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# A flexible loop-type flow modulator for comprehensive two-dimensional gas chromatography

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ABSTRACT

The present investigation is focused on a simple flow modulator (FM), for comprehensive two-dimensional gas chromatography (GC × GC). The interface is stable at high temperatures, and consists of a metallic disc (located inside the GC oven) with seven ports, which are connected to an auxiliary pressure source *via* two branches, to the first and second dimension, to a waste branch (linked to a needle valve) and to an exchangeable modulation loop (2 ports). The ports are connected *via* micro-channels, etched on one of the inner surfaces of the disc. Modulation is achieved using a two-way electrovalve, connected on one side to the additional pressure source, and to the two metal branches, on the other. An FM enantio-GC × polar-GC method (using a flame ionization detector) was optimized (a 40-μL loop was employed), for the analysis of essential oils. As an example, an application on spearmint oil is shown; the method herein proposed was subjected to validation. Finally, an FM GC × GC diesel experiment was carried out, using an apolar–polar column combination, to demonstrate the effectiveness of the modulator in the analysis of a totally different sample-type.



## 1. Introduction

In comprehensive 2D GC (GC × GC), a transfer device, normally a cryogenic modulator (CM), has the function of accumulating, reconcentrating, and launching primary-column bands, onto a second column [1,2]. Among many developed modulators, the first CM, the longitudinally modulated cryogenic system (LMCS), was introduced in 1998 [3]. Although cryogenic modulation has been used successfully throughout the last decade, all CMs suffer a disadvantage, namely the operational costs.

The first flow modulator was reported the same year as the LMCS [4]: a six-port diaphragm valve, located in the GC oven, was employed. A 0.5 s modulation period was used, with a 50-ms duration of the injection mode, causing a loss of 90% of the primary-column flow. A disadvantage of the FM method was the restricted operational temperature of the valve.

Although several flow modulators followed the first apparition, the FM mode has always been considered as a poor alternative to cryogenic modulation. The FM mode, most similar to twin-stage cryogenic modulation, should be characterized by an accumulation and a high-pressure injection step. Further desirable characteristics are: a duty cycle equal to unity, no thermal restrictions, "normal" modulation periods (3–6 s range) and an independent flow in each dimension. A number of FMs, with such features, have been reported: in 2004, Bueno and Seeley developed a differential flow modulator equipped with two accumulation loops [5]. The interface was constructed using four T-unions, segments of uncoated fused-silica capillaries, and a two-way solenoid valve. While the primary-column effluent filled one of the loops, an auxiliary flow flushed the contents of the other. The main disadvantage was the elaborate FM construction.

Another differential FM device was introduced in 2006 by Seeley et al. [6], and was a simpler version of that previously described [5]. The interface was constructed using three fused-silica columns, two microvolume T-unions and a 2-way solenoid valve (located outside the GC oven), connected to an auxiliary pressure source. The output ports of the solenoid valve were connected to the unions using two fused-silica segments. One of the T-unions was linked to the primary column, while the other was connected to

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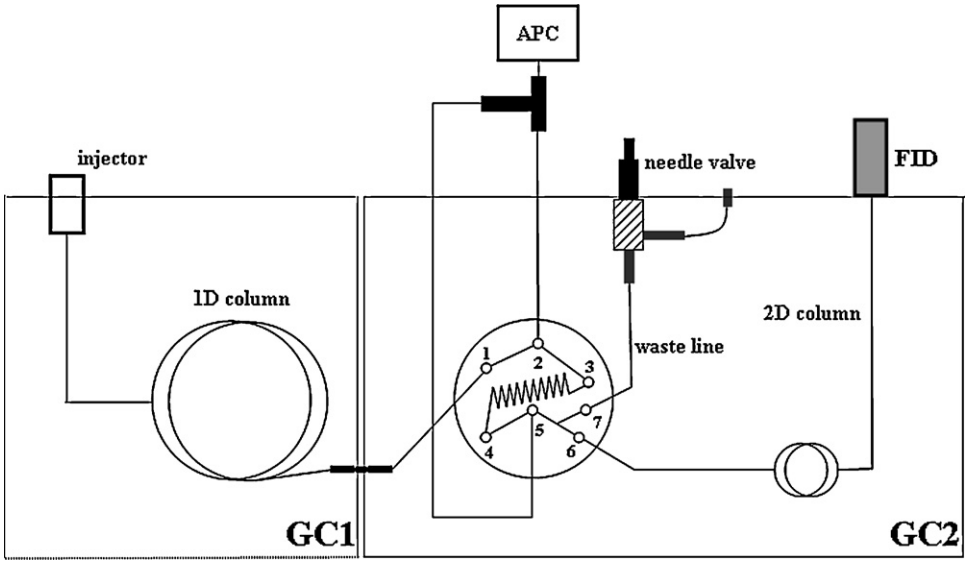


**Fig. 1.** Scheme of the FM GC × GC set-up.

the second dimension. A fused-silica segment, bridged between the two unions, acted as sample loop. During accumulation, the auxiliary flow was directed to the secondary-column head, and the primary-column effluent flowed within the loop; when the solenoid valve was switched for a brief period, the auxiliary flow flushed the loop content onto the second dimension. A series of FM works, based on Seeley et al.'s design [6], have been reported [7–9]. Agilent Technologies introduced a flow modulator [10] based on Seeley's work [6]: a planar metal structure contained the collection chamber, the connection points and valve transfer lines. The aim of the present investigation is the development of flexible loop-type FM with the characteristics and advantages of the two previously described devices [6,10], plus the possibility to optimize flows, using a waste branch bridging the interface (seventh-port) and a needle valve [11]. Evaluation of the interface was achieved through the optimization of an FM enantio-GC × polar-GC-flame ionization detection (FID) method, for essential oil analysis. A further method, using an apolar–polar combination, was exploited for diesel analysis.

## 2. Experimental

### 2.1. Samples and sample preparation

A sample of spearmint essential oil was diluted 1:10 (v/v) in *n*-hexane. All standard compounds used for modulator evaluation and calibration purposes (see Section 3), were supplied by Sigma–Aldrich (Milan, Italy). A diesel sample was attained from a local petrochemical station.

### 2.2. GC × GC analyses

All GC × GC applications were carried out using two Shimadzu GC2010 gas chromatographs (Kyoto, Japan), and an FID (280 °C). Data were acquired using the GCsolution software (Shimadzu), while bidimensional visualization was achieved using the ChromSquare software (Chromaleont S.r.l., Messina). Spearmint application: the primary column, a Dettsbeta (diethyl-*tert*-butylsilyl-β-CD) 20 m × 0.10 mm ID × 0.10 μm $d_f$ (MEGA, Legnano, Italy), was connected to position 1 of the interface (SGE, Ringwood, Victoria, Australia) (Fig. 1), after passing through a heated transfer line. A Supelcowax-10 2.5 m × 0.25 mm ID × 0.25 μm capillary (polyethylene glycol) was connected to port 6, while a 0.13 m × 0.25 mm ID uncoated capillary [both columns (Supelco, Bellefonte, PA, USA) were located in GC2], was linked to port 7. The uncoated column was linked to a manual needle valve, while the 2.5 m column was connected to the FID. The FID was operated as follows in all applications: $H_2$ flow: 50.0 mL/min, air flow: 400.0 mL/min. Sampling frequency: 125 Hz.

GC1 temperature program: 50–200 °C at 4 °C/min. GC2 temperature program: +10 °C offset. $H_2$ head pressure (constant linear velocity): 318.0 kPa. Auxiliary (APC: advanced pressure control) $H_2$ pressure (constant linear velocity): 24.9 kPa. Injection volume: 0.3 μL; split ratio: 1:10. Modulation: 3.2 s accumulation/0.2 s injection.

Diesel application: the primary column was an SLB-5 ms [silphenylene polymer] 20 m × 0.18 mm ID × 0.10 μm $d_f$ (Supelco); the secondary column was a Supelcowax-10 2.5 m × 0.10 mm ID × 0.10 μm. The waste line was a 0.10 m × 0.10 mm ID uncoated capillary. GC1 temperature program: 50–250 °C at 3 °C/min. GC2 temperature program: +10 °C offset. $H_2$ head pressure (constant linear velocity): 250.4 kPa. Auxiliary $H_2$ pressure (constant linear velocity): 229.4 kPa. Injection volume: 1.0 μL; split ratio: 1:50. Modulation: 4.8 s accumulation/0.2 s injection.

## 3. Results and discussion

### 3.1. Description of the flow modulator

A schematic of the FM is illustrated in Fig. 2: a two-way solenoid valve is located outside the GC oven and is connected to an APC unit, which generates a pressure defined as $P_2$. The valve is connected to two metallic branches, which are linked to the interface in positions 2 and 5. The disc is characterized by a diameter of 2.5 cm, a 7 mm thickness, and by internal rectangular channels (250 μm width/75 μm depth), which link ports 1-2-3 and 4-5-6/7. The first and second dimension columns are linked to positions 1 and 6, respectively. A 40 μL loop (20 cm × 0.71 mm OD × 0.51 mm ID stainless steel tubing) is located between positions 3 and 4; the loop size is chosen considering the modulation period, first-column flow and second-column dimensions.

During the accumulation stage, which is typically between 1.5 and 6 s (depending on the primary flow), the pressure at position 5

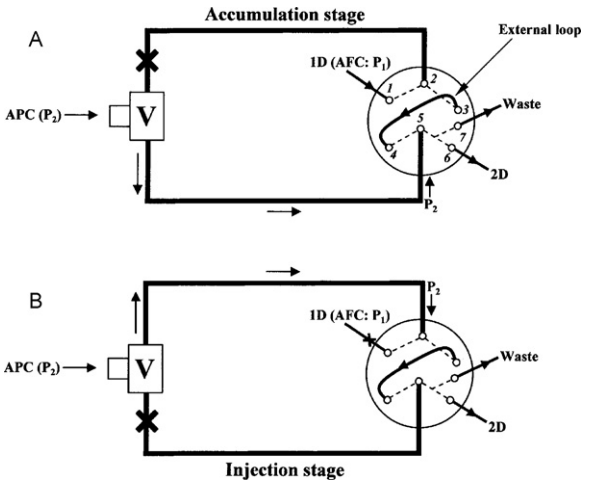


**Fig. 2.** Scheme of the FM accumulation (A) and injection modes (B). Abbreviations: V: 2-way solenoid valve; AFC: advanced flow controller.

equals $P_2$. Usually, pressure $P_2$ generates a high flow in the second dimension. The pressure at position 1, the first-dimension outlet, equals $P_1$ (inlet pressure)–$\Delta P$ (first-dimension pressure drop), a value slightly higher than $P_2$. It is obvious that the pressure drop $P_1 - \Delta P \rightarrow P_2$ generates a downstream flow in the loop, enabling the accumulation of a volume of first-dimension effluent.

During the injection stage (typically 0.1–0.3 s), the pressure at position 2 equals $P_2$. The pressure at position 1 is approximately equal to that of the accumulation stage ($P_1 - \Delta P$). Pressure $P_2$ enables rapid injection of the loop content onto the second dimension, briefly stopping the first-dimension flow. During switching from the accumulation to the injection stage, the former pressure $P_2$ at position 5 decreases rapidly because elution continues in the second dimension and waste line, and a pressure pulse at the head of the loop is generated. The analytes are separated on the secondary column throughout the following accumulation stage. It is noteworthy that, during loop flushing, the effluent is divided between the channels linked to ports 6 and 7. Flow division is dependent on regulation of the needle valve, remaining unaltered during the analysis because the valve restriction parts are contained within the GC oven (Fig. 1). Modulation parameters are controlled *via* a modulator key panel assembly; a PLC assembly is used for valve switching and to synchronize modulation with data acquisition, *via* "remote start".

### 3.2. Performance of the flow modulator

The optimized FM method was characterized by a 318.0 kPa inlet pressure; the first-dimension outlet pressure can be considered approximately equal to $P_2$, namely 24.9 kPa. Such a pressure drop generates a linear velocity and flow (50 °C) of *circa* 40.5 cm/s (constant velocity conditions) and 8.7 μL/s (0.52 mL/min), respectively. A micro-bore first dimension (0.1 mm ID) was employed because low flows generate optimum separation conditions and enable longer accumulation periods; moreover, efficiency increases with an ID reduction. However, the limited sample capacity of micro-bore capillaries does represent a disadvantage. A 40 μL loop was used, and various accumulation periods were tested: though a 3.2 s period was chosen, no breakthrough occurred up until 4 s. With regards to the second-dimension configuration, a 0.25 mm ID analytical column was chosen because high flows were generated. The reasons for using a waste line (the needle valve was maintained open for the essential oil experiments) were: (a) the

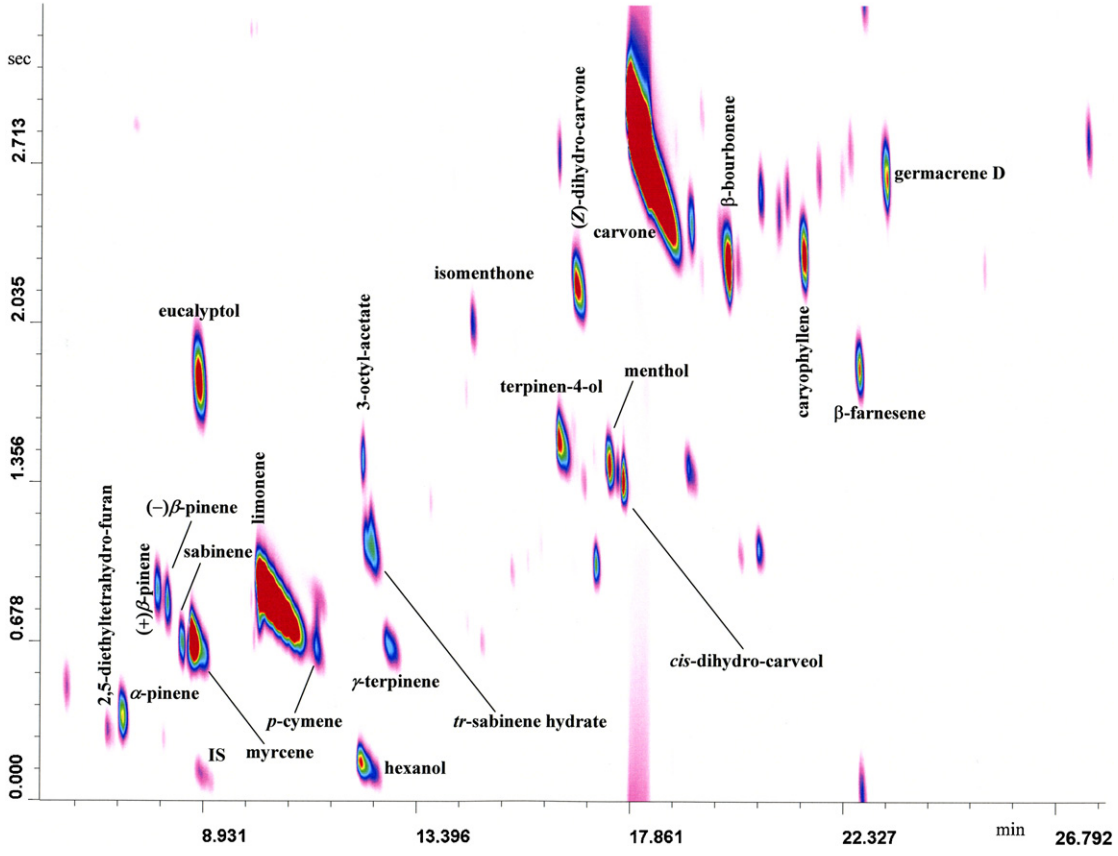


**Fig. 3.** FM GC × GC-FID spearmint oil analysis.

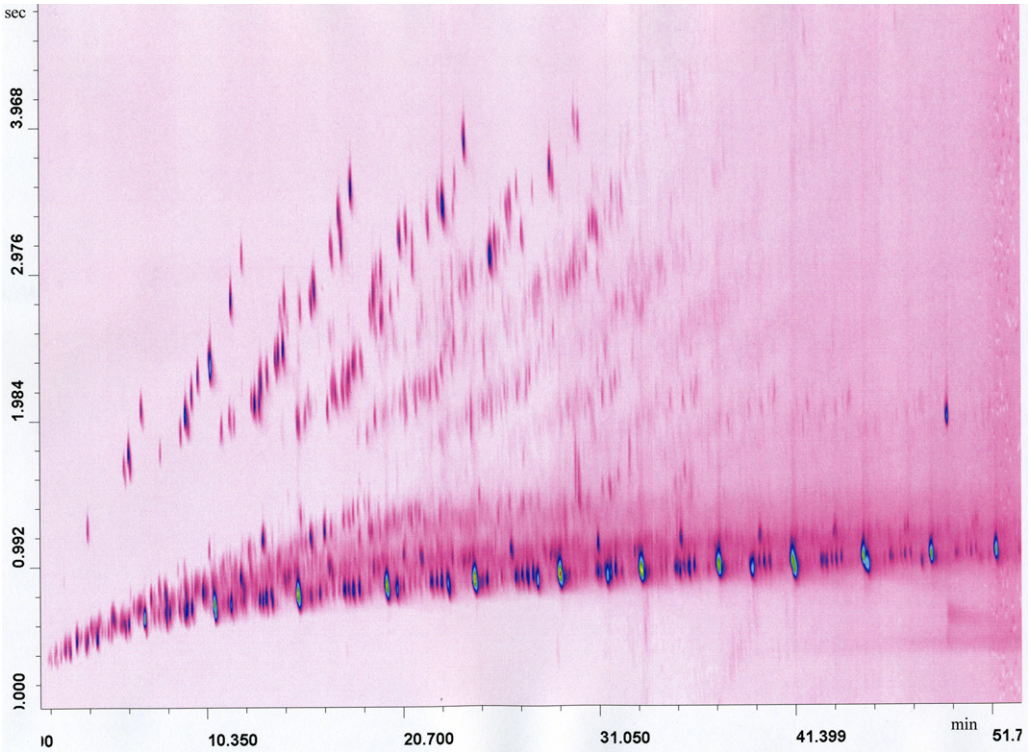


**Fig. 4.** FM GC × GC-FID diesel analysis.

loop pressure release was much more rapid during flushing; (b) to emulate a high split-ratio injection; and (c) to generate acceptable second-dimension linear velocities: a value of ∼180 cm/s (∼5.5 mL/min) was calculated [11], with about 95% of the effluent directed to waste. The inevitable sensitivity reduction (at the moment the main disadvantage) was counterbalanced by injecting higher sample amounts. However, care must be taken to avoid primary-column overloading. The loop flow was estimated to be 111 mL/min, while a 0.2 s injection period was sufficient for efficient flushing. The optimized FM method was applied to spearmint oil analysis (Fig. 3). Peak identification was carried out using pure standard compounds and by considering a GC–MS application on the spearmint sample, using the chiral capillary; altogether, 23 compounds were identified. Second-dimension peak widths ($4\sigma$), for the highest modulated peak, were measured for a series of chemically different compounds (mono- and sesquiterpenes): values in the 250–300 ms range were found for (+)β-pinene, isomenthone, menthol, caryophyllene, and farnesene. The peak width values are comparable to CM GC × GC, especially considering that a 2.5 m × 0.25 mm ID analytical column was used. Obviously, a comparative study between CM and FM systems would be necessary for conclusive data. A brief interruption of the second-dimension flow during valve switching, caused the presence of base-line dips (on average −600 μV). Cryogenic modulation can enhance sensitivity by a 10–50 factor, compared to monodimensional GC. In the present investigation, the FM effect on sensitivity was evaluated using the standard solutions and considering the highest modulated peak. Compared to unmodulated experiments, the sensitivity increases were similar for eucalyptol, β-pinene, isomenthone, terpinen-4-ol, and carvone, namely in the 9–11 factor range. However, such an evaluation is only indicative because in modulated and unmodulated experiments, the same amount of effluent was directed to waste. Peak intensities would have been much higher in a stand-alone single-column experiment. The optimized GC × GC method was subjected to validation, using the five spearmint constituents and a $C_9$ alkane internal standard (100 ppm). Six-point calibration curves were constructed at the 500, 300, 100, 50, 20, and 10 ppm level, with the lowest $R^2$ value equal to 0.998 (carvone). Method limits of detection (LOD) and quantification (LOQ) were calculated by multiplying the standard deviation of the response (at 20 ppm) by 3.3 and 10, respectively, and dividing the result by the calibration curve slope [12]. LOD and LOQ values were in the 0.9–2.1 mg/L and 3.1–7.0 mg/L range, respectively. Intra-day first and second-dimension retention time repeatability ($n = 3$), expressed as CV% values for the five constituents, were in the 0.0–0.4% and 0.4–1.6% range, respectively. Absolute quantification was carried out by subjecting the spearmint oil to 5 consecutive analyses; CV% values relative to the mean concentrations were satisfactory, with no result over 7.3%.

### 3.3. A different application

At the moment, FM research is being carried out using other columns sets, such as the apolar–polar combination. Attempts are also being made to: (a) limit sensitivity losses using a primary wider-bore column and (b) enhance second-dimension separation power and sensitivity using a micro-bore column. It is noteworthy that if both points are applied, then the column combination would near that employed in most GC × GC experiments (0.25 mm + 0.10 mm ID).

A preliminary FM application has been carried out on diesel, using an apolar primary 20.0 m × 0.18 mm ID × 0.10 μm $d_f$ column, and a secondary polar 2.5 m × 0.10 mm ID × 0.10 μm capillary (Fig. 4). As can be observed, the diesel analytes are nicely spread out over the 2D plane, in an ordered manner. The primary-column pressure drop (250.4 → 229.4 kPa) generated a first-dimension velocity of *circa* 10 cm/s, corresponding to an initial flow of about 0.5 mL/min (8.3 μL/s). A 40 μL loop was employed, with a 4.8 s accumulation period.

With regards to the second dimension, the 229.4 kPa initial pressure generated a linear velocity of *circa* 250 cm/s, corresponding to a flow of about 2.5 mL/min. About 96% of the effluent exit-

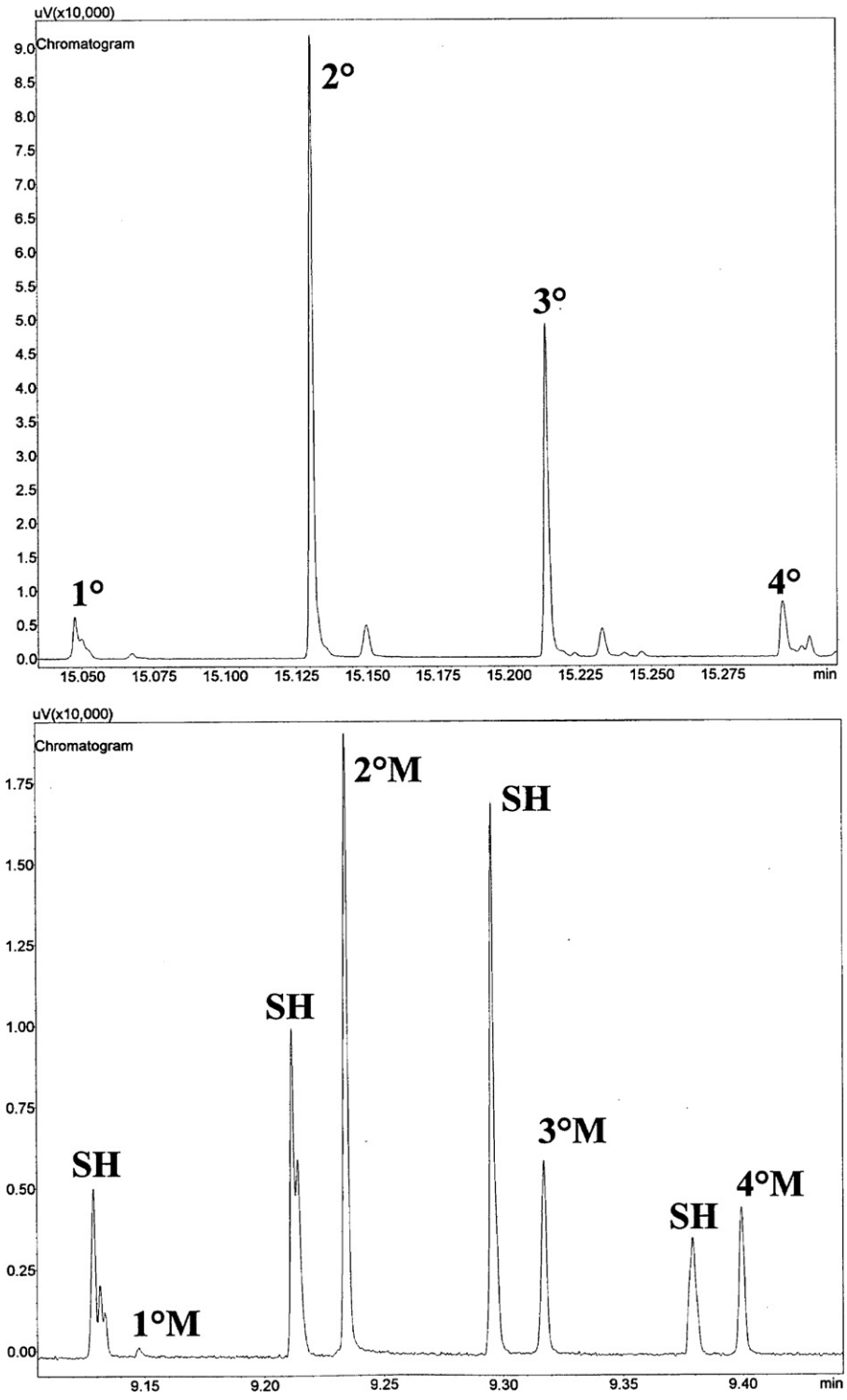


**Fig. 5.** Untransformed chromatogram expansions, relative to the diesel application; 1°, 2°, 3° and 4° refer to the modulation number. *Abbreviations*: SH, saturated hydrocarbon; MH, monoaromatic hydrocarbon.

ing the modulator was directed to waste (again, the needle valve was maintained open). Peak shapes were good, as can be seen for a linear alkane in the upper raw-chromatogram expansion in Fig. 5; the peak width ($4\sigma$) for the third modulated peak equalled 180 ms. The second-dimension resolution was satisfactory, as can be seen in the lower expansion, which illustrates the separation of a monoaromatic from saturated hydrocarbons. Obviously, peak widths increased with analyte polarity, with a value of 250 ms measured for the third modulated monoaromatic peak. As is evident, the base-line dip completely disappeared, probably due to the lower second-dimension flow. Research is underway to confirm such a hypothesis.

## 4. Conclusions

Until now, the FM device has provided satisfactory results. Considering the validated application, the second-dimension bands (250–300 ms, on average), generated under satisfactory gas veloc-

ity conditions, and after a lengthy column journey (2.5 m), are encouraging. Obviously, further research is necessary to improve the transfer system. In particular, the loop requires an adequate and precisely defined flushing flow, to generate good-shaped peaks. The waste branch is also necessary to enable both satisfactory loop-flushing, and optimum second-dimension velocities. Hence, a balance must be found to limit losses in sensitivity which, at present, is the greatest problem. Work is also required to define the best column dimensions, and to reduce the complexity of the optimization step. As shown, satisfactory results are also being attained using a 0.18 + 0.10 mm ID combination. Hopefully, the proposed improvements will help close the performance gap between flow and cryogenic modulation.

## Acknowledgement

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