

2.1. Instrumentation

A Finnigan TSQ-70B quadrupole mass spectrometer was used as a GC detector, running in either 70 eV electron-impact ionisation or methane moderated positive ion chemical ionisation mode, coupled with a HP 5890 series II gas chromatograph. The GC injector was maintained at 250°C, the GC transfer line at 320°C, the source at 150°C and the vacuum manifold at 70°C. The mass range was scanned from $m/z=10$ to 770 per 0.5 s for electron impact and from 70 to 770 per 0.5 s for chemical ionisation. The GC program was from 35°C to 320°C at 15°C/min, followed by 10 min isothermally at 320°C. The mass spectrometer electron multiplier was at +1100 V, the conversion dynode at −15 kV and the electron current at 400 μA. The Wiley library was the 118 137 entry edition (Finnigan part No. 70001-30098, version 1.00, 1992). All extracts were measured with simultaneous injection of C_5 to C_{32} *n*-alkanes, as retention index calibration standards. Supelco Thermogreen low-bleed (LB-1) GC injector septa were used. A poly(dimethylsiloxane) HP Ultra-1 capillary column with dimensions 25 m×0.32 mm I.D. and a film thickness of 0.52 μm, was used for consistency with the Sadtler compilation.

2.2. Measurement

Ten mg from a 38 mg residue of a cyclohexane extract of *Curcuma longa* L. (Allepey) fingers was dissolved in 500 μl of dichloromethane and 0.2 μl was injected in a purged splitless mode onto the GC–MS system to give the results in Table 2. For preparation of this extract see Ref. [12]. An equivalent volume of the cyclohexane used for extraction was concentrated, dissolved in dichloromethane and injected as a control.

2.3. Retention index calibration

The base/neutrals mixture for US Environmental Protection Agency (EPA) method 625 (Sigma part No. 38 463-1; 41 structures with retention index 952 to 3199), was manually injected, simultaneously with a C_5 to C_{32} *n*-alkane mixture and the retention indices measured by simple linear interpolation between bracketing *n*-alkanes, using the van den Dool and Kratz equation [6]. This was used to find the ''pseudo-Sadtler'' conditions. Of the 41 structures, 29 are reliably in the Sadtler data set. Various temperature programming rates were evaluated between 15 and 18°C/min together with various helium carrier gas flow-rates (between 45.0 to 70.0 kPa head pressure) to find the ''pseudo-Sadtler'' conditions. A

Table 2
GC–MS of *Curcuma longa* L. cyclohexane extract analysis (composite of results in Ref. [12])

I_{exp}	I_{bleed}	M_r	Identity	I_{Sad}
1472.96±0.28	1471.14	202	α-Curcumene	–
1489.28±0.35	1487.70	204	α-Zingiberene	–
1503.99±0.51	1502.51	204	β-Bisabolene	–
1518.34±0.65	1516.69	204	β-Sesquiphellandrene	–
1638.36±0.56	1635.69	216	ar-Turmerone	–
1648.84±0.22	1646.22	218	α-Turmerone	–
1680.82±0.08	1678.86	218	β-Turmerone	–
1724.25±0.55	1723.17	220	1-Bisabolon	–
1737.27±0.46	1736.39	228	Tetradecanoic acid	–
1753.93±0.00	1753.48	218	Zerumbone	–
1916.29±0.49	1911.79	254	*cis*-9-Hexadecenoic acid	–
1938.97±0.99	1933.17	256	Hexadecanoic acid	–
2106.20±0.72	2101.79	280	*cis*–*cis*-9,12-Octadecadienoic acid	2105.07
2113.48±1.35	2109.79	282	*cis*-9-Octadecenoic acid	–
2138.54±0.68	2134.66	284	Octadecanoic acid	2139.86

The ± values in the I_{exp} column refer to the standard deviation from six measurements. The I_{bleed} values approximate to I_{Sad} values, though due to the gaps in the bleed series and some error propagation, the I_{bleed} values are not as close to I_{Sad} values as the I_{exp} values, e.g., in the third last and last rows.

least-squares unweighted fit of these 29 retention indices with those in the 8°C/min on OV-1 subset of the Sadtler compilation allowed adjustment of the temperature programming and helium flow-rate to find the best alignment i.e., the ''pseudo-Sadtler'' conditions. The best combination found was a linear temperature programming rate of 15°C/min from 35 to 300°C and a HP 5890A GC column head-pressure of 70.0 kPa helium (Table 1 of Ref. [12]). The retention index calibration from the artifacts in Table 1 was generated using the cubic spline option in the program Kaleidagraph, version 3.0.5, Abelbeck Software, Reading, PA, USA.

3. Results and discussion

Table 1 only includes the artifacts that were present in all samples and which have been observed consistently on the Thermogreen LB-1 septa/HP-Ultra-1 column combination over several years in our laboratory. The presence of hexamethylcyclotrisiloxane was observed in all the experimental samples, but it was not possible to measure a retention index due to its broad and non-symmetrical chromatographic peak characteristics. Since this is the most abundant of these siloxanes, this may be an overloading effect. Their mass spectra have been reported in Ref. [14]. The first three eluting siloxanes in Table 1 have already been described as analytical artifacts in Ref. [7] as was hexamethylcyclotrisiloxane. The remaining siloxanes in Table 1 have not.

In the presented work the polymethylcyclosiloxane series tapers out above octadecamethylcyclononasiloxane, although eicosamethylcyclodecasiloxane, with molecular mass (M_r) 740 has been reported to elute from a capillary column ($I_{Lee\ Smith}$ = 2031) when deliberately injected [7].

The I_{exp} values in Table 1 were measured under ''pseudo-Sadtler'' conditions and so are very close to Sadtler indices (>99.9%). The $I_{Lee\ Smith}$ values [8] were run on a poly(dimethylsiloxane) J&W capillary column with dimensions 30 m×0.25 mm I.D. and a film thickness of 1.0 μm, programmed from 50°C to 250°C at 4°C/min [9,10]. From Table 1 it can be seen that the $I_{Lee\ Smith}$ values [8] are also close to the Sadtler indices (estimated as >99.5%), at least in the range from 1000 to 1855. Squalene is in Table 1 because of its useful late eluting position and its ubiquitous nature [15,17], although its primary source may be the adhesives used to secure capliners in screw caps [11], rather than the LB-1 septa/HP-Ultra-1 column combination. Two polyphenylpolymethylsiloxanes, putatively tetra-

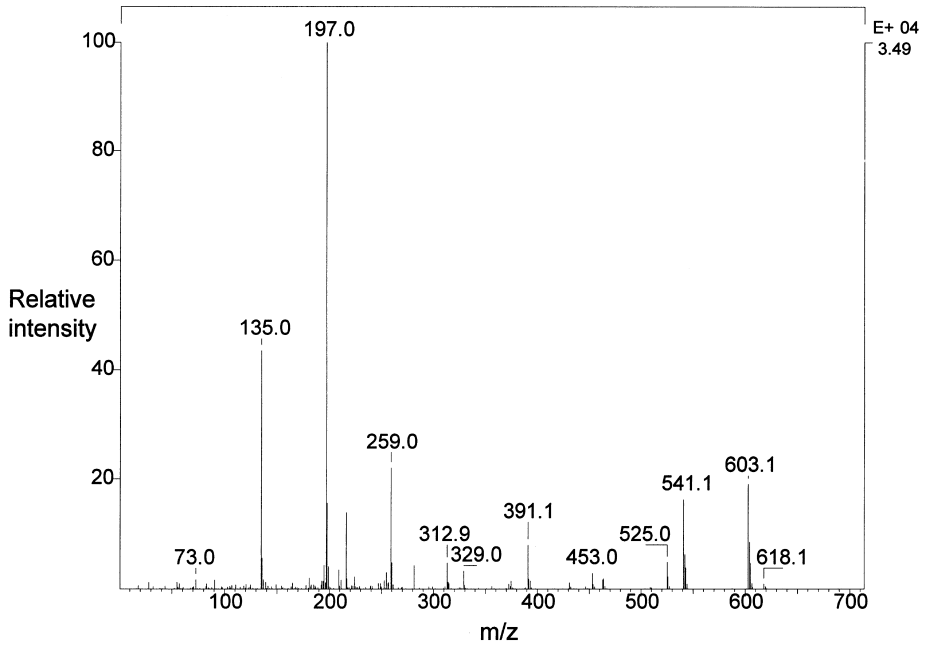

Fig. 1. Electron impact MS spectrum of polyphenylpolymethylcyclosiloxane structure with putative formula $C_{30}H_{38}Si_5O_5$ and M_r=618, having an I_{exp}=2955.26±0.96 (isomer 2). The ion at m/z=197 argues for a diphenylmonomethyl-silyl side group outside the cyclosiloxane ring, making the latter either three or four silicon membered.

phenylhexamethylpentasiloxane isomers, formula $C_{30}H_{38}Si_5O_5$ (see Fig. 1), have apparently not been reported before. Indeed, three more novel polyphenylpolymethylsiloxanes (all isomers with M_r= 692) exist, but are not described in Table 1 as they elute between I_{exp}=2985 and 3125 i.e., above the linear temperature programming range, and so cannot be used for retention index calibration. The source of the three polyphenylpolymethylsiloxanes in Table 1 may be the deactivation chemicals used to mask residual silanol group in capillary column manufacture e.g., 1,1,2,2-tetraphenyl-1,3-dimethylsilazane [16].

The actual source of the majority of these artifacts i.e., the polycyclosiloxanes is not definitely known. It has been attributed to silicon rubber septum bleed [13] for instance Supelco Thermogreen LB-1 injector septa or to poly(dimethylsiloxane) stationary phases, for instance the Hewlett-Packard Ultra-1 capillary columns.

To obtain I_{bleed} values, the first step is the identification of the approximate elution positions of the bleed series in Table 1. For instance by 70 eV electron-impact library identification or by chemical ionisation-derived molecular mass. Then the already established I_{exp} values in Table 1 establish the calibration table i.e., I_{exp} versus the retention times of the bleed/artifact series. Interpolation between I_{exp} values gives the I_{bleed} values for the relevant GC eluting compounds. These should be close to I_{Sad} values (see Table 2). In the I_{exp} range between 1300 and 1800, the calibration is close to the conventional *n*-alkanes method (Table 2). A disadvantage is the relative absence of artifacts in the I_{exp} range from 1900 to 2800. This problem can be seen in Table 2, e.g., most fatty acids in the *Curcuma longa* L. sample have an I_{exp} above 1875 and therefore lie away from the region where the majority of calibrating artifacts elute. This forces a reliance on cubic spline fits rather than conventional linear interpolation for the calculation of retention indices. No external calibration standards need to be added for I_{bleed} values to be derived. Therefore these reproducible bleed artifacts may prove uniquely useful for calibration in certain circumstances e.g., when coelution with an *n*-alkane hinders conventional cali-

bration or as a retrospective method for analysing old GC–MS data that was not calibrated for retention index measurement.

4. Conclusions

The I_{exp} values of a series of GC artifacts were determined under ''pseudo-Sadtler'' conditions. These values are almost identical to Sadtler values (>99.8% accuracy) and can be used in turn to generate I_{bleed} values that likewise approximate Sadtler retention indices between 1000 and 1850 retention index units, without the need for *n*-alkane co-injection. The application of this technique to an underivatised cyclohexane extract of *Curcuma longa* L. illustrates the proximity of the I_{bleed} to the I_{exp} values within the retention index range 1300 to 1850. This turns a previously considered detrimental feature of GC to the advantage of the analyst.

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