GLOBAL ANALYTICAL SOLUTIONS

GERSTEL

AppNote 2/1999

Automated Sample Fractionation and Analysis Using a Modular LC-GC System

Frank David
Research Institute for Chromatography, Kennedypark 20, B-8500 Kortrijk Belgium

Pat Sandra
Department of Organic Chemistry, University of Gent, Krijgslaan 281 S4, B-9000 Gent, Belgium

Andreas Hoffmann
Gerstel GmbH & Co. KG, Eberhard-Gerstel-Platz 1, D-45473 Mülheim an der Ruhr, Germany

Introduction

For the separation of complex mixtures or for the detection of trace analytes in complex matrices, a single (one-dimensional) chromatographic separation is often not adequate. The peak capacity (number of peaks that can be separated in one run) in capillary gas chromatography (CGC), using standard columns and conditions, is in the order of 100 to 300, while the peak capacity in high performance liquid chromatography (HPLC) is in the order of 50-100 (1). The complexity of samples, such as petroleum fractions or natural product extracts, largely exceeds these numbers. The availability of selective detectors for CGC or HPLC and even the combination of chromatography with spectroscopic detection (Mass Spectroscopy, Fourier Transform Infra Red, Atomic Emision detection or Inductively Coupled Plasma detection) can also not guarantee sufficient sensitivity and specificity in cases where the target solutes are masked by

a very complex matrix. From this experience, the need for multidimensional chromatographic techniques became obvious.

Multidimensional capillary gas chromatography, and, in its ultimate form, comprehensive CGC, result in the highest obtainable peak capacity and overall resolution. The combination of CGC techniques is however restricted to the analysis of volatile (GC-amenable) compounds and the separation mechanism is mostly based on volatility (boiling point separation). High molecular weight compounds or very polar compounds cannot be analysed or will contaminate the analytical system. The potentials of multidimensional HPLC, in this respect, are much larger and very specific separations based on hydrophobicity, polarity, ion strength, molecular size or affinity can be obtained. HPLC, on the other hand, cannot offer the same peak capacity and overall resolution as CGC. HPLC and CGC are thus quite complementary to each other and therefore the on-line combination of the two techniques is very interesting [1].

Recently on-line HPLC-CGC equipment became commercially available [2,3]. In this paper, a new automated and modular system based on a flow cell and large volume PTV injection is presented.

Experimental Set-Up

The automated on-line LC-GC system can be build using standard HPLC and GC equipment. The GC must be equipped with a programmable temperature vaporizing inlet (PTV).The LC-GC interface consists of a modified large volume sampler (MultiPurpose Sampler, Gerstel, Mülheim, Germany). The system configuration is illustrated in Figure 1. The flow exiting the LC detector is send via a well defined capillary tubing to a flow cell. The flow enters the flow cell from the bottom and exits from the side. This cell is equipped with a septumless head, through which a syringe can be introduced by the large volume sampler. To transfer a defined fraction from the LC to the GC, the heart-cut window (start and stop time of transfer) is introduced in a software program, together with the flow rate (mL/min) used in the HPLC analysis. At the moment that the beginning of the fraction enters the flow cell, the syringe from the sampler penetrates the septumless head and samples the LC fraction at a speed equal to the LC flow rate. The time delay between the HPLC detector and the flow cell is automatically taken into account by the software program (using the mobile flow rate and the capillary tubing dimensions), so that the heart-cut times can be measured on the LC chromatogram. At the end of the heart-cut time, the total LC fraction is collected in the syringe. Depending upon the syringe size, volumes up to 1 or 2 mL can be collected. After collection, the flow cell rotates away from the syringe and the sampler makes a speed controlled injection into the PTV inlet of the GC. The PTV injector is used in the solvent vent mode. During injection, the inlet is kept at low temperature while the split vent is open (eventually with increased split flow and reduced inlet pressure). During this vent time, the largest part of the solvent is evaporated. After solvent venting, the split valve is closed, the pressure is set to the normal head pressure and the injector is heated to evaporate the compounds of interest, whereby they are introduced (in splitless mode) into the GC column.Injection parameters (speed of injection, vent time,...) are calculated by the software program.

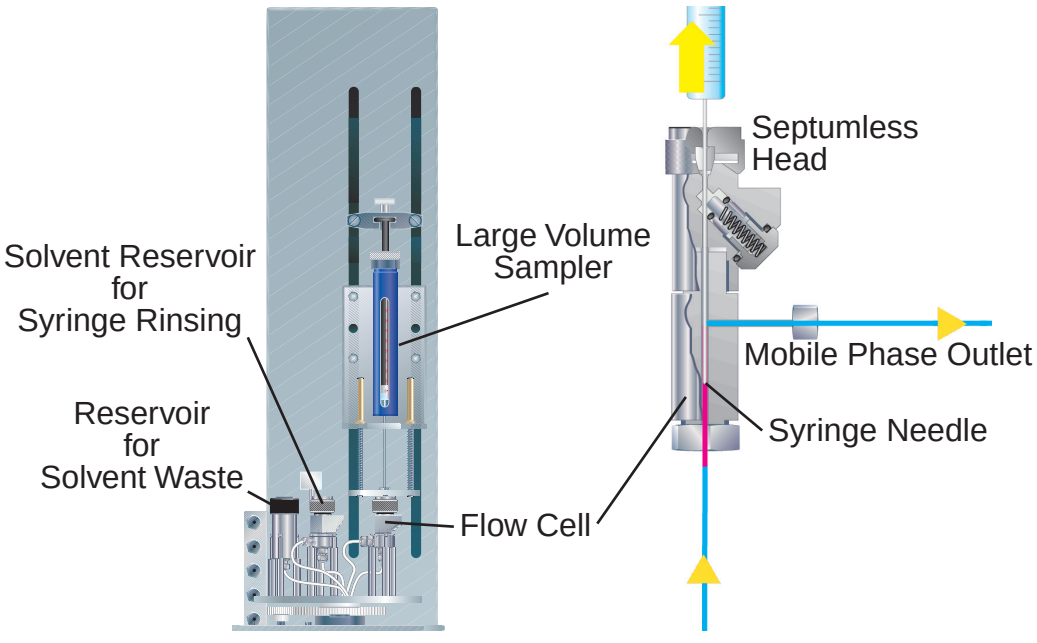

Figure 1. Schematic drawing of the LC-GC interface (left: general view, right: detail of the flow cell).

APPLICATIONS

Analysis of Dibenzothiophenes in Crude Oil. Methylbenzothiophenes can be used as markers of oil pollution [4]. Direct analysis by CGC is not possible due to the complexity of the matrix and the presence of a non-volatile fraction. For this reason a LC-prefractionation is used [5].

A crude oil sample was diluted at 1% in hexane and separated on an aminopropyl column (Hypersil APS, 25 cm L x 4.6 mm i.d. x 5 µm). The mobile phase was 100 % hexane at 0.8 mL/min. On this column a separation according to number of aromtic rings is achieved. This is illustrated below by the chromatograms of a crude oil sample and of an aromatic hydrocarbon test mixture.

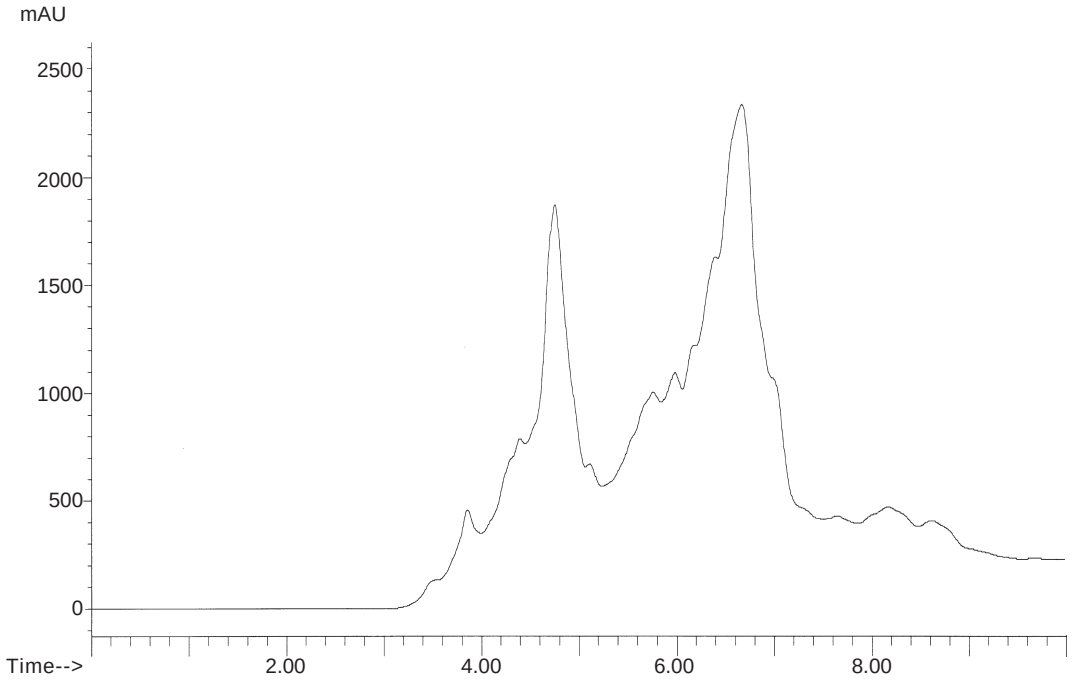

Figure 2. HPLC chromatogram of a crude oil sample.

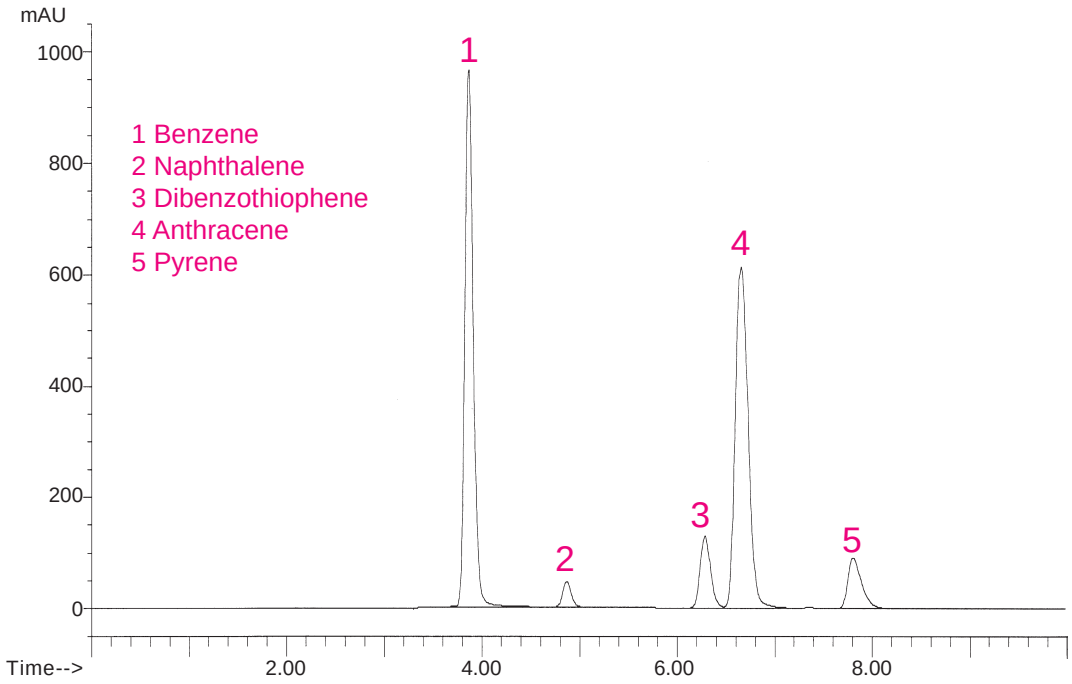

Figure 3. HPLC chromatogram of an aromatic hydrocarbon test mixture.

The fraction eluting from 6 to 6.5 min (volume = 0.4 mL) was automatically transferred to the GC inlet. The LC-GC interface was programmed to take the sample at a 800 µL/min sampling speed (= HPLC flow rate). The complete fraction was then injected in the PTV in solvent vent mode at 250 µL/min injection speed. The column was a 30 m x 0.25 mm i.d. x 0.25 µm HP-5MS and was programmed from 50°C to 250°C at 20°C/min and to 320°C at 5°C/min. Detection was done by FID.

The GC chromatogram is shown below. Good peak shapes are obtained. In the chromatogram dibenzothiophene (peak 1), the mono-methyl isomers (2 = 4-methyl, 3= 2-methyl, 4= 3-methyl and 5=1-methyl) and the higher alkyldibenzothiophenes (6) are easily detected.

This example also demonstrates the enormous complexity of the sample. The GC chromatogram contains more than 100 peaks and this is only a fraction of the LC preseparation.

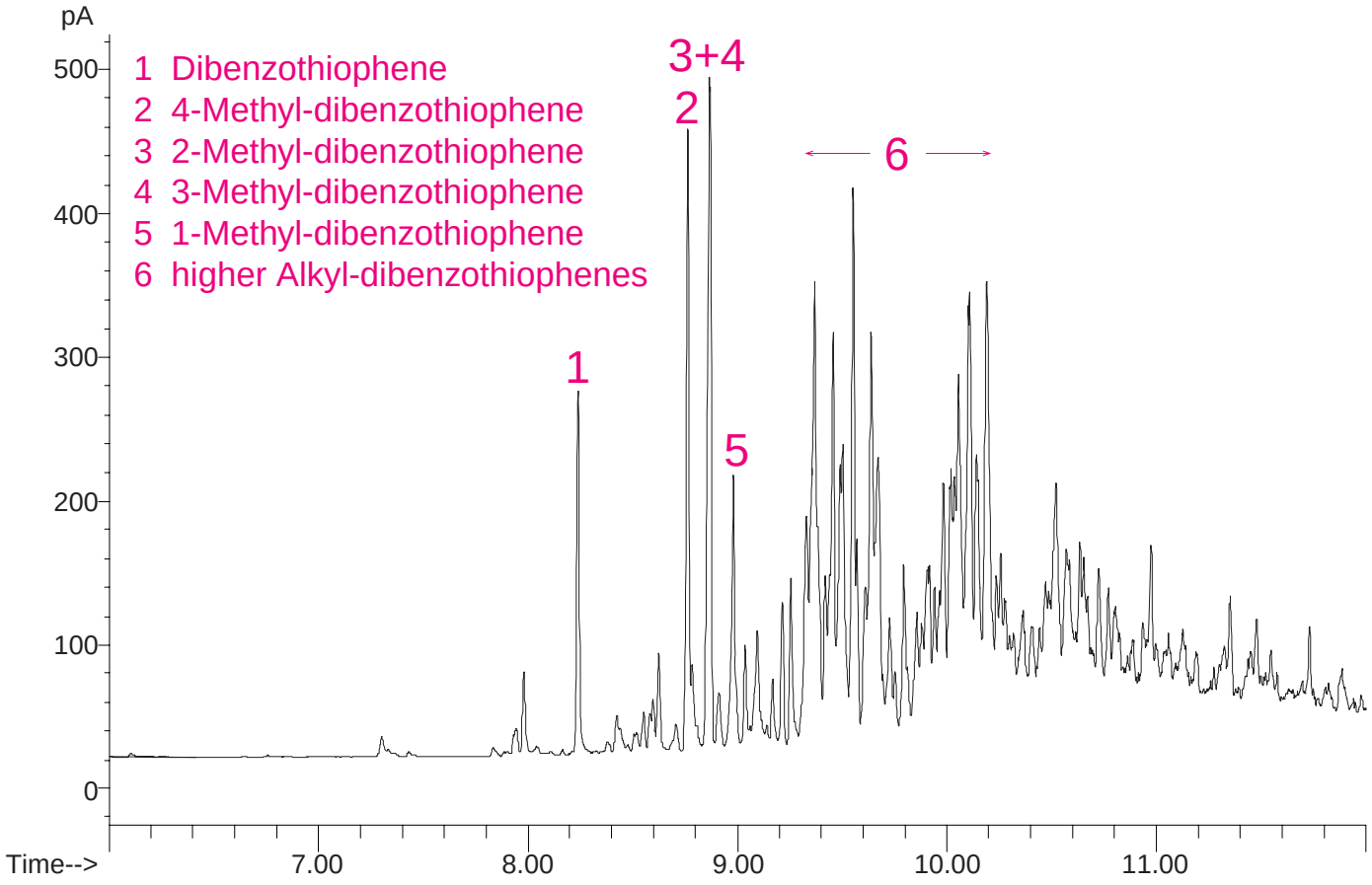

Figure 3. GC/FID-chromatogram of the transferred LC-fraction.

Analysis of Pesticides in Essential Oils. Recent studies have demonstrated that essential oils can contain trace levels of pesticides [6,7]. Since essential oils are a complex and concentrated matrix, sample preparation is more challenging than for classical food (fruits and vegetables) matrices. In the on-line LC-GC method, the diluted essential oil sample is injected on a LiChrolut DIOL column. A hexane:isopropanol gradient is used. A typical profile obtained for an orange oil is shown below.

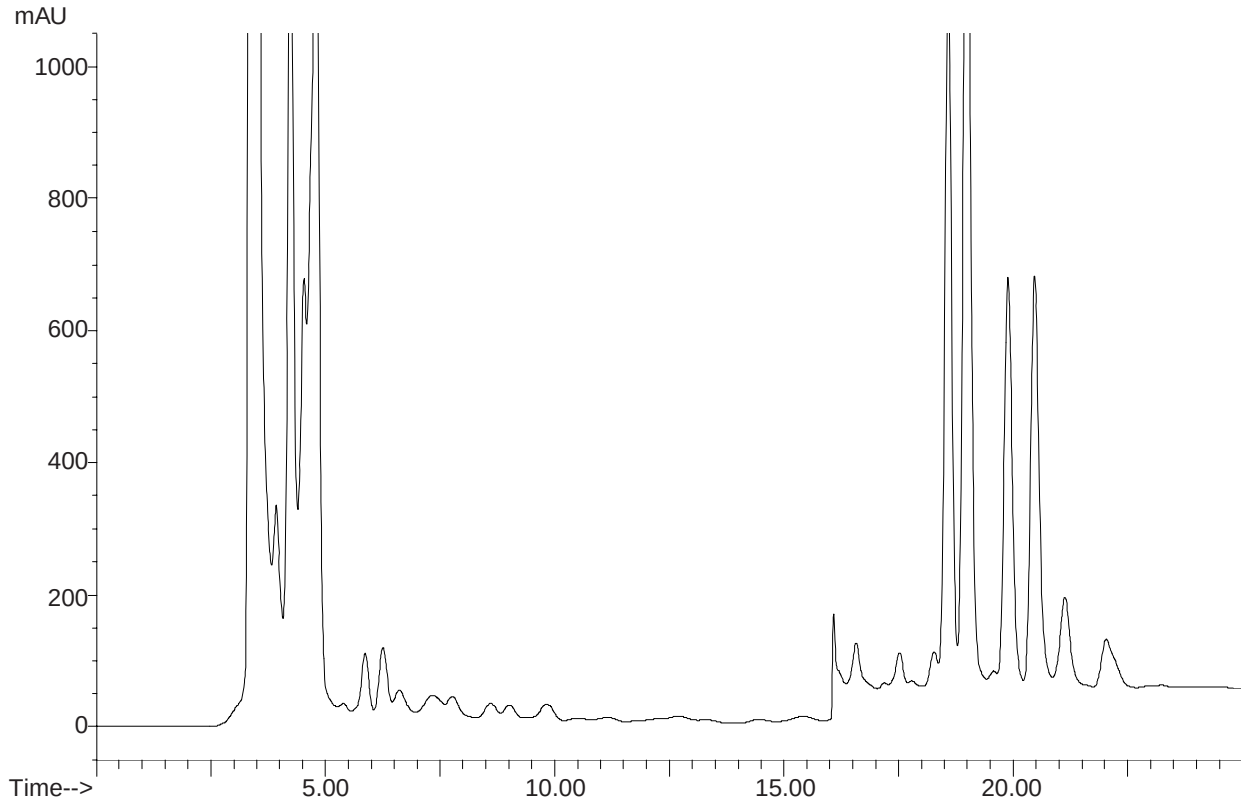

Figure 5. HPLC-chromatogram of an orange-oil.

The compounds are separated according to polarity. First the mono- and sesquiterpenes elute, followed by terpenoids and finally the flavanoids. Especially these last compounds interfere with the pesticide determination.

Organophosphorous pesticides elute around 4.5 min. The fraction eluting from 4.4 to 4.9 min (500µL)was transferred to the GC inlet. Injection in the PTV inlet was performed at 250 µL/min. The analysis was done on a 30 m x 0.25 mm i.d. x 0.25 µm HP-5MS column. NPD detection was used. The resulting chromatogram is shown below.

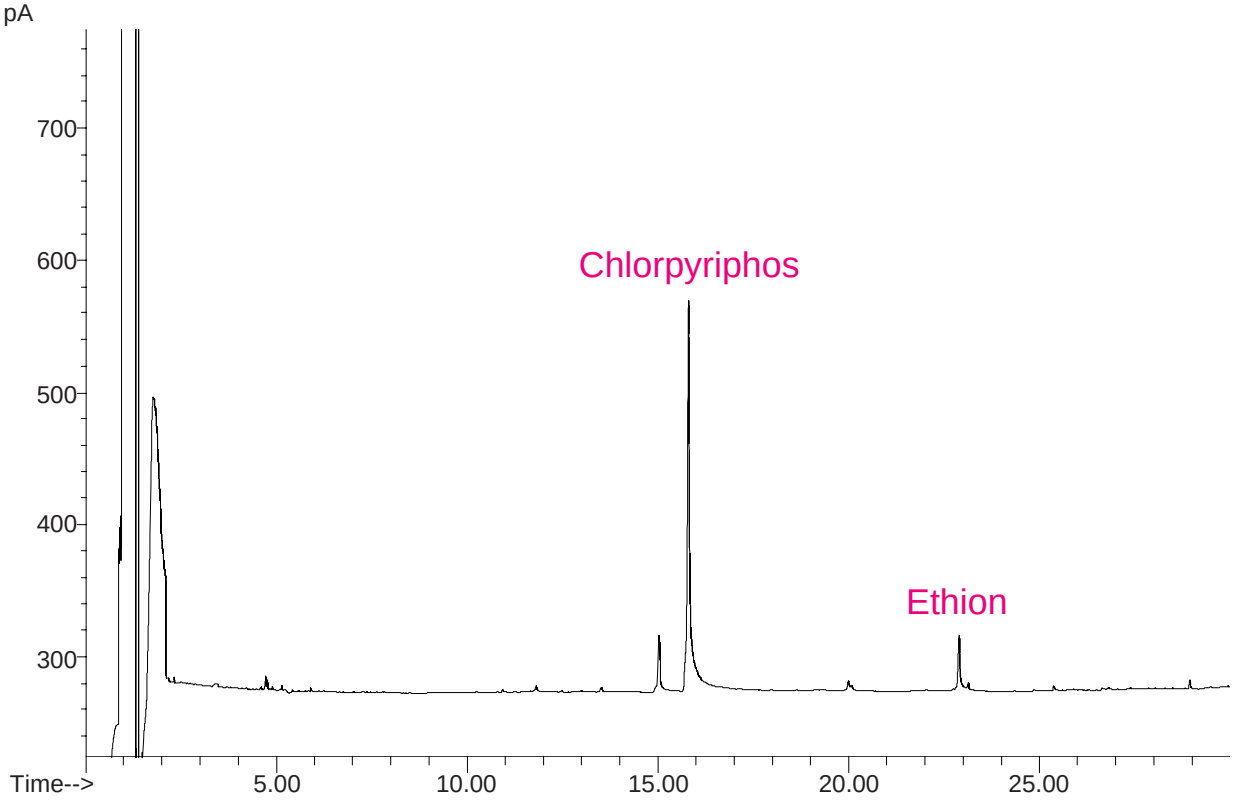

Figure 6. GC/NPD-chromatogram of the transferred LC-fraction.

In the orange oil, both chlorpyriphos and ethion are detected. The chromatogram is very clean and no interferences are present. This demonstrates the excellent selectivity of the LC-GC combination. The solvent vent also allows the introduction of a large amount (500 µL) on the GC-NPD system.

Conclusions

The combination of liquid-chromatography and gas chromatography offers very high efficiency and selectivity for fractionation of complex mixtrures and for the isolation of trace solutes in complex matrices. A new on-line LC-GC system, based on a modified large volume sampler and PTV injection in the solvent vent mode, allows full automation of the analysis and reduced total analysis time in comparison to standard methods.

References

[1] K. Grob, On-Line Coupled LC-GC, Chromatographic Methods Series, Hüthig, Heidelberg, 1991.

[2] F. Munari, G. Dugo and A. Cotroneo, in Proc. Tenth Int. Symp. on Capillary Chromatography, Riva del Garda,1989, P. Sandra and G. Redant (Eds), Huthig, Heidelberg, p. 736.

[3] F. David, P. Sandra, D. Bremer, R. Bremer, F. Rogies and A. Hoffmann, Labor Praxis 21 (1997) 5.

[4] F. Berthou, Y. Dreano and P. Sandra, J. High Resolut. Chromatogr. 7 (1984) 679.

[5] F. Berthou and Y. Dreano, J. High Resolut. Chromatogr. 11 (1988) 706.

[6] V. Leoni and E. D'Alessandro De Luca, Essenz Deriv Agrum. 48 (1978) 39.

[7] G. Dugo, M. Saitta, G. Di Bella and P. Dugo, Perfumer & Flavorist 22 (1997) 33.

GERSTEL GmbH & Co. KG
Eberhard-Gerstel-Platz 1
45473 Mülheim an der Ruhr
Germany
+49 (0) 208 - 7 65 03-0
+49 (0) 208 - 7 65 03 33
gerstel@gerstel.com
www.gerstel.com

GERSTEL Worldwide

GERSTEL, Inc.
701 Digital Drive, Suite J
Linthicum, MD 21090
USA
+1 (410) 247 5885
+1 (410) 247 5887
sales@gerstelus.com
www.gerstelus.com

GERSTEL AG
Wassergrabe 27
CH-6210 Sursee
Switzerland
+41 (41) 9 21 97 23
gerstelag@ch.gerstel.com
www.gerstel.ch

GERSTEL K.K.
1-3-1 Nakane, Meguro-ku
Tokyo 152-0031
SMBC Toritsudai Ekimae Bldg 4F
Japan
+81 3 5731 5321
+81 3 5731 5322
info@gerstel.co.jp
www.gerstel.co.jp

GERSTEL LLP
Level 25, North Tower
One Raffles Quay
Singapore 048583
+65 6622 5486
+65 6622 5999
SEA@gerstel.com
www.gerstel.com

GERSTEL Brasil
Av. Pascoal da Rocha Falcão, 367
04785-000 São Paulo - SP Brasil
+55 (11)5665-8931
+55 (11)5666-9084
gerstel-brasil@gerstel.com
www.gerstel.com.br

Awarded for the
active pursuit of
environmental sustainability