ELSEVIER

Journal of Chromatography A, 962 (2002) 127–134

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Effects of temperature and flow regulated carbon dioxide cooling in longitudinally modulated cryogenic systems for comprehensive two-dimensional gas chromatography

Peter Haglund[a,*], Mikael Harju[a], Conny Danielsson[a], Philip Marriott[b]

[a]*Environmental Chemistry, Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden*

[b]*Department of Applied Chemistry, Royal Melbourne Institute of Technology, Melbourne 3001, Australia*

Received 28 November 2001; received in revised form 25 March 2002; accepted 12 April 2002

Abstract

Two different modes of temperature regulation in longitudinally modulated cryogenic systems (LMCSs) for comprehensive two-dimensional gas chromatography (GC×GC) were compared. Carbon dioxide was used as coolant. In the first mode of operation, the temperature of the trap was regulated to a pre-set temperature using a digital temperature controller (''the constant temperature mode''). In the second, the temperature was regulated to a fixed negative offset to the oven temperature by using a constant flow of CO_2 (''the constant flow mode''). A number of problems were occasionally observed using the constant temperature mode: (1) severe band broadening of high boiling analytes in the second dimension; (2) non-Gaussian reconstructed first-dimension peak profiles; (3) high background due to modulation of first-dimension column bleed. It was concluded that these problems were associated with inefficient solute remobilization at low LMCS trap temperatures (1 and 2) or large trap temperature fluctuations (3). These problems could be avoided or significantly reduced by using the constant flow mode. Best results were obtained as the trap temperature was kept about 70 °C below the oven temperature.

Keywords: Gas chromatography, comprehensive two-dimensional; Cryogenic systems; Liquid crystals; Peak shape; Polychlorinated biphenyls

1. Introduction

During the last decade, a technique called comprehensive two-dimensional gas chromatography (GC×GC) has evolved. In GC×GC two columns are connected in series using an interface known as the modulator. The modulator is the most critical component of a GC×GC system. The three most widely used modulator types are the ''sweeper'' modulator [1], the longitudinally modulated cryogenic modulator [2,3], and the cryo-jet modulator [4,5]. In a recent study a sweeper and a longitudinally modulated cryogenic system (LMCS) were compared and shown to yield equivalent results within the scope of the applications studied [6]. The present study specifically addresses the working principle of the LMCS.

In an LMCS the capillary column is fed through a narrow tube and by providing cryogenic cooling to the tube it is possible to trap compounds eluting from

*Corresponding author. Tel.: +46-90-7866667; fax: +46-90-128133.

E-mail address: peter.haglund@chem.umu.se (P. Haglund).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00433-8

the first column (Fig. 1). That is, accumulation of solutes occurs at the inlet of the cold trap. By simply moving the trap along the capillary (upstream) to expose the column section (and trapped material) to the oven temperature, the focused band is launched to the second-dimension capillary column. Only one column connector is needed to connect the first and second-dimension columns. When the speed of the second separation is high enough it is possible to perform multiple modulations over a first-dimension peak profile. This is a sequential process and thus all material that is injected onto the column reaches the detector. From the chromatogram obtained a true three-dimensional chromatogram, or a two-dimensional projection of the three-dimensional data (a contour plot), is produced by specially designed software.

An LMCS trap is typically operated at constant temperature. Cooling is obtained using liquid CO_2. Its flow is controlled using an on–off valve and a digital temperature controller (DTC), see Fig. 1A. The target temperature is set using the DTC. Trap cooling may then be started at a pre-set time during the GC×GC run using an external contact closure, which opens the CO_2 valve. The trap will cool until the pre-set temperature is passed. At this moment the DTC sends a signal that closes the on–off valve. The oven temperature will cause the trap temperature to rise and as it passes the set temperature, the CO_2 flow is again turned on. The process continues until the CO_2 flow is switched off using an external contact closure.

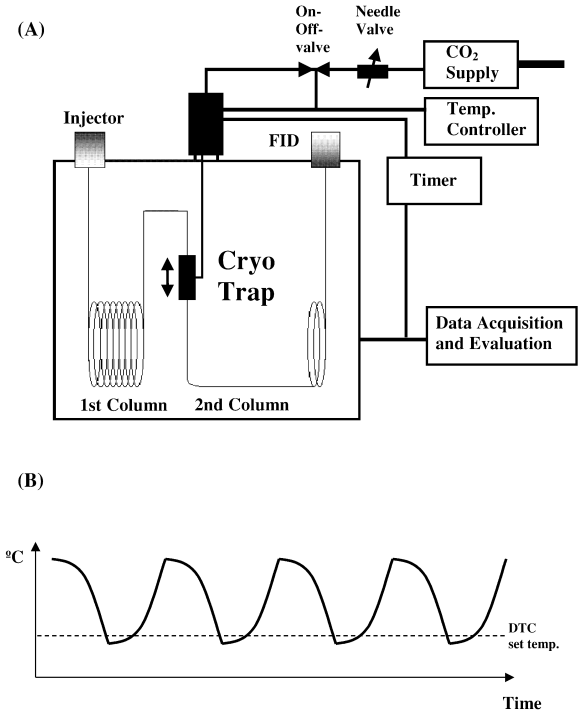

Fig. 1. (A) Schematics of the LMCS system in a single oven. (B) Illustration of a temperature profile of an LMCS trap operated in the constant temperature mode.

Consequently, the temperature of the trap will vary in a cyclic manner, shown schematically in Fig. 1B. The fluctuation is often rather large as it takes a considerable time for the CO_2 spray to form, for the trap to cool (or heat), for the thermocouple circuit to react, etc. As long as the maximum trap temperature is low enough this does normally not affect the operation of the system, and all target compounds will be efficiently modulated. A number of problems have however been observed that may be attributed to this mode of operation: (1) high boiling analytes sometimes exhibit severe band broadening in the second dimension; (2) unexpected, and unusual, non-Gaussian reconstructed first-dimension peak profiles are sometimes obtained; (3) the column bleed (and other system impurities) are efficiently modulated, which sometimes causes interfering signals—especially at elevated temperatures and low levels of target analytes. Note that this latter point will only be observed when the modulator can effectively trap or collect column bleed, and hence may not be seen in the thermal sweeper studies.

The aim of this study has been to seek logical explanations to the above-mentioned phenomena as a means of better understanding modulator operation, and for troubleshooting purposes. It has also been investigated if some of them may be avoided by using a constant flow of CO_2 for cooling, instead of a constant trap temperature and, finally, an attempt has been made to optimise the trapping conditions. The results obtained will also have some validity for other types of cryogenic modulators, e.g. cryo-jet modulators.

2. Experimental

2.1. Standards

Technical-grade Clophen A50 was provided by the Institute of Applied Environmental Research (ITM), Stockholm University, Sweden, and solutions of PCB180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl) and racemic PCB132 (2,2′,3,3′,4,6′-hexachlorobiphenyl) were obtained from Ultra Scientific (North Kingstown, RI, USA) and AccuStandard (New Haven, CT, USA), respectively. The standards were diluted to a final concentration of 10 ng/μl (in *n*-hexane or toluene), 55 pg/μl and 250 pg/μl (in isooctane), respectively.

2.2. GC and GC×GC analysis of Clophen A50

The GC was an Agilent HP6890 system retrofitted with an LMCS Everest longitudinally modulated cryogenic system (LMCS; Chromatography Concepts, Doncaster, Australia) and equipped with a flame ionisation detection (FID) system. A 10 m× 0.15 mm column, coated with 0.1 μm poly(50% liquid crystalline–50% dimethyl) siloxane (LC-50; J&K Environmental, Sydney, Canada), was used as the first-dimension column followed by a 0.5 m×0.1 mm second-dimension column coated with 0.1 μm 5% phenylmethylsilphenylene (BPX-5; SGE International, Australia). Cryotrapping was performed on the second-dimension column. Hydrogen was used as carrier gas at a constant flow of 1.0 ml/min. The CO_2 flow into the LCMS cryo-trap was either controlled using an on–off valve and a DTC circuit or adjusted using a needle valve to a temperature ≈100 °C below the oven temperature. Aliquots (0.2 μl) of *n*-hexane or toluene solutions of the polychlorinated biphenyls (PCBs) were injected in the splitless mode (at 250 °C) using an autosampler. The initial oven temperature was either 60 or 90 °C depending on which solvent was used. The oven temperature was then raised as given for individual chromatograms (see Results) to a final temperature of 275 °C. During the GC×GC experiments the CO_2 flow was turned on and the LMCS modulation was started 4 min after injection. Modulation periods between 1.8 and 9.9 s were tested to find the appropriate modulation frequency for each oven temperature program. With this unit, the trap time in the release position is fixed to 0.5 s, and so trapped components must be re-launched in about 0.5 s if they are to be fully expelled within this time frame to the second column. The FID was kept at 250 °C and was operated at either 5 or 100 Hz depending on whether the GC or GC×GC mode was utilised. The Agilent ChemStation software was used for system control and data acquisition. The resulting data were exported as a comma separated value file, converted to a text matrix format using the Comprehensive GC File Converter 2.0 software of Chromatography Concepts, and imported to Transform 3.3 (Fortner Research, USA) for data visualisation.

2.3. GC×GC analysis of PCB132 enantiomers

The GC×GC system consisted of two gas chromatographs connected using a laboratory-made interface (250 °C). The first-dimension GC column was housed in an Agilent HP5890, and the second-dimension GC column in an Agilent HP6890. The first-dimension column was passed through the interface and extended 0.4 m into the second GC oven. The two GC ovens were used with differential temperature ramping, with the second-dimension GC column maintained at higher temperature. The column set consisted of a 3 m×0.25 mm deactivated retention gap, a 10 m×0.10 mm, 0.1 μm Chirasil-Dex CB column (Chrompack, Middelburg, The Netherlands), and a 2 m×0.10 mm, 0.1 μm LC-50 column. Standard pressfits where used to connect the columns. The LMCS trap was positioned at the end of the first column (0.2 m from the interface). It was activated 48 min after injection and was then modulated for 50 min. The DTC was set to 60 °C and the modulating period was 4 s.

Splitless injections (1 μl) were performed at 250 °C. The first GC oven was temperature programmed as follows: 80 °C for 2 min, raise at 10 °C/min to 110 °C, raise at 0.5 °C/min to 160 °C, and finally raise at 10 °C/min to 250 °C, isothermal for 5 min. The second GC oven was ramped in a similar way, but with a +40 °C offset. The same final temperature (250 °C) was however used. Hydrogen was used as carrier gas at a head pressure of 60 p.s.i.,

and the PCBs were detected using an Agilent micro electron-capture detection (μECD) system (260 °C; 150 ml/min of N_2 make up gas). The data collection rate was 50 Hz.

2.4. Optimisation of the trap temperature

The trap temperature was optimized using PCB180 as test probe; the instrument was set up as described in the previous section, and experimental conditions according to Fig. 6. The trap was operated in the constant flow mode. In order to set the trap temperature the oven temperature was raised to the expected elution temperature for PCB180 (195 °C) and the CO_2 flow was adjusted to the desired temperature offset (ΔT) using the needle valve. Temperature offsets ranging from 20 to 220 °C were tested.

3. Results and discussion

3.1. Modulation of high boiling analytes

The PCBs of Clophen A50 exhibited severely distorted peak shape when the LMCS was operated in the constant temperature mode with the DTC set at −30 °C, see Fig. 2A. In fact, it was difficult to identify individual peaks. The contour plot was instead dominated by a number of diagonal bands, all with negative slopes. It was postulated that this was due to inefficient remobilization of analytes from the trapping region. As the temperature of the trap was increased by 60 °C to +30 °C individual peaks started to appear in the contour plot (Fig. 2B) although still broad.

These observations may be explained in the following way: when a relatively cold LMCS trap (in this case −30 °C) moves to its release position the column section in the trapping region may not heat fast enough to allow the trapped analyte or analytes to be remobilized before the trap returns to begin the next trap-and-release cycle, see Fig. 3A–C. During the following cycles some of the previously trapped material may escape the trapping region but some may be still located in the trapping zone, whilst additional material is sequestered at the beginning of the trapping region (Fig. 3D–G). In this way analytes may take longer to elute from the second column than expected, and may also exhibit multiple pulsing inconsistent with the regular modulated pulsing. Materials that elute late from the trap will elute at a higher oven temperature than materials that elute early and will therefore have a shorter second-dimension retention time. This would explain the parallel bands with negative slope in the chromatogram (Fig. 2B). By increasing the trap temperature to +30 °C the separation was greatly improved (Fig. 2C). The chromatography was further improved when the trap temperature was regulated to 100 °C

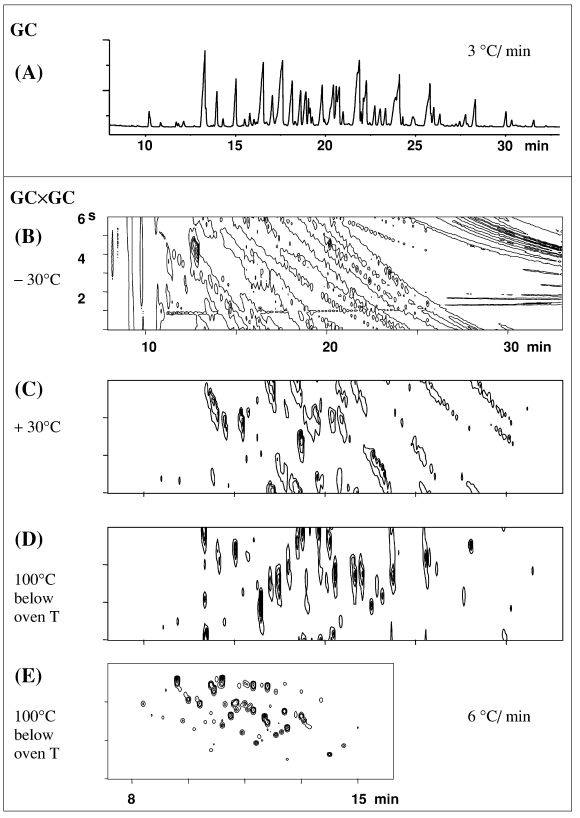

Fig. 2. (A) Unmodulated GC chromatogram of Clophen A50. Oven temperature program: 60 °C for 1 min, raise at 20 °C/min to 120 °C, raise at 3 °C/min to 230 °C, hold for 5 min. (B–E) illustrate the effect of various trap cooling conditions on the GC×GC chromatograms (6 s modulation period). (B) Constant temperature modulation at a set temperature of −30 °C; (C) constant temperature modulation at +30 °C; (D) modulation using a constant flow of CO_2 and a trap temperature of 100 °C below the oven temperature; (E) same as (D), but 6 °C/min, oven temperature program rate.

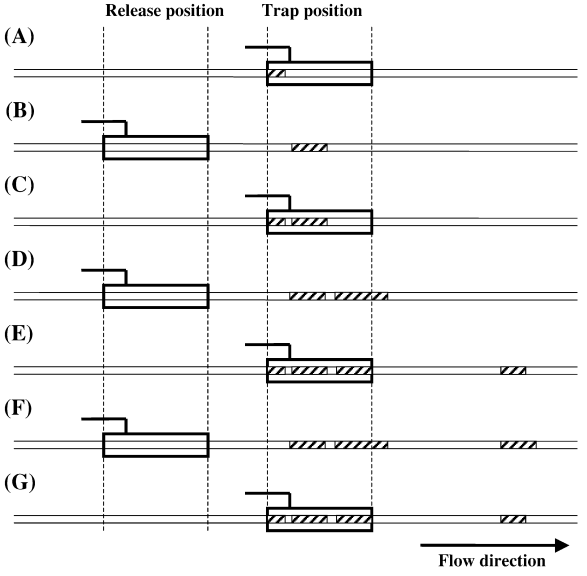

Fig. 3. Illustration to the conceptual model to explain the inefficient modulation of high boiling analytes by a low temperature LMCS trap: analytes are trapped in the beginning of the LMCS trap (A), the trap is moved to release position (B), but the analyte(s) do not escape the trapping region before the trap returns (C); during the following cycles additional material is sequestered in the trap whilst some of the material is launched to the second column (D–G).

below the oven temperature by quenching the CO_2 flow, see Fig. 2D. Now the diagonal bands were eliminated, but a significant ''wrap-around'' was observed. By increasing the oven ramp rate (to 6 °C/min) and thereby the elution temperature, the wrap-around was avoided and the peak-shape improved further (Fig. 2E).

The reason for this phenomenon not being previously described is most probably that the majority of applications reported so far for the LMCS technique are dealing with relatively volatile analytes. Furthermore, in the application described above, the analytes elute from the first column at unusually low temperatures due to the narrow bore thin-film column. The partial exclusion of non-planar PCBs from the rigid liquid crystal structure further reduces the retention, making this a non-standard application.

Whatever the reason for the low elution temperatures there are (at least) three principle ways to overcome the problems associated with incomplete remobilization: (1) to increase the trap temperature, (2) to increase the desorption period, and (3) to decrease the wall-thickness of the column. The first option has been tested with success, as described above. The other options have been difficult to explore since the desorption period was not variable (fixed to 0.5 s in the system available for this study) and thin-wall columns were not available. It is likely however that the remobilization could be improved by increasing the time the trap is held in the release position, but this measure is not feasible when operating the LMCS system at short modulation periods. During fast operation a significant portion of the total modulation period is spent moving the trap between the two positions and the time available for analyte remobilization is limited.

3.2. Unexpected first-dimension peak profiles

The above mentioned conceptual model may also explain the unusual, non-Gaussian peak profiles that occasionally have been observed, see Fig. 4. Initially, the two atropisomeric forms of PCB132 were successfully separated using a chiral (permethylated β-cyclodextrin) first-dimension column (Fig. 4B). The two enantiomers were well separated and each of the two peak envelopes were Gaussian in shape. Immediately following that analysis the CO_2 cylinder ran empty and had to be changed. Once the system was operational again the analysis was

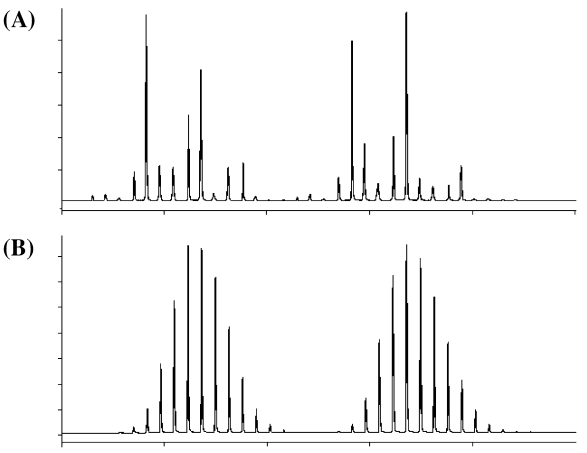

Fig. 4. GC chromatograms obtained during GC×GC analysis of PCB132, using (A) a full cylinder of CO_2, and (B) using an almost empty CO_2 cylinder.

repeated, but with a completely different result, see Fig. 4A. Now the shape of the peak envelopes was far from Gaussian.

A plausible explanation may be that the CO_2 flow increased (and the average trap temperature decreased) as a full cylinder was installed and that the desorption efficiency of the LMCS system varies over time due to short-term variations in trap temperature. The temperature variation of the trap (Fig. 1B) in this system typically ranges ±30 °C. Following this line of argument the analytes may be trapped so efficiently while the trap is cooling that all of the focused material is not desorbed from the trapping region once the trap is moved to its release position. If the trap temperature increases during the next cycle all of the material residing in the trapping region may now be desorbed. In both cases, the recorded peak areas will be biased as compared to the quantities that were eluting from the first column per cycle time. Therefore, the first of these LMCS cycles will result in a too low peak area whilst the second will result in a too high area.

3.3. Optimisation of the trap temperature

The results of the optimisation experiments are summarised in Table 1 and Figs. 5 and 6. It agrees very well with the line of argument presented above. The trap temperature clearly has a major influence on the second-dimension peak profiles. If the difference between the trap temperature and the oven temperature (ΔT) is small the analyte will not be efficiently trapped (ΔT=20 °C). As sufficient cooling is applied to the trap all of the analyte will be efficiently trapped and remobilized (ΔT=70 °C). However, if the trap temperature is too low the analyte will not be completely remobilized during the short time (0.5 s) the trap is held in the release position (Fig. 3). It will only move a short distance within the trap. The

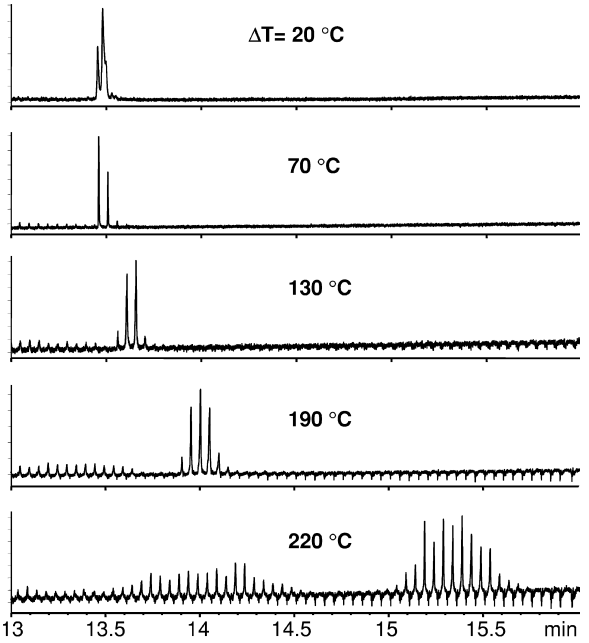

Fig. 5. Optimisation of the trap temperature using PCB180, which elutes at 195 °C (215 °C for the ΔT=220 °C experiment). Experimental conditions: LMCS modulation period, 3 s; split injection, 1 μl, split ratio 1:10; constant carrier gas (H_2) flow, 0.6 ml/min; oven temperature program 80 °C for 2 min, 10 °C/min to 240 °C; μ-ECD at 50 Hz.

Table 1
Overview of the results obtained from the trap temperature optimisation experiments

Trap temperature[a] (°C)	ΔT[b]	First-dimension retention time (min)	Reconstructed first-dimension (base) peak width (s)	Second-dimension (half-height) peak width (s)
155	40	13.46	9	0.27
125	70	13.46	9	0.20
95	100	13.51	9	0.27
65	130	13.56	12	0.37
35	160	13.61	15	0.34
5	190	13.91	18	0.33
−10	205	14.79	30	0.32
−25	220	15.05	42	0.28

[a] Refers to the trap temperature upon elution of the target, PCB180.
[b] Difference between elution and trap temperature. The elution temperature of the target was 195 °C.

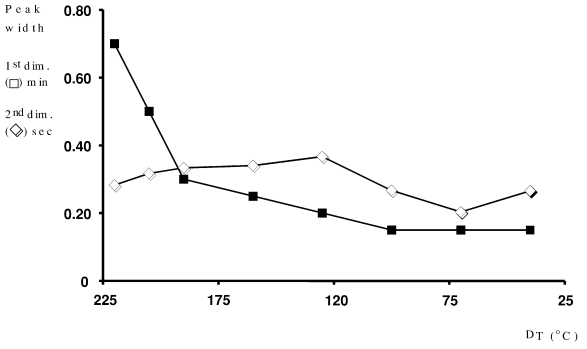

Fig. 6. Graphs of the trap temperature (as ΔT, the temperature difference between trap and oven) vs. the first and second-dimension peak widths denoted with filled squares and open diamonds, respectively. The target analyte elutes at 195 °C (and consequently, at ΔT=20 °C the trap is at 175 °C).

earlier trapped material will therefore be released from the trap during subsequent trap-and-release cycles. Consequently, the first-dimension retention time will increase, as well as the first-dimension peak width (ΔT=130–220 °C).

Optimum first-dimension and second-dimension peak widths were obtained at a ΔT of 70 °C (Table 1 and Fig. 6). The first-dimension peak widths increased with ΔT (within the range −25 to 155 °C) and became very broad at trap temperatures below 0 °C. However, the relationship between ΔT and second-dimension peak width seems to be more complicated. If ΔT is increased above its optimum (the trap gets cooler) the second-dimension peak width will first increase (ΔT=70 to 130 °C) and then gradually decrease (Fig. 6). A plausible explanation may be that the first-dimension retention times increase with ΔT and that the analytes therefore elute at higher temperatures (temperature programming was applied), which results in compression of the second-dimension peak profiles.

3.4. Modulation of first-dimension column bleed

The way the cryo-trap is operated does also influence the baseline noise level, see Figs. 5 and 7A. The observation of cryogenically modulated bleed has been previously noted [7]. The noise is higher in the constant temperature mode than in the constant flow mode, simply because of the higher

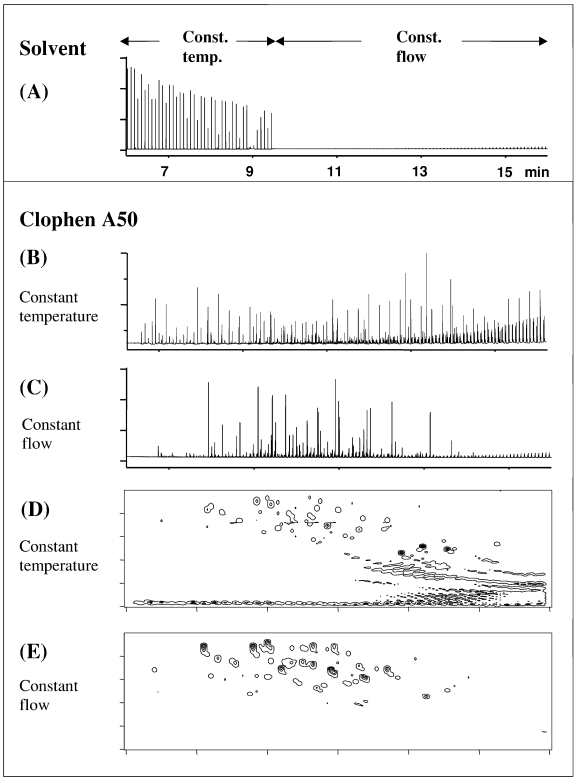

Fig. 7. Comparison of two trap cooling modes: (1) constant temperature mode, (2) constant flow mode. Oven temperature program: initial temperature 90 °C for 2 min, raise at 20 °C/min to 140 °C, raise at 10 °C to 250 °C, hold for 5 min. (A) Baseline during constant temperature, 6 to 9.5 min (DTC at +40 °C; thermocouple reading −20 °C to +60 °C) and constant flow (ΔT= 100 °C) operation, 9.5 to 16 min, respectively. (B and D) Pulsed GC chromatogram and GC×GC contour plot, respectively, of Clophen A50, constant temperature mode. (C and E) Corresponding pulsed GC chromatogram and GC×GC contour plot obtained using the constant flow mode.

trap temperature fluctuations of the former. Consequently, system impurities such as carrier gas impurities, septum and column bleed, etc., will be modulated. This has serious consequences for qualitative as well as quantitative analyses of trace level contaminants. It is much more difficult to interpret the chromatograms resulting from injections of small amounts of Clophen A50 when the constant temperature setting mode is used, cf. Fig. 7B and C, and Fig. 7D and E, respectively. It will also affect the limits of detection for target components that coelute, or partially coelute, with system peaks.

4. Conclusions

In using the current design of the LMCS system there seems to be few advantages in using the (traditional) constant temperature mode. Instead there seems to be a number of disadvantages: it produces more modulation of system impurities; it sometimes results in severe peak broadening of compounds that are highly retained in the second-dimension column; and it sometimes distorts the first-dimension peak profiles.

Naturally, there may be ways to avoid or partially overcome these disadvantages. If, for instance, the trap and temperature controller design was modified in such a way that the temperature stability increased, a better baseline would certainly be obtained. A new temperature controller has been shown to provide better thermal stability, although the above observations will allow interpretation of inappropriate temperature settings should they arise.

However, the problems caused by the increasing temperature difference, between LMCS trap and GC oven, in temperature programmed GC runs will remain as long as the trap temperature is kept isothermal. If, on the other hand, the DTC set temperature were ramped (increased) during the analysis these problems would be circumvented. However, then it would not be much different to the constant flow mode. In both cases, the trap temperature would increase during the analysis. On the other hand, if it for some reason were desirable to ramp the trap temperature at a different rate to the oven it would however be easier, we believe, to achieve this by programming the set temperature of the trap.

Acknowledgements

The authors would like to thank Dr. Naikwadi of J&K Environmental Ltd. for supplying us with the LC-50 liquid crystal column and MISTRA (Foundation for Strategic Environmental Research) for financial support as part of the national research program COLDREM (‘‘Soil remediation in a cold climate’’).

References

[1] J.B. Phillips, R.B. Gaines, J. Blomberg, F.W.M. Van Der Wielen, J.-M. Dimandja, V. Green, J. Granger, D. Patterson, L. Racovalis, H.-J. De Geus, J. De Boer, P. Haglund, J. Lipsky, V. Sinha, E.B. Ledford Jr., J. High Resolut. Chromatogr. 22 (1999) 3.
[2] P.J. Marriott, R.M. Kinghorn, Anal. Chem. 69 (1997) 2582.
[3] R.M. Kinghorn, P. Marriott, P.A. Dawes, J. High Resolut. Chromatogr. 23 (2000) 245.
[4] E.B. Ledford Jr., C.J. Billesbach, J. High Resolut. Chromatogr. 23 (2000) 202.
[5] J. Beens, M. Adahchour, R.J.J. Vreuls, K. van Altena, U.A.Th. Brinkman, J. Chromatogr. A 919 (2001) 127.
[6] P. Marriott, P. Haglund, M. Harju, R. Kinghorn, R. Ong, P. Morrison, J. High Resolut. Chromatogr. 23 (2000) 253.
[7] R.M. Kinghorn, P.J. Marriott, P.A. Dawes, J. Microcol. Sep. 10 (1998) 611.