Optimization of time-flow parameters for thermal modulation in comprehensive two-dimensional gas chromatography

Chiara Cordero[1], Carlo Bicchi[1], Erica Liberto[1], Patrizia Rubiolo[1], Gianluca Stani[2], Armando Miliazza[2]

[1] Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, 10125 Torino, Italy
[2] SRA Instruments Italia S.r.l. Viale Assunta 101, 20063 Cernusco S/N (Milan), Italy

AIM AND SCOPE

The role of the modulator in GCxGC is to trap and accumulate analytes eluting from the first dimension (^{1}D), refocus and then rapidly release them as a narrow band in the second dimension (^{2}D) [1]. Gas jet thermal modulators consist of a capillary where bands eluting from the ^{1}D are trapped in a short portion of it by a cold jet, and then rapidly re-injected into the ^{2}D by a pulsed hot jet. The effectiveness of a GCxGC separation is conditioned by modulator performance, in consequence band re-injection should take few milliseconds to obtain a chromatographic system with the highest peak capacity [2].

The liquid nitrogen-cooled loop modulator in combination with a suitably programmed cold flow control was shown to be effective with analytes in a wide range of volatilities (C_4-C_{47}) in a single run [3]. This study concerns the simultaneous control of cold-jet flow and hot-jet pulse duration by a programmable device with a dual stage thermal loop modulator. The optimized combination of these two parameters improve the modulation efficiency preventing break-through of the highly volatiles at the same time avoiding anomalous or irreversible cold-trapping for medium-to-low volatiles in a single chromatographic run.

[1] M. Adahchour, J. Beens, R.J.J. Vreuls, U.A.Th. Brinkman, TRAC, Vol. 25, No. 6, 2006
[2] L.M. Blumberg, J. Chromatogr. A 985 (2003) 29
[3] R. B. Gaines, G. S. Frysinger. J. Sep. Sci. 2004, 27, 380–388

EXPERIMENTAL

Samples
Pure standard samples n-alkanes (C_5 to C_{28}) were supplied by Analytical-Controls BV, Rotterdam, NL.
Solvents (cyclohexane, n-hexane, acetone) were all HPLC-grade from Riedel-de Haen (Seelze, Germany).
Roasted Coffee samples (*Coffea canephora* var. *robusta*) were supplied by Lavazza SpA, Turin, Italy.

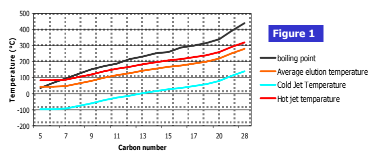
Instrumental Set-up
Comprehensive GCxGC/qMS analyses were carried out on a **Agilent 6890N GC** coupled with a **5975 MS detector** (Agilent, Little Falls, DE, USA) operating in E.I. mode at 70 eV. Ion source temperature: 230 °C, Quadrupole temperature 150°C, Transfer line: 280 °C. An automatic tuning was used. Scan range was from 35 m/z to 300 m/z with a scan rate of 10000 amu/s.
The modulator was a **Dual Stage Thermal Modulator Zoex KT-2005 GCXGC,** Hot Jet temp.: 145°C (5min) to 320°C, rate 2.5°C, Modulation Period: 4 sec and 8 sec, Liquid Nitrogen cooling system.
The **Mass Flow Controller** was a Bronkhorst Hi-Tech Mass Flow Controller 0-50 *SLPM* Nitrogen connected with a Programmable Logic Controller Horner XLE (Horner APG, Cork, Ireland)

GCxGC Column Set-up:
^{1}D column: HP-1, 30 m x 0.32 mm ID, 0.25 µm df; ^{2}D columns: BPX-50, 2.5 m x 0.1 mm ID, 0.1 µm df. Loop modulator: 1 m x 0.1 mm ID deactivated fused silica. Oven Programme for the n-alkanes analysis: 35°C (5min) to 320°C, rate 5°C/min, Secondary oven Programme: 60°C (5min) to 340°C, rate 5°C
Oven Programme for HS-SPME analysis of Coffee: 50°C (1min) to 240°C, rate 4°C/min.

RESULTS

Temperature requirements

The temperatures to trap analytes between C_5 and C_{28} effectively, with a deactivated capillary in a dual-stage loop thermal modulator should be from 120°C to 140°C colder than analytes elution temperatures [1]. Liquid nitrogen-cooled loop modulator has been successful in trapping highly volatile components, such as C_4 (boiling point -0.5°C), although the cold jet flow conditions suitable for such volatiles result in broadened peaks along the 1D time axis or in irreversible trapping for higher boiling analytes.
The optimization of the thermal modulation temperatures implies that cold jet flow is adapted to the analyte elution temperature over the chromatographic run [1,2] by optimizing the nitrogen flow.
Figure 1 reports the boiling points profile of n-alkanes test mixture, the optimal cold and hot jet temperatures estimated on the basis of the average elution temperatures of each of them under the chromatographic conditions adopted.



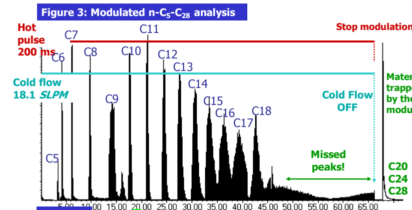
On the other hand, hot jet temperature and duty cycle (i.e. pulse time) must be adapted to the variation of cold flow conditions to divert the cold nitrogen stream at the modulation point quickly and produce sharper and symmetrical 2D peaks at every time cycle during the chromatographic run. Heating of hot jet block and increasing of duty cycle time are two further interesting features.

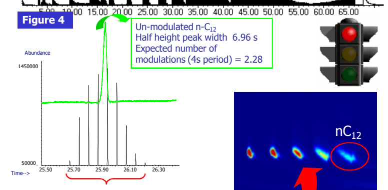
[1] R. B. Gaines, G. S. Frysinger. J. Sep. Sci. 2004, 27, 380–388
[2] W. Rathbun, J. Chrom Sci 2007, 45, 636-642

Cold-jet flow and hot-jet pulse time optimization

The cold-jet nitrogen flow has been programmed by means of a Digital Mass-Flow Controller calibrated over a range from 0 to 54.1 *SLPM* (Standard Liter Per Minute) to comply temperature requirements for an efficient focusing of the highly volatile analytes, meanwhile avoiding irreversible trapping of the low-volatiles. The linear decrease of the nitrogen flow let the temperature at the modulation point to increase and enables to cover a wider elution temperature range.
Figure 3 reports the GCxGC profile of the C_5-C_{28} n-alkanes test mixture analyzed with cold-jet flow conditions optimized for an efficient trapping of the C_5 (i.e. 18.1 *SLPM*) and with an hot jet duty cycle of 200 ms. Analytes with a carbon number > 9 results broadened along the 1D time axis or irreversibly trapped by the modulator, as shown in **Figure 4** for the n-C12 .

Figure 3: Modulated n-C_5-C_{28} analysis

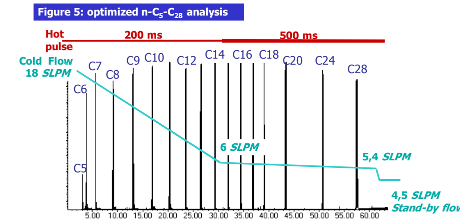


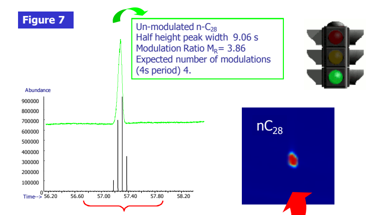


Experimental number of modulations= 9 (above S/N 10) : n-C_{12} resulted broadened because of an unappropriate modulation temperature.

Figure 5 reports the C_5-C_{28} n-alkanes profile analyzed with cold-jet flow conditions optimized for an efficient trapping over the chromatographic range and with a variable hot jet duty cycle. Analytes are now properly focused without breakthrough, or irreversible trapping, and efficiently released because of both optimized hot jet temperature and pulse time.

Figure 5: optimized n-C_5-C_{28} analysis

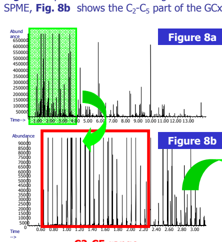




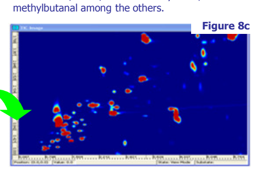
Experimental number of modulations= 4 (above S/N 10)

Headspace analysis of the highly volatile fraction of coffee

The effectiveness of the programmable device to properly focus/trap the highly volatiles components, eluting before n-pentane, is here shown by the separation of the volatile fraction of a sample of *Robusta* roasted coffee. Analytes between C_2-C_5 contribute to "odor fingerprint" of roasted coffee and some of them with a low odor threshold are key-aroma markers.
The C_2-C_{15} volatile fingerprint can be characterized by GCxGC, thanks to its high separation power and sensitivity; the optimization of the modulation temperatures, even in a relatively narrow b.p. range, is here challenging also because it produces perfectly focused 2D peaks suitable for accurate quantitation and mass spectral identification. **Fig. 8a** report the raw chromatogram of the volatile fraction of the investigated roasted coffee sampled by HS-SPME, **Fig. 8b** shows the C_2-C_5 part of the GCxGC profile and **Fig. 8c** its 2D plot.



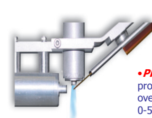
Identified components: Acetaldehyde, Acetic acid, Methyl acetate, 1-hydroxypropanone, 2,3-butanedione, furan, 2 and 3-methylfuran, 2 and 3-methylbutanal among the others.



Programmable device features

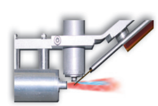
To obtain an optimal modulation ratio of 3-4 [1], the cold jet flow [5] and the duty cycle of the hot jet pulse must change during the GC run for those application that requires the simultaneous determination of either very volatile and low volatility compounds. The optimized combination of these two parameters has been shown to improve modulation effectiveness resulting in preventing break-through of the high volatility compounds and avoiding trapping of semi-volatile compounds causing band broadening along the 1D axis and peak tailing. An independent programmable device is here used as an accessory for the thermal modulator to control the cold flow during and after the GC run with a mass flow controller. An additional feature controls and programs the hot pulse valve activity.

[1] W. Khummueng, J. Harynuk and P. J. Marriott. "Modulation ratio in Comprehensive Two-dimensional Gas Chromatography". Anal. Chem. 2006, 78, 4578-4587



Cold Jet: immobilizes and traps the compounds by rapid cooling

- ***PROGRAMMABLE N_2 COLD FLOW***: allows proper trapping of a wide range of boiling points over the chromatographic run. Nitrogen flow range: 0-55 *SLPM.*
- ***STAND-BY-FLOW:*** a minimum N_2 flow is maintained between each run or after the modulation time within an analysis. It reduces the N_2 gas flow from operation rate (flow 30-15 *SLPM*) to about 3 *SLPM,* without transfer-line iceing. Reduced consumption of gas & liquid N_2.
- ***N2 COLD FLOW:*** reliable and precise, controlled by a digital MFC



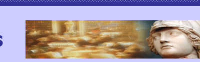
Hot Jet: flash heating to diverge cold flow and launch trapped compounds

- ***VALVE PULSE & POWER :*** independent from the GC
- ***HOT PULSE TIME PROGRAMMABLE:*** for proper re-mobilization of heavy compounds
- ***PROGRAMMABLE PULSE TIME*** **:** programmable within a run
- ***VARIABLE MODULATION PERIOD*** **:** programmable within a run

CONCLUSIONS

The optimization of the analyte trapping and remobilization with a dual jet loop modulator has here been achieved with an automated programmable device, controlling cold-jet flow and hot-jet pulse time contemporarily during the chromatographic run.
The temperatures required for a proper modulation of n-alkanes (b.p. range 36-440°C) eluting between 35°C to 300°C are in the interval between -95°C and 141°C, the optimal conditions were achieved by decreasing the N_2 cold flow from 18 to 5.4 *SLPM* following a linear profile. The hot jet pulse was simultaneously tuned and its duration increased to improve the efficiency of the analyte remobilization since it depends on the heat exchange during the hot-pulse duty cycle.
The combination of hot and cold jet conditions has resulted in a successful and reliable trapping of highly volatile components, in the range between C2-C5, in a real world sample (*Coffea canephora* var. *robusta*) and in a proper modulation of a C5-C28 n-alkanes mixture, without breakthrough and band broadening or irreversible trapping.
A further interesting feature of this device is to make possible the setting-up of a "stand-by flow" to save gaseous and liquid nitrogen between runs.

Acknowledgments



This research was carried out within the project entitled: "Sviluppo di metodologie innovative per l'analisi di prodotti agroalimentari" (FIRB Cod: RBIP06SXMR_002) of the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) (Italy).