# CONVENTIONAL AND ENANTIOSELECTIVE GC MICROFABRICATED COLUMNS VERSUS FSOT COLUMNS IN THE ANALYSIS OF REAL-WORLD SAMPLES IN THE FLAVOUR AND FRAGRANCE FIELD

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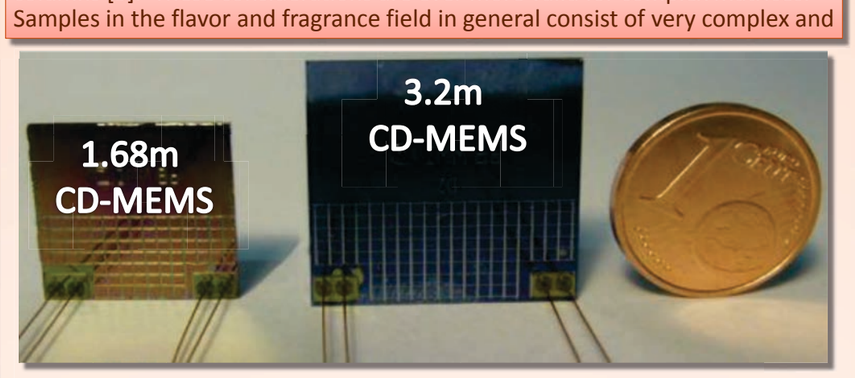
## INTRODUCTION

Instrument miniaturization in all its parts (columns, injection system, detector) is one of the fields where gas chromatography is expected to progress dramatically provided that the same or improved performances of conventional instruments are guaranteed. Compact instrumentation has obvious benefits in saving energy, materials and laboratory space [1,2]. Portable gas chromatographs for "in-field" analyses is another area where miniaturized GC can play a fundamental role. In-field gas chromatography affords in-situ analysis immediately after (or on-line to) in-field sampling thus avoiding sample alteration and drastically reducing analysis time. These analyses are of high interest for environmental control, in mineral oil industry, studies of ecological biochemistry and toxicological chemistry.

Micro-electromechanical system (MEMS) technologies play a fundamental role for portable and/or micro-GCs in all their parts (injectors, columns, detectors).

Many efforts are therefore devoted to the development of microcolumns with efficiencies and selectivities comparable to those of conventional capillary columns [3]. These considerations are ever more valid for complex matrices. Samples in the flavor and fragrance field in general consist of very complex and homogeneous mixture containing hundredths of components with quite similar structures and physicochemical characteristics, relatively different polarities and medium-to-high volatilities. Their separation can therefore be obtained only with a very high efficiency chromatographic system.




Chiral recognition is a further fundamental step in this field since enantiomers often have different sensory properties. High chromatographic efficiency microcolumns coated with highly effective chiral selector and are therefore necessary for chiral recognition of markers in complex real-world samples.

In this study, the performance of two chiral etched silicon MEMS columns statically coated with **30% 2,3 diethyl – 6 t-butyldimethylsilyl-β-cyclodextrin in PS-086** of different lengths (**1.68 and 3.20 m**) is investigated and compared under the same analysis conditions to that of conventional capillary columns and of comparable length and phase ratio. Comparison is carried out by analyzing a set of test standard mixtures and real-world samples from the flavours and fragrance field and essential oils from different plant species.

***AIMS:* Comparison of MEMS chiral column performance to those of conventional and narrow bore chiral columns for enantioselective GC in the flavour and fragrance field.**

## MATERIALS AND METHODS

**Columns-** Two CD-MEMS columns of different lengths (1.68 and 3.20 m) (**1.68 CD MEMS and 3.2 CD-MEMS**) statically coated with 30% $6^{I\text{-}VII}$-O-TBDMS-$3^{I\text{-}VII}$-O-ethyl-2I-VII-O-ethyl-β-cyclodextrin in PS-086 were tested. The 1.68 CD-MEMS column has a nominal film thickness of 0.1 µm coated on a square channel of 80 µm sides; the 3.2 CD-MEMS column is coated with 0.075 µm $d_f$ of stationary phase on a circular channel of 75 µm $d_c$. The performances of the CD-MEMSs were compared to those of conventional and narrow bore FSOT capillary columns coated with the same stationary phase, i.e. a 25m × 0.25mm $d_c$ × 0.25µm $d_f$ (conventional) and a 5m × 0.10 m $d_c$ ×0.10 µm $d_f$ (5m narrow bore) columns. All columns were from MEGA (Legnano, Italy).

**Test samples** - Column performances were evaluated by analyzing ethyl dodecanoate isothermally at 110°C at different flow rates. Each column was tested with a chiral test developed in the authors' laboratory, consisting of ten compounds with different structures and polarities (see caption to figure 2) [4]. Standard mixtures of racemic $C_6$ to $C_{12}$ γ- and δ-lactones solubilized in cyclohexane at a concentration of 100 ppm and bergamot (*Citrus bergamia* Risso et Poiteau), lavender (*Lavandula angustifolia* P. Mill.)and peppermint (*Mentha* x *piperita* L.) essential oils (e.o.) were also analyzed. The e.o.s, obtained by hydrodistillation in agreement to the European Pharmacopoeia (8th ed.), were diluted in cyclohexane 1:200 for the 25m conventional column and 1:500 for the other columns before analysis.

**Instrumental set-up** - Analyses were carried out on a Shimadzu GC-FID 2010 system provided with a Shimadzu GC Solution 2.53SU software (Shimadzu, Milan, Italy). Temperatures: injector: 220°C, detector: 230°C; carrier gas: H2, flow control mode: constant linear velocity. FID sampling rate: 40ms.

**Analysis conditions -** Isothermal analyses: temperature: 110°C, flow rates: 0.4, 0.6, 0.8, 1, 1.4, 1.8, 2 and 2.5 mL/min for the 25m conventional column; 0.14, 0.29, 0.44, 0.58 and 0.72 mL/min for the 5m narrow bore column; 0.15, 0.2, 0.25, 0.3, 0.4 and 0.5 mL/min for the two MEMS columns. Temperature programmed analyses: different flow rates were set for each column, i.e. the "efficiency optimizing flows" (EOF), that was obtained with the approach developed by Blumberg [5]; in particular the EOF was 1mL/min for the conventional column, 0.3 mL/min for the 5m narrow bore column and 0.2 mL/min for the two MEMS columns. Three temperature rates (2, 5 and 10°C/min) were tested for each sample.

## RESULTS AND DISCUSSION

### *Evaluation of chromatographic efficiency and enatioselectivity*

Efficiency of the investigated MEMSs was first evaluated by analyzing ethyl dodecanoate in isothermal conditions (110°C) at different flow rates (see experimental). Figure 1 reports the Van Deemter plots for the two MEMSs and the two capillary columns and table 1 the number of theoretical plates (N) and the number of plates per meter (N/m) for each column. The Van Deemter curves of 5m narrow bore (NB) column and 3.2 CD-MEMS almost overlap and their minimum relative plate heights, *h*, are similar. The minimum *h* of these columns is considerably lower than that of both 25m conventional column and 1.68m MEMS. The number of theoretical plates per meter (N/m) shows a similar trend: the highest value was obtained for the 5m NB column followed by the 3.2 CD-MEMS and then by the 25m column and the chip 1.68m MEMS. However, the true chromatographic efficiency of the investigated columns is given by the total number of theoretical plates (N) that depends on their total lengths. As expected, the highest value of N was obtained for the conventional 25m column followed by the 5m NB and by the MEMS 3.2m; N for MEMS 1.68m column was considerably lower.

| | N | N/m | $S_{chiral\ test}$ |
|---|---|---|---|
| 25m conventional | 67188 | 2687 | 848 |
| 5m narrow bore | 28093 | 5618 | 711 |
| 3.2 m MEMS | 15780 | 4932 | 521 |
| 1.68 m MEMS | 3249 | 1934 | 247 |

Table1: number of theoretical plates (N), plates per meter (N/m) and separation measure (S) on chiral test for the two MEMS columns and the two capillary columns.

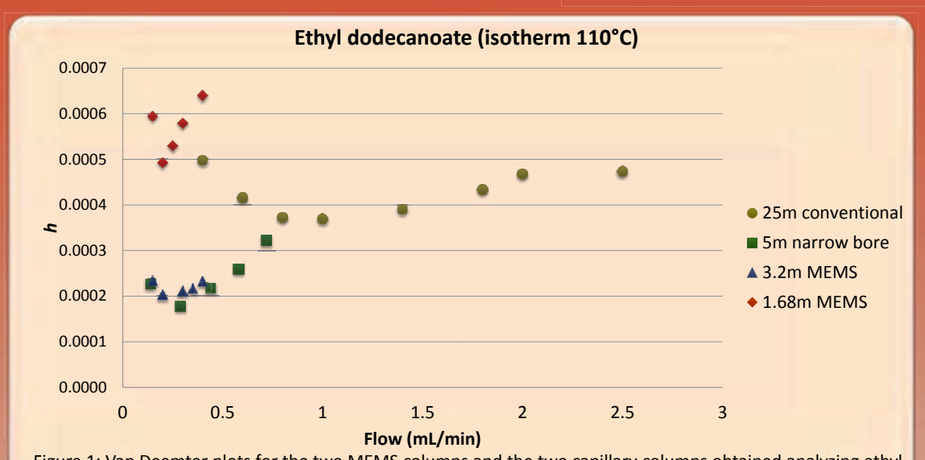



Figure 1: Van Deemter plots for the two MEMS columns and the two capillary columns obtained analyzing ethyl dodecanoate at 110°C at different flow rates (see experimental).

Separation efficiency and enantioselectivity of the MEMS columns were then investigated by analyzing the chiral test (see experimental) with the four columns, measuring the "efficiency optimizing flow, EOF" (in agreement with Blumberg [5]) at the same temperature rate of 2°C/min. Figure 2 shows the profiles of the chiral test and table 1 the separation measures (S) for the four investigated columns calculated in the time range between the retention times of the same analytes ((S)-limonene/(S)-δ-decalactone). The results show that the enantioselectivities of the conventional and 5m narrow bore columns and the 3.2 MEMS are comparable. The separation measures (S) of 5m and MEMS 3.2m columns are slightly lower than those of the conventional 25m column taken as a reference (15% and 38% respectively) but they afford an analysis time reduction of 14% and 24% respectively.

The enantioselectivity and the separation efficiency of the MEMS 1.68m column are considerably lower showing that this column can only be used for moderately complex mixture and for the separation of the chiral compounds whose enantiomers are very well discriminated.

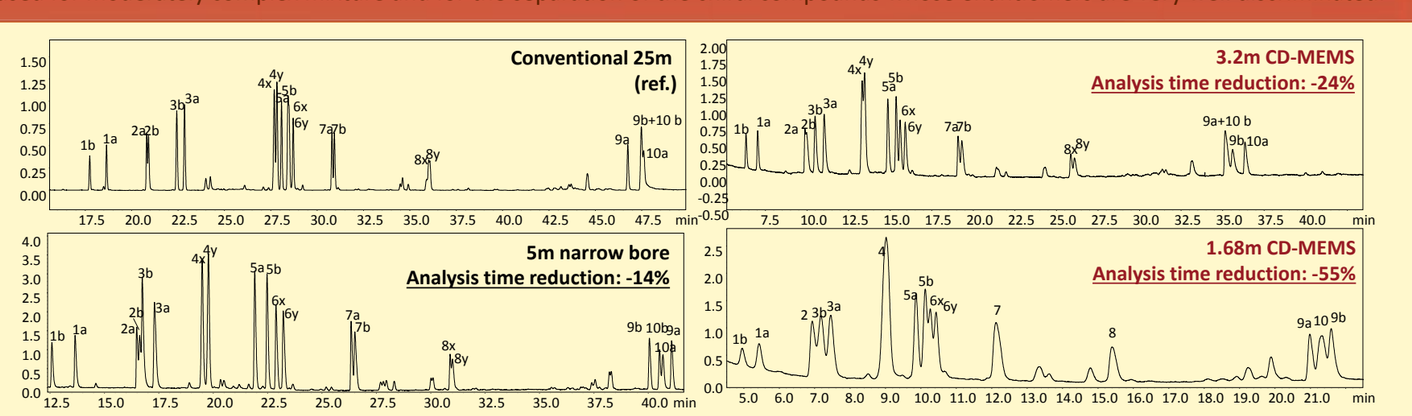



Figure 2: profiles of the chiral test on the two MEMS columns and the two capillary columns. Analysis conditions: see text. Peak identification: 1: limonene, 2: 2-octanol, 3: camphor, 4: isobornyl acetate, 5: linalyl acetate, 6: 2-methyl-3-(*Z*)-hexenyl butyrate, 7: menthol, 8: hydroxycitronellal, 9: γ-decalactone, 10: δ-decalactone; a: (*R*) enantiomer, b: (*S*) enantiomer, x and y: enantiomer configuration not assigned.

### *Analysis of real word samples*

Standard mixtures of racemic γ- and δ- lactones (both from $C_6$ to $C_{12}$) and a set of different essential oils (bergamot, lavender and peppermint essential oils) were also analyzed with the four columns at different temperature rates (i.e. 2, 5, 10°C/min). Figure 3 reports resolutions and elution temperatures of a C6-C12 δ-lactones standard mixture obtained with the 25m conventional (taken as a reference), 5m narrow bore and 3.2 CD-MEMS columns at different temperature rates. Figure 4 reports ES-GC profiles of the C6-C12 δ-lactones obtained with the three above columns at 2°/min. Resolutions of δ-lactones on the 5 m narrow bore and, even more, on the 3.2 CD-MEMS are considerably higher than those of the 25m conventional columns; the better resolution is due to the decrease elution of temperature that improves the separation capability of cyclodextrins because enantiomer separation is thermodynamically driven. Figure 5 compares the profiles of a lavender e.o. with the three columns above. Only 25m and 3.2 CD-MEMS separate all markers but analysis time with MEMS is reduced by 60%.

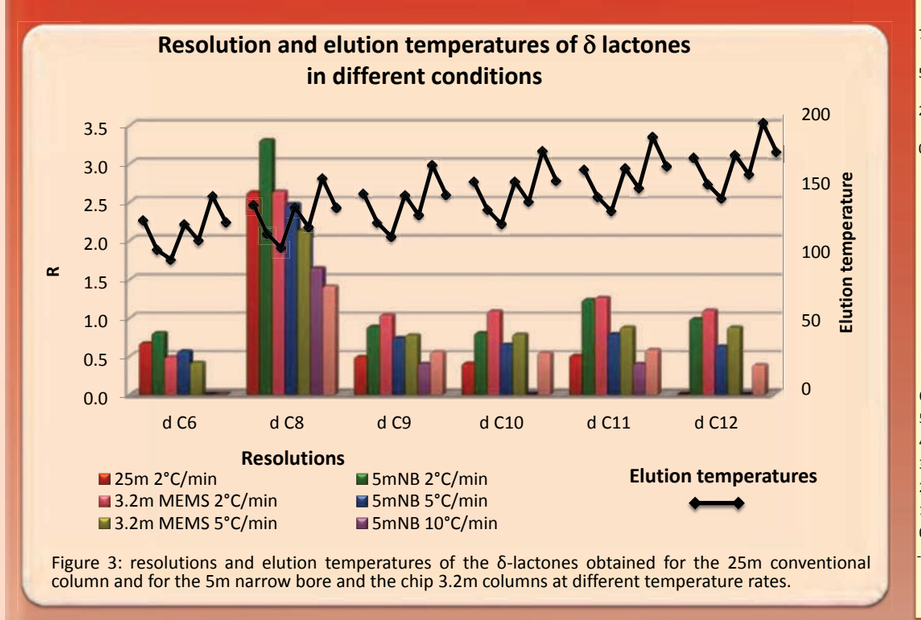



Figure 3: resolutions and elution temperatures of the δ-lactones obtained for the 25m conventional column and for the 5m narrow bore and the chip 3.2m columns at different temperature rates.

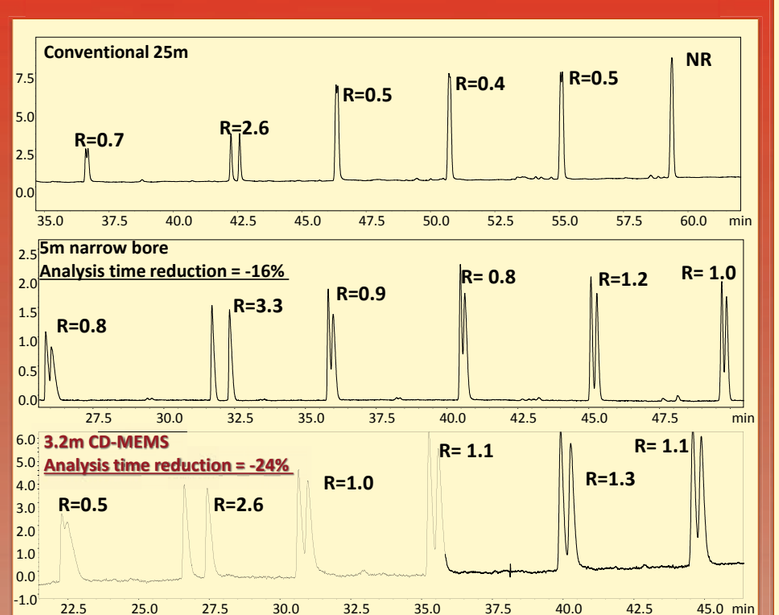



Figure 4: profiles of δ-lactones standard mixture (from $C_6$ to $C_{12}$) on the 25m conventional, 5m narrow bore and chip 3.2m columns. Analysis conditions: see caption to figure 3.

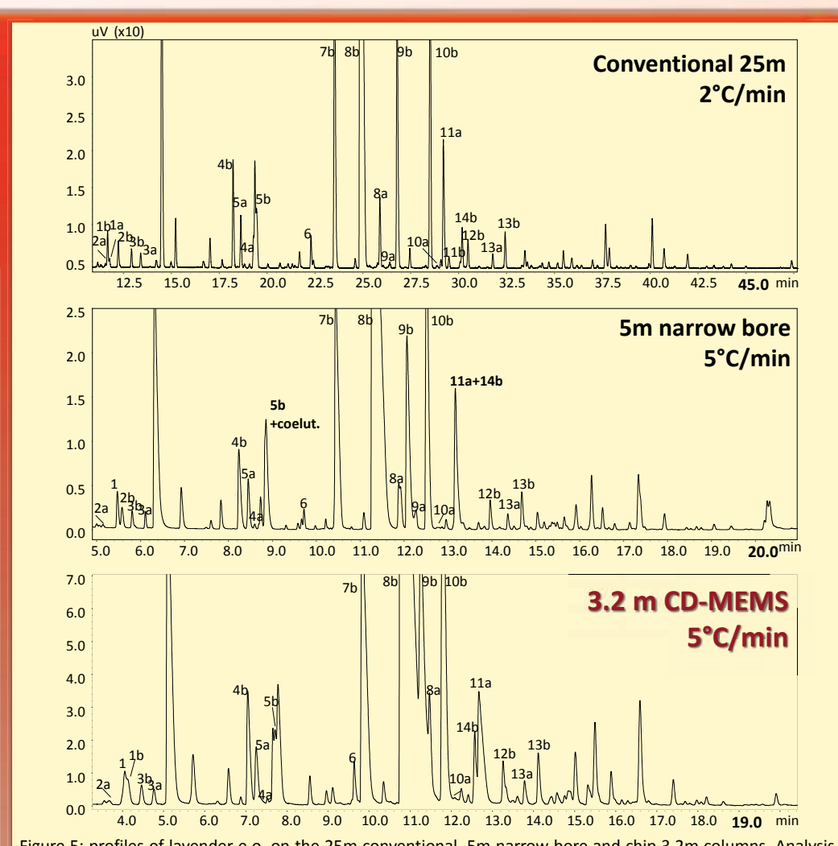



Figure 5: profiles of lavender e.o. on the 25m conventional, 5m narrow bore and chip 3.2m columns. Analysis conditions: flow rate: see caption to figure 3, temperature rate: 2°C for the 25m conventional column, 5°C/min for the 5m narrow bore and the 3.2m MEMS columns. Peak identification: 1: α-pinene, 2: camphene, 3: β-pinene, 4: β-phellandrene, 5: limonene, 6: 1-octen-3-ol, 7: camphor, 8: linalool, 9: borneol, 10: linalyl acetate, 11: 4-terpineol, 12: lavandulol, 13: α-terpineol, 14: lavandulyl acetate; a: (S) enantiomer, b: (R) enantiomer.

## CONCLUSION AND FUTURE PERSPECTIVES

The 3.2 CD-MEMS shows chromatographic efficiency and selectivity comparable to those of a capillary 5m narrow bore column and can therefore be used for chiral recognition of medium to high complex samples such as those in the flavour and fragrance field. CD-MEMSs show enantioselectivity similar (or even higher for high boiling compounds) to that of a conventional long column with an average analysis time reduction of about 25%.

However, MEMS columns still show a limited chromatographic efficiency and further efforts must therefore be made for their optimization, also by decreasing void volumes due to the present need to use capillary tubing to connect them to conventional injectors and detectors.

The use of highly efficient MEMS column together with the possibility to connect them directly to injector and detector and the possibility to heat them directly on chip through metal filaments deposition [6] open a concrete perspective to the everyday use of micro-GC for routine analyses.

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