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Short communication

Retention time reproducibility in comprehensive two-dimensional gas chromatography using cryogenic modulation II. An interlaboratory study

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Abstract

An interlaboratory survey was conducted to determine the reproducibility of retention times in both the first (^{1}D) and second dimension (^{2}D) axes of the two-dimensional separation space, using the longitudinal cryogenic modulation comprehensive two-dimensional gas chromatographic approach. Intralaboratory reproducibility has been demonstrated in part 1 of this investigation [J. Chromatogr. A 968 (2002) 161]. Confidence in absolute retention times (hence component positions) in the two-dimensional separation space is critical to component identification. Comparing data from four independent laboratories, five independent gas chromatographs, five independent LMCS units, and numerous operators has determined that the LMCS cryogenic modulation approach provides reliable comprehensive two-dimensional GC results.

Keywords: Retention time; Cryogenic modulation; Reproducibility; Gas chromatography, comprehensive two-dimensional

1. Introduction

Comprehensive two-dimensional gas chromatography (GC × GC) is a relatively recent technique which offers increased peak capacity and improved resolution capabilities over pre-existing GC techniques. Various methods of achieving the GC × GC experiment, and primarily the modulation process, which is the key technical requirement, are reviewed in Ref. [2]. An indication of the expanding GC × GC applications base is provided in Ref. [3]. The improved resolution of GC × GC over single-column techniques, and the opportunities for fingerprinting were important in recent studies on the source, transport,

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and fate of organic compounds in the environment [4,5]. The previous year's International Symposium on Capillary Chromatography saw great interest in GC × GC [6], where key questions, such as those regarding the transportability and reliability of GC×GC data were raised. Reichenbach et al. [7] discussed the requirements of data processing and comparison software, and has proposed the use of reference "chromatogram images" to assist in the characterisation of complex chemical mixtures. With respect to this concept, the use of GC × GC for the fingerprinting of essential oils [8] is noteworthy, as is its use for oil spill source identification [9], for the detection of ignitable liquids in fire debris [10], and also the pattern recognition approaches described by Johnson and Synovec [11]. It should be noted that in all cases, it is only the access to the GC × GC two-dimensional chemical separation "map" that permits these studies to progress. The improved resolution offered by GC × GC should provide genuine opportunities for improved fingerprinting. It is also desirable that individual peak positions within the 2D separation space can be used to assign component identity with a high level of confidence; however, there is a critical requirement that the modulation technique can provide reproducible component retention times. The key to reproducible peak positions in GC × GC is a well-controlled and reproducible modulation event start time throughout the GC × GC analysis. In Part 1 [1] of this study, the LMCS cryogenic modulation approach was shown to produce reproducible start times and consistent modulation phase profiles [12] for individual components in a range of experiments over a space of days, and on a variety of instruments, and with different column sets comprising the same stationary phases, of matched dimensions. Reproducible 2D peak distribution should also allow comparison of GC × GC–FID results with well-characterised chromatogram images from GC × GC–MS analysis, which is likely to be important to future GC × GC studies. The previous study has been extended to an interlaboratory study to further demonstrate the suitability of the LMCS cryogenic modulation approach for fulfilling these requirements. Hopefully by extension, other modulation methods will also be shown to provide a high degree of absolute retention reliability, which will support further opportunities for sample characterisation using GC×GC.

2. Experimental

GC × GC analysis was carried out in four different laboratories, using either an Agilent Technologies 6890 model gas chromatograph (Agilent Technologies, Forest Hill, Australia) or a Shimadzu GC-2010 gas chromatograph (Shimadzu, Milan, Italy). Each GC was equipped with flame ionization detection (FID; operated at 100 Hz (Agilent), or 50 Hz (Shimadzu) data acquisition frequency). The GCs were retrofitted with Everest model LMCS units (Chromatography Concepts, Doncaster, Australia) comprising a stepper motor drive for movement of a cryo-trap. A modulation frequency of 0.2 Hz was applied in all analyses and the thermostatically controlled cryo-trap was maintained at ∼0 °C. The electronic modulation control unit was instructed to commence modulation by the events control function of the Chemstation (Agilent) or GC Solution (Shimadzu) GC software. Four identical column sets were used for the GC × GC experiments. Each set comprised of two columns, which were serially coupled by a zero dead-volume fitting. These column sets were used in a previously described intralaboratory study [1], and one set was distributed to each of the four participating laboratories for the present study. The primary column in each set was a low-polarity BPX5 (5% phenyl equiv. polyphenylmethyl siloxane; 0.25 μm film thickness) fused-silica capillary column of dimensions 30 m × 0.25 mm. The second column in each set was a polar BP20 (polyethylene glycol; 0.10 μm film thickness) fused-silica capillary column of dimensions 1.0 m × 0.10 mm. All columns were from SGE International (Ringwood, Australia). The 1 m BP20 columns were taken from a single 10 m column. Each analysis was carried out by temperature-programmed analysis from 60 to 210 °C at 2 °C min^{-1}, then to 260 °C at 20 °C min^{-1}. An injection volume of 1.0 μl was employed in all analyses using an autosampler with a split ratio of 100:1. The carrier gas was hydrogen, with a column head pressure of 7.5 p.s.i. No further steps were taken to adjust operating conditions, or to correct any settings.

The sample used for all analyses was a mixture of common essential oil type components, including α-pinene, 3-octanone, γ-terpinene, linalool, linalyl acetate, borneol, bornyl acetate, nerol, neryl acetate, neral (*Z*-citral), geranial (*E*-citral), geranyl acetate,

camphor, hexyl butyrate, and methyl salicylate. All reference standards were provided by Australian Botanical Products (Hallam, Australia). The sample was prepared in *n*-hexane such that the final concentration of each was ~0.5% (v/v).

3. Results and discussion

A typical GC × GC chromatogram of the 15 standards listed above is given in Fig. 1. In Part 1 of this study [1], run-to-run, day-to-day, instrument-to-instrument, and column set-to-column set first dimension retention times were demonstrated to have good reproducibility to within a few retention index units and calculated peak maxima retention times were generally within plus or minus one modulation period (0.08 min). The positions of most second dimension component contour positions were found to be reproducible within $\pm 1\sigma$ of the second dimension peak widths. The mean absolute retention times of 15 components analysed by each laboratory in the present investigation are reported in Table 1, for the first dimension (^{1}D) and second dimension (^{2}D) retentions. The data are also presented in Fig. 2 as a scatter plot of the components' peak apex positions within the 2D separation space. The results are consistent with the previous intralaboratory study; excellent run-to-run retention time reproducibility was observed from each of the three participating laboratories. Comparison of the data from the four laboratories, including the reference laboratory, demonstrates that a chromatogram image obtained by one laboratory can be reliably compared with those obtained elsewhere, using the same experimental conditions (Fig. 2). It is also evident that the results acquired on gas chromatographs from different manufacturers can be directly compared.

The results from the laboratory in Italy gave consistently longer retentions in the first and second dimensions compared to all other laboratories, thus the squares representing that laboratory's data are plotted to the upper right of the cluster for a given compound. This will be consistent with the column being used in this laboratory having a lower carrier gas flow-rate through the columns (assuming that the temperature program is not the source of error). A reduced flow

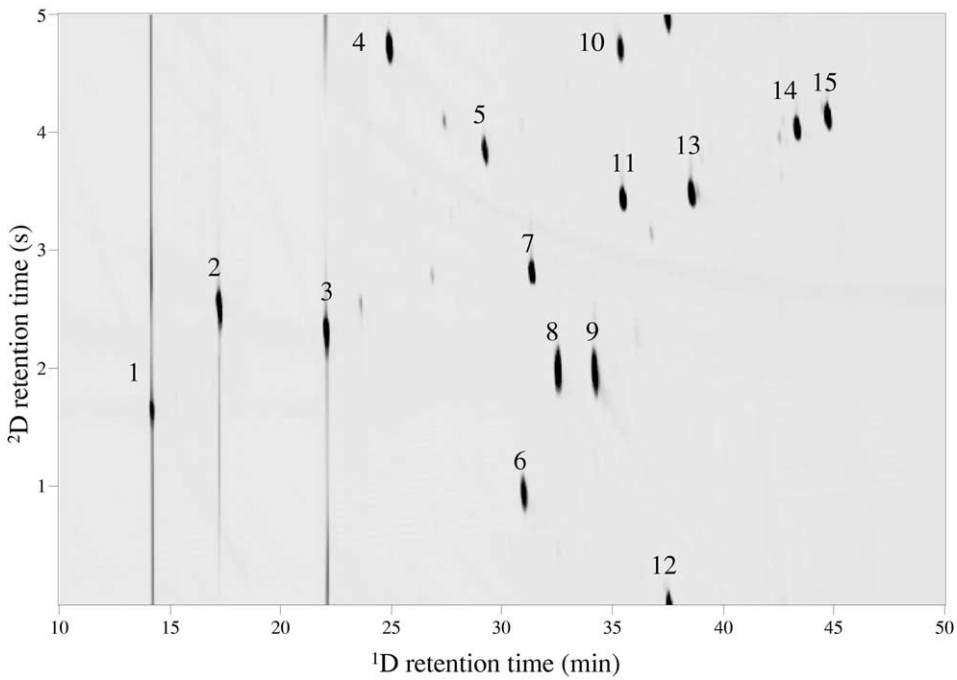

Fig. 1. Typical GC × GC chromatogram illustrating the separation of the 15 components listed in Table 1.

Table 1
Comparison of first dimension (^{1}D) and second dimension (^{2}D) retention times from the three test laboratories with the average retention times from the reference laboratory

	Reference laboratory				Test laboratory 1, NZ				Test laboratory 2, USA				Test laboratory 3, Italy			
	^1t_R	SD	^2t_R	SD	^1t_R	SD	^2t_R	SD	^1t_R	SD	^2t_R	SD	^1t_R	SD	^2t_R	SD
1. α-Pinene	14.13	0.09	1.68	0.04	14.00	0.09	1.64	0.03	13.86	0.00	1.66	0.02	14.57	0.01	1.77	0.00
2. 3-Octanone	17.13	0.07	2.61	0.10	17.04	0.03	2.55	0.04	16.97	0.00	2.55	0.02	17.82	0.01	2.81	0.01
3. γ-Terpinene	22.10	0.10	2.39	0.11	21.77	0.11	2.34	0.01	21.77	0.09	2.32	0.01	22.69	0.09	2.59	0.01
4. Linalool	24.92	0.11	4.79	0.08	24.71	0.04	4.68	0.05	24.52	0.00	4.80	0.00	25.56	0.01	5.05	0.03
5. Camphor	29.19	0.00	3.94	0.13	28.98	0.01	3.83	0.02	28.67	0.00	3.94	0.01	29.79	0.07	4.20	0.03
6. Borneol	30.85	0.00	5.99	0.08	30.59	0.07	5.91	0.07	30.43	0.00	6.05	0.02	31.52	0.01	6.25	0.02
7. Hexyl butyrate	31.40	0.00	2.92	0.13	31.09	0.09	2.89	0.02	30.99	0.00	2.92	0.01	31.91	0.07	3.18	0.03
8. Methyl salicylate	32.38	0.13	7.04	0.07	32.17	0.12	6.94	0.05	32.05	0.01	7.13	0.01	33.12	0.00	7.29	0.02
9. Nerol	34.14	0.00	6.96	0.05	33.84	0.09	6.95	0.17	33.82	0.01	7.06	0.00	34.78	0.03	7.27	0.08
10. Neral	35.40	0.01	4.08	0.11	35.02	0.00	4.73	0.02	34.85	0.01	4.83	0.01	36.05	0.01	5.10	0.04
11. Linalyl acetate	35.45	0.01	3.53	0.13	35.19	0.01	3.49	0.02	35.05	0.00	3.56	0.01	36.15	0.01	3.85	0.03
12. Geranial	37.44	0.01	5.10	0.07	37.18	0.09	4.96	0.07	37.07	0.00	5.09	0.01	38.14	0.09	5.35	0.02
13. Bornyl acetate	38.53	0.02	3.58	0.12	38.31	0.01	3.53	0.02	38.01	0.00	3.61	0.01	39.21	0.00	3.89	0.01
14. Neryl acetate	42.55	0.17	4.11	0.01	43.05	0.00	4.06	0.01	42.76	0.01	4.15	0.02	43.95	0.00	4.47	0.02
15. Geranyl acetate	44.69	0.01	4.23	0.10	44.49	0.00	4.19	0.01	44.22	0.00	4.27	0.01	45.39	0.00	4.56	0.01

^1t_R are given in minutes, and ^2t_R are given in seconds. Average peak widths were of the order of 150 ms in ^{2}D. The reference laboratory and test laboratories 1 and 2 used an Agilent 6890 GC. Test laboratory 3 used a Shimadzu GC-2010.

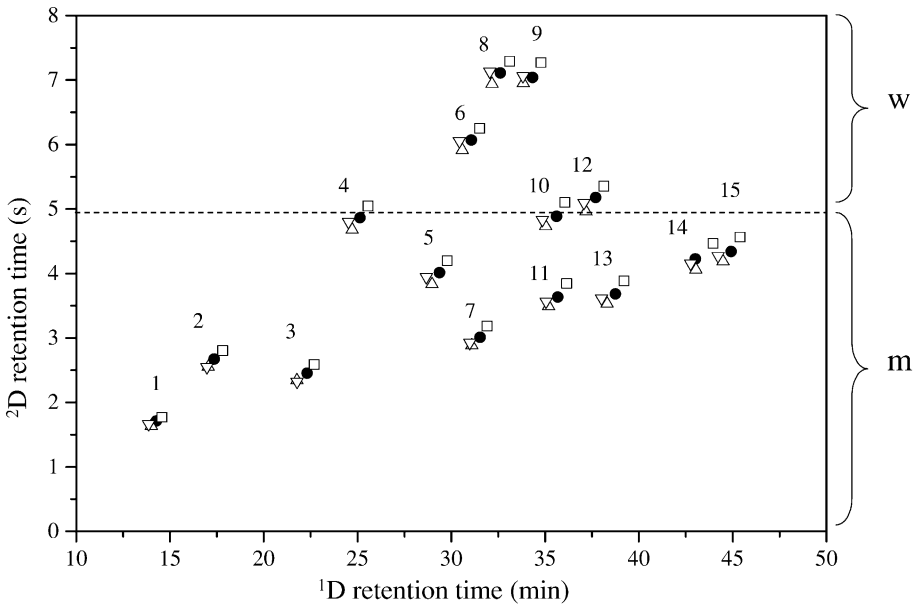

Fig. 2. Apex plot representation of the 2D separation space, showing the peak positions of the 15 components. Circle, averaged results from reference laboratory (Australia); up triangle, test laboratory 1 (NZ); down triangle, test laboratory 2 (USA); square, test laboratory 3 (Italy); m, modulation period; w signifies the extent of wrap around, which was recognized based on previous studies on similar samples which contained these components.

causes the compound to elute from ^{1}D at a later time, and in turn enter and elute from ^{2}D at a higher temperature. Generally if a compound is introduced to ^{2}D at a higher temperature, this would suggest that the compound would elute with a smaller $^{2}t_{R}$. But if the flow rate is sufficiently lower, then $^{2}t_{R}$ may be expected to increase. Thus these two effects cause contrary trends in $^{2}t_{R}$ values. Note again that no data or experimental conditions were corrected or adjusted to account for possible column or system differences and this again supports the contention that the data here exhibit good correlation. Incorporation of test samples between laboratories, with suitable correction strategies should further improve reproducibility. The results from New Zealand and the USA are in very good agreement with one another, and the positions of the peak apexes from the analyses carried out in Australia indicate that a flow variation is apparent, since these peak positions fall between those from Italy and the NZ/USA couples.

The completion of this two-part investigation has determined that the LMCS cryogenic modulation approach provides reliable GC × GC results. A comparison of data from four independent laboratories, five independent gas chromatographs, five independent LMCS units, and numerous operators has now been made. The experimental observations indicate that the retention time reproducibility should be appropriate for chromatogram matching, “image comparisons”, and for identification based on the two independent GC × GC retention times defining peak positions. An understanding of long-term retention time reproducibility however is unclear, and the use of relative retention times rather than absolute retention times may be necessary. In order to archive relative retention data, it may be useful to have a classification system based on (for example) retention indices. These may take the form of a linear temperature programmed retention index–Kovats’ index couple, as described by Beens and co-workers in their model for predicting GC × GC chromatograms [13]. Alternatively, the retention correlation maps proposed by Western [14] provide an interesting development to follow.

One can now propose that a “reference laboratory” equipped with a (capital intensive) GC × GC–TOFMS facility may in the future be used to characterise and locate target compounds within the 2D space, for a network of analytical laboratories, which in turn can then perform GC × GC analysis on a routine basis, using the two independent GC × GC retention times, and comparison with the reference laboratory data, as a basis for component identification. This is likely to require an additional method translation or correction procedure to ensure “exactly” coincident data, although a recent attempt by Shellie and co-workers [15] has indicated that the peak distribution in the 2D separation space for GC × GC–MS and GC × GC–FID experiments is consistent, so reliable method translation should be achievable. Note that as an alternative, a column set might be calibrated in one laboratory, and then used in another to permit more reliable correlation of data in subsequent cooperative studies.

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