

One-Minute Essential Oils Characterization by Gas Chromatography through Nanovolume Injection

Thermo Fisher Scientific Inc., Milan, Italy

Key Words

- 1-minute Essential Oils Analysis
- Nanovolume Injection
- Plunger-in-needle Syringe
- Ultra Fast GC



Thermo Scientific TRACE GC Ultra™ with AS3000 Autosampler

Introduction

For some GC applications, the possibility to inject sample volumes as small as 10-20 nanoliters is extremely attractive. This is particularly true with very concentrated samples, such as essential oils, where the main constituents easily overload the capillary column even at the highest achievable split ratios. Column overloading results in peak broadening with the consequent deterioration of the separation power. This aspect is even more critical in Fast and Ultra Fast GC where narrow bore columns, characterized by limited sample capacity, are typically used. Dilution with a solvent is generally not desired since it requires an additional sample preparation step, and some of the components of interest may be hindered by the large solvent peak.

Injection of very small volumes may be also desirable for diluted samples when a splitless injection has to be performed in combination with Fast GC. In fact, a classical 1 μL splitless injection is hardly compatible with narrow bore columns since it requires a long splitless period to complete the transfer at the low flow rates used. Additionally, the large amount of solvent will easily produce peak deformation due to a flooding effect.

Plunger-In-Needle Syringe Type

Ordinary GC syringes of 5-10 μL are not suitable for injecting sample volumes smaller than 0.1 μL since they cannot provide enough volume accuracy.

The use of plunger-in-needle syringes (Figure 1) allows an accurate measurement of ten times smaller volumes than ordinary ones, although their conventional use in combination with a hot inlet has serious shortcomings. The problems are mainly related to premature evaporation of the sample during needle insertion in the inlet, evaporation of sample from the annular space between the internal needle wall and the plunger, and sample discrimination due to distillation inside the needle [1]. These drawbacks essentially come from the classical use of a split-splitless injection where the sample is introduced through the syringe needle to the place where it will evaporate (hot needle technique). In this application, plunger-in-needle syringes have been used in combination with a hot inlet exploiting the liquid band formation technique (cold needle, see next section) [2], which allows the liquid sample to be “shot” into the vaporization chamber without significant evaporation from the needle (Figure 2). Sample volumes as low as 10-20 nL have been injected with high accuracy and precision.

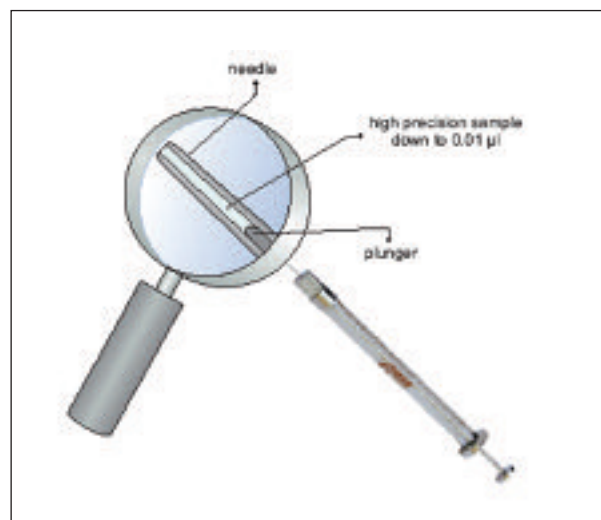


Figure 1: Plunger-in-needle syringe schematic diagram. The plunger is extended to the tip of the needle (zero needle volume) and when liquid is pulled up, it fills only a portion of the needle with no glass contact.

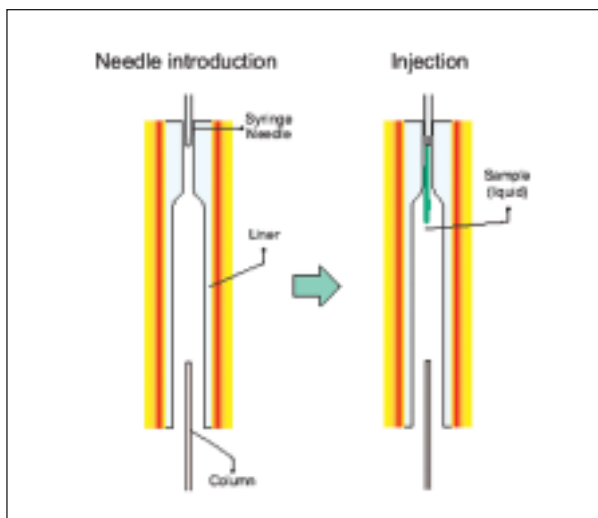


Figure 2: Cold Needle Injection Technique into hot inlets.

Injection Techniques into Hot Inlets

By means of visual experiments, it has been demonstrated that two mechanisms are involved in sample injection inside a hot injector^[2]: thermospray and liquid band formation. Depending on the injection mode, one of these two mechanisms will predominate strongly influencing the sample evaporation process and the sample transfer to the separation column. Namely, a hot empty needle injection mode will provide a thermospray while a cold needle will provide a liquid band formation.

These two injection modes can be automatically achieved through the Thermo Scientific AS3000 Autosampler by selecting “standard mode” or “minimum mode”, with respect to the needle penetration depth in the inlet^[3]. Achieving independent mechanisms of vaporization is the key to obtain good data accuracy and repeatability.

In “standard mode” the needle is programmed to fully enter the injector for a preset dwell time, typically 3-5 seconds. Adequate heating of the needle is obtained, and a thermospray formation is achieved. In “minimum mode” the needle is programmed to enter the inlet for a limited depth with no dwell time. Heating of the needle is avoided, and a liquid band formation is achieved. Possible lack of reproducibility due to the mixed vaporization modes is, in this way, eliminated.

Experimental

Reagents

A standard mixture containing 13 n-alkanes, ranging from *n*-C5 to *n*-C23, at approximate equal percentage (around 7-8 % each) has been used without dilution to test system performance. The same solution has been diluted to a concentration of around 10 ng/μL using carbon sulfide to perform the on-column injections used for reference. A pure sample of lavender essential oil (undiluted) has been analyzed.

Instrumentation

Analyses are performed in Ultra Fast GC mode using a TRACE GC Ultra (See front page) equipped with a Split/Splitless injector (SSL) and a Digital Pressure and Flow Controller, as well as a Fast FID detector. The GC is equipped with an Ultra Fast Module (UFM) featuring an OV5, 5 m x 0.1 mm i.d., 0.1 μm f.t. separation column. The UFM consists of a metal cage containing the fused silica column combined with a heating element and a temperature sensor to ensure a direct resistive heating of the capillary column. The UFM module is housed inside the GC oven (Figure 3) and is capable of heating rates as high as 1200 °C/min^[4]. The same GC unit has been used in conventional mode after quick removal of the module.



Figure 3: UFM column module housed in the TRACE GC Ultra oven.

Split injections from 10 nL (10^{-2} μL) to 0.2 μL are performed with a AS3000 Autosampler using a 0.5 μL plunger-in-needle syringe. A minimum penetration depth in the injector (cold needle mode) is set, and 0.3 μL of air are automatically withdrawn after the sample to ensure that the part of needle inserted into the injector is empty. A 3 mm i.d. upper-tapered empty liner with an 8 mm long and 1 mm wide restriction at the top is installed. SSL injector is set to 225 °C and the FID to 320 °C. Helium carrier gas is supplied at 0.5 cc/min in constant flow mode and split ratio is set to 1:1000 (unless differently specified). Column temperature is programmed from 50 °C (0.1 min) to 330 °C (0.1) at 300 °C/min for the hydrocarbon analysis, and from 50 °C (0.1 min) to 230 °C (0.1) at 180 °C/min for the lavender oil analysis.

Results and Discussion

Alkanes Standard Mix

Figure 4 shows the chromatogram obtained from the injection of 20 nL of the undiluted standard mixture of *n*-alkanes. The sample is injected with a split ratio of 1:1000, which corresponds to a total amount of about 16 ng of sample into the column. Consequently, each peak corresponds to 1-2 ng. Using the described system, the 0.10 mm i.d. column does not undergo overloading, and a perfect peak shape is observed (Figure 5). Peak widths are around 100 ms (at half height) as expected for this ultra fast analysis.

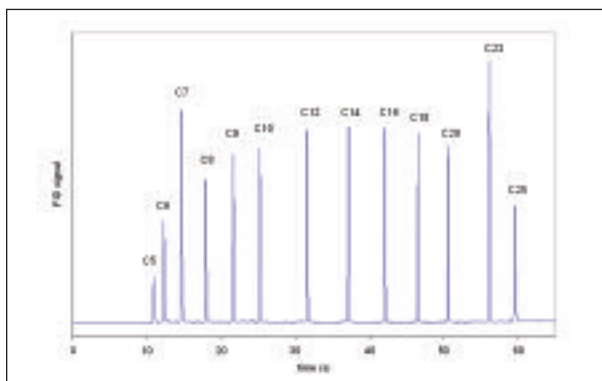


Figure 4: Ultra Fast chromatogram: 20 nL injection of an undiluted mixture of hydrocarbons. Difference in peak heights is due to difference in concentration. Conditions: see text.

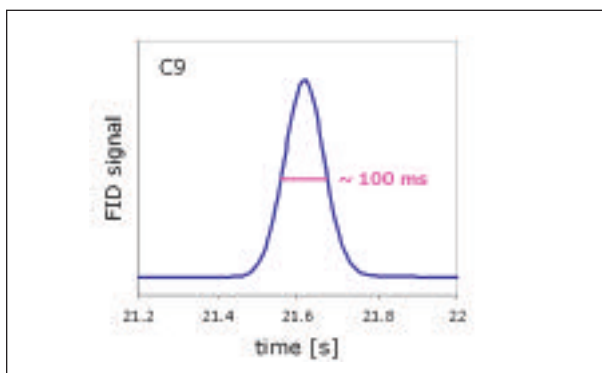


Figure 5: *n*-C9 peak zoom of the Ultra Fast chromatogram reported in Figure 5. Peak width at half height is around 100 ms.

Linearity with Sample Volume

Injections at increasing volumes are performed to verify the accuracy of the sample volume injected. Figure 6 plots the peak area of *n*-C12 and *n*-C20 versus the injection volume from 10 nL to 150 nL. Correlation factors of the linearity curves for the components are respectively 0.999 and 0.998.

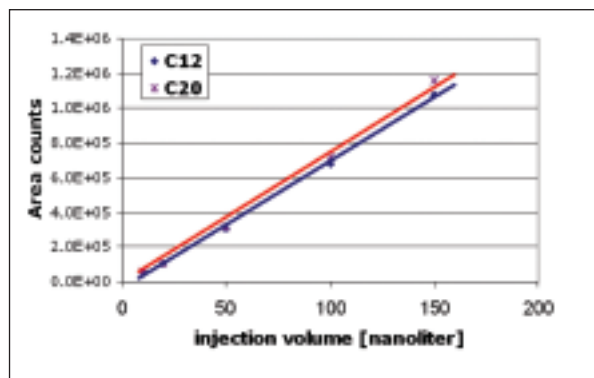


Figure 6: *n*-C12 and *n*-C20 linearity curves in sub microliters range (undiluted hydrocarbons mix split 1:1000).

A very good linearity, considering the very small volumes, is observed. It should also be pointed out that the curves' regression passes very close to the axes origin proving the accuracy of the volume introduced.

Sample Integrity

Eventual discrimination due to the different component boiling point is evaluated comparing the relative peak areas (using C14 as reference) to those obtained by On-column injection of the diluted solution. The recoveries versus On-column obtained at different injection volumes are reported in Table 1. Recoveries very close to 1 are generally found even at the lowest volumes.

COMPOUND	10 nL	20 nL	50 nL	100 nL	150 nL
C6	1.08	1.07	1.05	1.00	0.93
C7	1.14	1.12	1.09	1.04	0.98
C8	1.16	1.14	1.10	1.06	1.00
C9	1.14	1.12	1.09	1.06	1.01
C10	1.07	1.07	1.03	1.01	0.97
C12	1.02	1.00	1.00	1.00	0.99
C14	1.00	1.00	1.00	1.00	1.00
C16	1.00	1.00	1.00	1.01	1.01
C18	0.98	1.00	1.01	1.02	1.03
C20	1.00	1.00	1.01	1.04	1.04
C23	0.99	0.99	1.00	1.03	1.01
C25	0.99	0.98	0.98	1.02	1.01

Table 1: Nanoliters injection recoveries relative to cold On-column (C14 internal standard).

Repeatability

Peak area and retention time statistics for 20 and 50 nL are reported in Table 2. Average values and deviations are calculated on ten consecutive measurements. Peak area RSD % is proportionally inversed to the volume injected: around 6 % at 20 nL and around 3 % at 50 nL.

Retention Times standard deviations are found to be in the range of 50 ms.

COMPOUND	20 NANOLITER		50 NANOLITER		20 NANOLITER		50 NANOLITER	
	AREA (COUNTS)	RSD%	AREA (COUNTS)	RSD%	RT (SECONDS)	DEV. ST. (SECONDS)	RT (SECONDS)	DEV. ST (SECONDS)
C5	3.44E+04	8.0%	1.10E+05	2.8%	10.96	0.04	10.96	0.07
C6	7.71E+04	7.8%	2.41E+05	2.7%	12.29	0.04	12.29	0.06
C7	1.49E+05	7.7%	4.62E+05	2.8%	14.68	0.04	14.67	0.05
C8	9.01E+04	7.3%	2.75E+05	2.9%	17.98	0.04	17.99	0.05
C9	9.18E+04	7.2%	2.80E+05	2.9%	21.69	0.04	21.70	0.05
C10	9.08E+04	7.0%	2.76E+05	3.1%	25.26	0.05	25.28	0.05
C12	1.01E+05	6.3%	3.15E+05	3.4%	31.65	0.05	31.67	0.05
C14	1.07E+05	6.2%	3.28E+05	3.3%	37.18	0.05	37.20	0.05
C18	1.08E+05	6.4%	3.33E+05	3.4%	46.58	0.05	46.59	0.05
C20	1.06E+05	6.4%	3.28E+05	3.5%	50.67	0.05	50.69	0.05
C23	1.79E+05	6.7%	5.64E+05	3.4%	56.26	0.05	56.32	0.06
C25	9.52E+04	6.7%	2.97E+05	3.4%	59.69	0.06	59.75	0.06

Table 2: 20 nL and 50 nL repeatability of undiluted hydrocarbons standard mix.

Linearity versus Split Ratio

Split ratios from normal values, as 1:200, to extremely high as 1:3000 are tested. Figure 7 reports the peak areas versus the nominal split ratios (1/split ratio) for a 50 nanoliter injection. An excellent correlation between the peak areas and nominal split ratio is found.

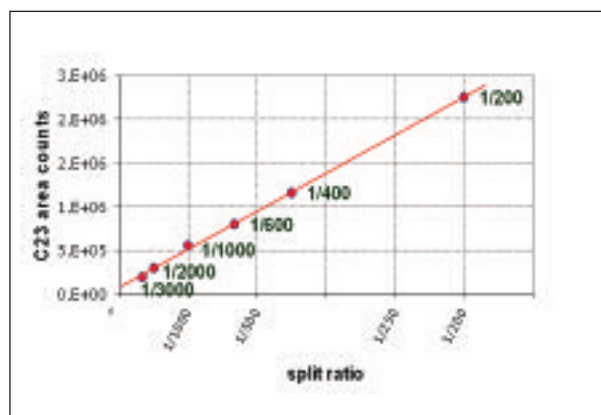


Figure 7: C23 peak areas at different split ratios (50 nL injection volume).

To reach extreme split ratios (1:2000 or 1:3000), split flow is kept constant while the column flow is decreased for a limited amount of time (time corresponding to the sample transfer in the column, see Figure 8). Since this time is minimal, the components retention times are not significantly affected.

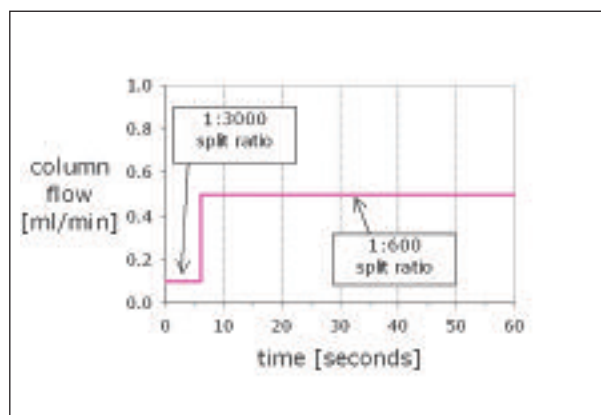


Figure 8: Column flow programming to reach extreme split ratios (Here split flow is 300 mL/min).

The choice of the split ratio is determined by the conditions in which peaks exhibit symmetrical shape. Figure 9 reports the gain in peak symmetry from using high split ratios when injecting 10 nL and 50 nL of undiluted standard mix. Perfect symmetry is displayed at 1:2000 with 50 nL and at 1:400 with 10 nL.

Application to the Ultra Fast GC analysis of Essential Oils

Nanovolume injection is a very useful tool for the analysis of pure essential oils particularly in combination with Ultra Fast GC [5-7] that implies the use of narrow bore columns having a limited sample capacity. Figure 10 shows the chromatogram obtained by the injection of 30 nL of pure lavender essential oil. Injection of pure sample reveals some very volatile compounds that would be hindered by solvent in diluted sample (like compounds 1 and 2, which are likely contaminants from the manufacturing process or storage of the lavender essential oil).

Repeatability evaluated on ten consecutive injections of 30 nL is reported in Table 6 together with the comparison with the conventional GC analysis, achieved by injecting 1 µL of the same essential oil diluted 200 times in iso-octane.

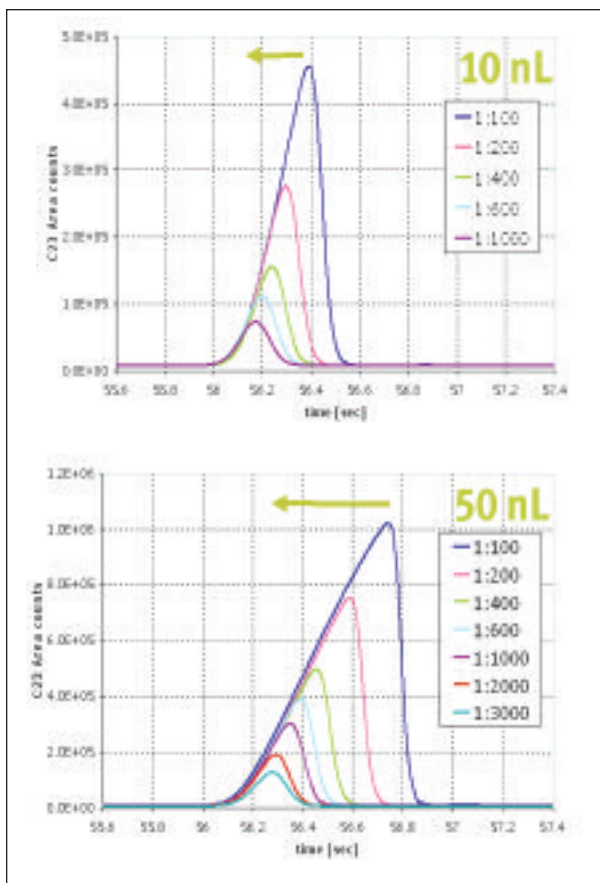


Figure 9: C23 peak related to 10 and 50 nanoliters injections at various split ratios.

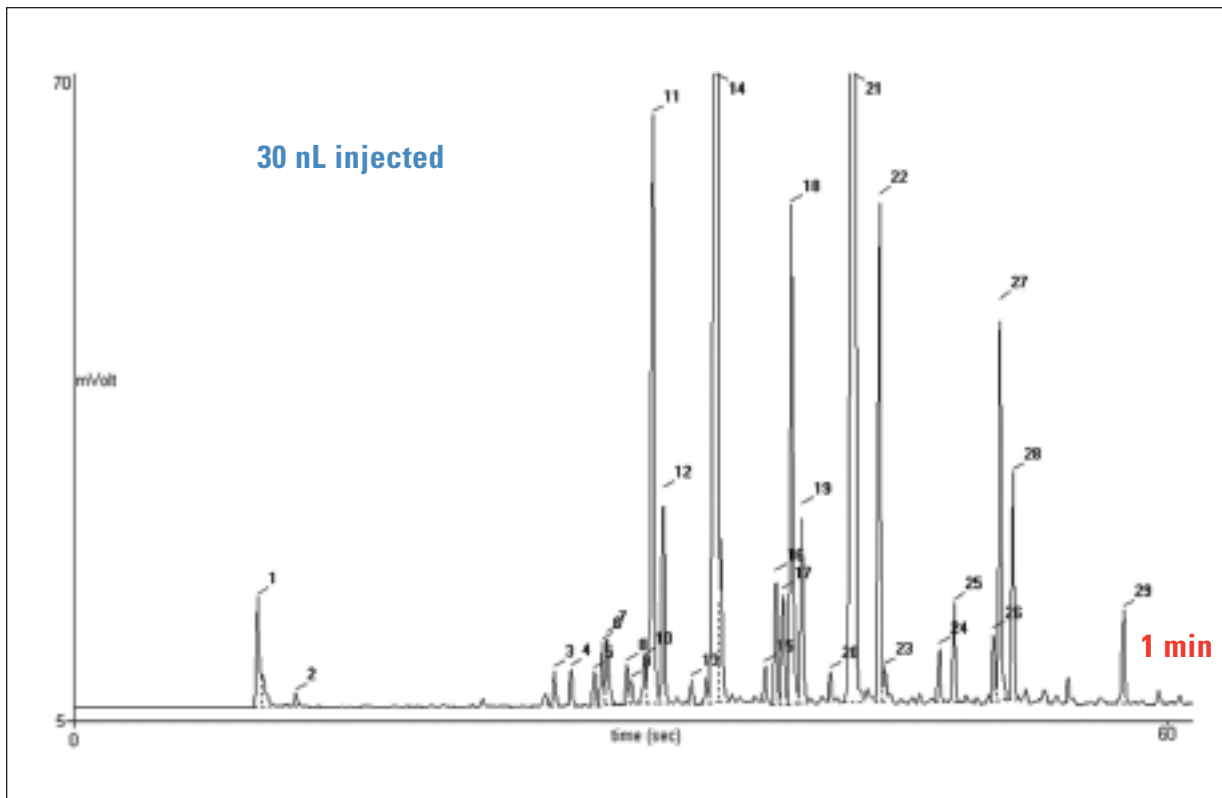


Figure 10: Characterization of pure lavender oil in less than 1 minute by a 30 nL injection (split 1:1000). Peaks identification: 1 Ethanol; 2 Ethyl acetate; 3 alpha-Pinene; 4 Camphene; 5 1-Octen-3-ol; 6 Sabinene; 7 beta-pinene; 8 Myrcene; 9 delta-3-Carene; 10 para-Cymene; 11 Limonene; 12 beta-Ocimene; 13 cis-Linalool oxide; 14 Linalool; 15 Camphor; 16 Lavandulol; 17 Borneol; 18 4-Terpeneol; 19 alpha-Terpeneol; 20 Geraniol; 21 Linalyl acetate; 22 Lavandulyl acetate; 23 Isobornyl acetate; 24 Neryl acetate; 25 Geranyl acetate; 26 alpha-Santalene; 27 Caryophyllene; 28 beta-Farnesene; 29 Cadinol.

COMPOUND	PRECISION		ACCURACY	
	PEAK AREAS		AREA %	
	MEAN	RSD %	UFGC UNDILUTED NANO INJECTION	CONV. GC DILUTED SAMPLE
α -Pinene	6519	4.0 %	0.29 %	0.28%
Limonene	119220	2.7 %	5.25 %	5.08 %
Linalool	653206	2.6 %	28.78 %	28.60 %
Lavandulol	22784	2.6 %	1.00 %	0.98 %
Borneol	21839	2.6 %	0.96 %	0.98 %
α -Terpineol	37464	2.5 %	1.65 %	1.65%
Linalyl acet.	887027	2.7%	39.08%	38.60%
Isobornyl acet.	7025	4.2%	0.31%	0.30%
α -Santalene	13170	2.4%	0.58%	0.58%
Caryophyllene	78071	2.6%	3.44%	3.40%
Cadinol	20051	4.7%	0.88%	0.71%

Table 3: Performances of a 30 nanoliters injection of a pure lavender essential oil.

Conclusions

Optimization of the injection method, on the basis of the mechanism of vaporization inside a hot SSL injector, allows reduction of the injection volume to the sub microliter range using a standard autosampler. The key elements are the use of a low capacity syringe (0.5 μ L) with in-needle-plunger (zero volume needle), combined with an upper-tapered liner and with the use of the cold needle injection technique. Injections in the range of 10-50 nL with split ratio around 1:1000 allow for the analysis of pure samples without overloading the column, also in the case of narrow bore columns as used in Fast and Ultra Fast GC. Excellent linearity, repeatability, and recovery relative to On-Column analysis are shown both for an undiluted standard mixture of hydrocarbons and for real samples.

Pure Essential Oils can be characterized by Ultra Fast GC in about one minute without need of dilution significantly simplifying sample preparation and allowing for the detection of any volatile components.

References

- ¹ K. Grob, *Split and Splitless Injection for Quantitative Gas Chromatography*, Wiley-VCH, Weinheim, 2001, ISBN 3-527-29879-7
- ² K. Grob, M. Biedermann, *J. Chromatogr. A* 2000, 897, 237-246 and 247-258.
- ³ T. Porzano and P. Magni, Proceedings of 25-th International symposium on Capillary Chromatography, D24, Riva del Garda, Italy, May 13-17, 2002 ed. P. Sandra.
- ⁴ P. Magni, R. Facchetti, D. Cavagnino and S. Trestianu, Proceedings of 25th International Symposium of Capillary Chromatography, KNL05, Riva del Garda, Italy, May 13-17, 2002, ed. P. Sandra
- ⁵ AN 10024: Characterization of Essential Oils by Gas Chromatography in One Minute. Thermo Electron Corporation.
- ⁶ C. Bicchi et al., *The Journal of Chromatography-A* 2004, 1024:1-2, 195-207: Direct resistively heated column gas chromatography (Ultrafast module-GC) for high-speed analysis of essential oils of differing complexities.
- ⁷ T. Porzano, F. Bedini, P. Magni, Proceedings of 27th International Symposium of Capillary Chromatography, D16, Riva del Garda, Italy, May 30-June 03, 2004, ed. P. Sandra

Acknowledgement

Authors: Thomas Porzano, Andrea Cadoppi

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa
+43 1 333 5034 127

Australia
+61 2 8844 9500

Austria
+43 1 333 50340

Belgium
+32 2 482 30 30

Canada
+1 800 530 8447

China
+86 10 5850 3588

Denmark
+45 70 23 62 60

Europe-Other
+43 1 333 5034 127

France
+33 1 60 92 48 00

Germany
+49 6103 408 1014

India
+91 22 6742 9434

Italy
+39 02 950 591

Japan
+81 45 453 9100

Latin America
+1 608 276 5659

Middle East
+43 1 333 5034 127

Netherlands
+31 76 587 98 88

South Africa
+27 11 570 1840

Spain
+34 914 845 965

Sweden/Norway/Finland
+46 8 556 468 00

Switzerland
+41 61 48784 00

UK
+44 1442 233555

USA
+1 800 532 4752

www.thermo.com

Legal Notices

©2007 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

ISO 9001
DNV-CERT-00203-94-AD
Thermo Electron Italia S.p.A
is ISO certified.

AN10100_E 12/07C