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Micro-bore Column Fast Gas Chromatography-Mass Spectrometry in Essential Oil Analysis

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The present research is focused on the use of micro-bore column fast gas chromatography in combination with rapid-scanning quadrupole mass spectrometry for mediumly-complex essential oil analysis. A basil essential oil sample was initially subjected to conventional GC-MS (analysis time: 25 min) under optimum analytical conditions. Peak identification was carried by using a dual-filtered MS library search procedure: the first filter deleted "hits" with a less than 90% spectral similarity, while the second filter eliminated matches with a linear retention index (LRI) outside a pre-defined LRI window. The same essential oil sample was analyzed under optimized fast GC-MS conditions by using a micro-bore column, with the same aforementioned MS library search process (analysis time: 5.3 min). Resolution was altogether similar in both applications, with the same number of compounds reliably-identified, namely fifty-nine. The experiment demonstrated the usefulness of the rapid GC approach in this type of experiment.

Keywords: fast gas chromatography; mass spectrometry; micro-bore column; essential oil.

The main objective of any gas chromatographic analysis is the satisfactory separation of the most critical compounds with the minimum time expenditure. Obviously, it is preferable to deliver totally-resolved analytes to the detection system, mass spectrometer included. The employment of a conventional capillary column in GC-MS experiments generally provides acceptable separations on simple-to-mediumly complex matrices; conventional GC-MS analyses times usually range between 0.5 and 1.5 hours. Such analytical time requirements become a considerable disadvantage for laboratories with a high daily sample throughput or where there is a need for rapid and reliable results.

There has always been a constant interest within the chromatographic community for the development and introduction of high-speed GC methodologies. Several approaches have been theorized and introduced with various degrees of success and application: multicapillary columns [1], micro-particle packed capillary columns [2], low pressure outlet conditions (LP-GC) [3], shorter conventional column lengths [4], resistive heating [5], reduced column I.D. and stationary phase thickness (micro-bore capillaries) [6].

The employment of micro-bore columns is probably the most effective way of increasing GC analysis speed. It is well-known that the reduction of the column I.D. limits band broadening, thus enhancing resolving power. The main advantage is that column lengths may be reduced while separation power is essentially maintained if compared to conventional columns. Further benefits are related to higher ideal gas linear velocities and to the fact that the ascending part of the Golay curve rises more gradually. The main disadvantages relative to the employment of micro-bore columns are a reduced sample capacity

and the need for more drastic experimental conditions. The former aspect is essentially counterbalanced by the generation of narrower peaks, while with regards to the second issue, modern GC systems guarantee fast GC requirements.

The present investigation is focused on the use of micro-bore column fast gas chromatography-mass spectrometry for the analysis of a mediumly-complex essential oil. A basil oil sample was subjected both to conventional and fast GC-MS analysis under ideal conditions.

Initially, a basil oil sample was subjected to a volatility-based GC separation using a conventional apolar column under ideal analytical conditions: helium was employed at a constant linear velocity of 30 cm/s; the most appropriate gas velocity was determined in preliminary tests, while the optimum temperature program rate was calculated considering 10°C per void time [7]. The essential oil experiment was carried out in about 25 min: the TIC MS chromatogram relative to this application is shown in Figure 1. The use of a mass spectrometer enabled the identification of 59 peaks (Table 1) by using an in-lab constructed MS library and a dual-filtered search procedure: a) MS library matches with a lower than 90% similarity were eliminated; b) hits with a calculated linear retention index (LRI) outside a pre-defined LRI window (+/- 10 LRI units) were deleted. This type of search process increases the reliability of peak assignment. Two pairs of co-elutions are evident in Figure 1, *viz.*, peaks 24/25 and 35/36. Reliable identification in these cases was made possible by observing the acquired full-scan spectra across the peak profile. The number of identified peaks and the GC-MS run time can be considered as quite normal in this specific field of research.

At this point, a micro-bore column fast GC-MS method was developed: a 10 m x 0.1 mm I.D. capillary with a 0.1 μm film of the same stationary phase as that employed in the conventional application was used. The rapid experiment was carried out also under ideal conditions: a helium linear velocity of 45 cm/s and a 27°C/min temperature program rate were applied.

The fast GC-MS essential oil experiment was carried out in about 5.3 min: the TIC MS chromatogram relative to this application is shown in Figure 2. On the basis of the GC run-time, the term "fast GC-MS" can be used to define the experiment. In our personal opinion GC analyses times between 3 and 12 min can be defined as "fast", between 1 and 3 min as "very

Table 1: Peak identification, LRI values, and spectral similarities in both applications.

Peak	Name	Conv. LRI-Simil.%	Fast LRI-Simil.%
1	α-Thujene	927-95	928-93
2	α-Pinene	937-98	937-97
3	Camphene	954-96	955-97
4	Sabinene	976-98	976-98
5	Vinyl amyl carbinol	978-94	983-98
6	β-Pinene	983-97	987-91
7	Myrcene	989-97	990-97
8	Dehydro-1,8-cineole	994-91	995-92
9	*(Z)*-hex-3-enyl acetate	1004-94	1006-97
10	α-Terpinene	1020-96	1021-97
11	*p*-Cymene	1028-96	1028-98
12	Limonene	1033-96	1034-98
13	Eucalyptol	1037-92	1038-96
14	(*E*)-β-Ocimene	1047-98	1047-97
15	γ-Terpinene	1061-95	1061-96
16	(*Z*)-Sabinene hydrate	1075-94	1076-94
17	Terpinolene	1088-93	1090-96
18	6,7-Epoxymyrcene	1092-91	1094-93
19	Linalool	1103-95	1102-98
20	(*E*)-Myroxide	1141-94	1142-95
21	Camphor	1156-97	1157-98
22	δ-Terpineol	1176-96	1177-94
23	Terpinen-4-ol	1187-95	1187-94
24	α-Terpineol	1201-96	1202-93
25	Estragole	1202-92	1204-93
26	Octyl acetate	1209-96	1210-95
27	Linalyl acetate	1250-90	1253-95
28	Bornyl acetate	1291-97	1289-97
29	δ-Elemene	1341-90	1338-90
30	Triacetin	1345-94	1343-95
31	α-Cubebene	1355-97	1353-96
32	Eugenol	1362-95	1358-96
33	β-Cubebene	1386-91	1384-92
34	β-Elemene	1385-95	1389-93
35	β-Bourbonene	1395-95	1393-96
36	β-Elemene	1398-96	1397-95
37	Methyl-eugenol	1402-95	1403-90
38	(*Z*)-α-Bergamotene	1421-97	1419-95
39	(*E*)-Caryophyllene	1434-96	1431-96
40	(*E*)-α-Bergamotene	1441-96	1440-97
41	α-Guaiene	1444-96	1445-93
42	α-Humulene	1469-96	1467-94
43	(*E*)-Muurola-4(14),5-diene	1475-98	1472-97
44	β-Acoradiene	1479-96	1476-95
45	β-Chamigrene	1487-90	1486-92
46	ψ-Amorphene	1495-91	1492-93
47	α-Bulnesene	1503-91	1500-94
48	Bicyclogermacrene	1509-97	1507-95
49	α-Bulnesene	1514-97	1512-93
50	Zonarene	1526-94	1525-94
51	δ-Cadinene	1529-94	1527-96
52	(*E*)-Calamenene	1533-95	1530-92
53	(*E*)-Nerolidol	1564-98	1563-95
54	Viridiflorol	1586-92	1583-92
55	Caryophyllene oxide	1592-92	1589-91
56	1-*epi*-Cubenol	1630-95	1628-97
57	α-Muurolol	1656-94	1656-91
58	Cadin-4-en-10-ol	1669-95	1667-94
59	α-Bisabolol	1696-94	1694-94

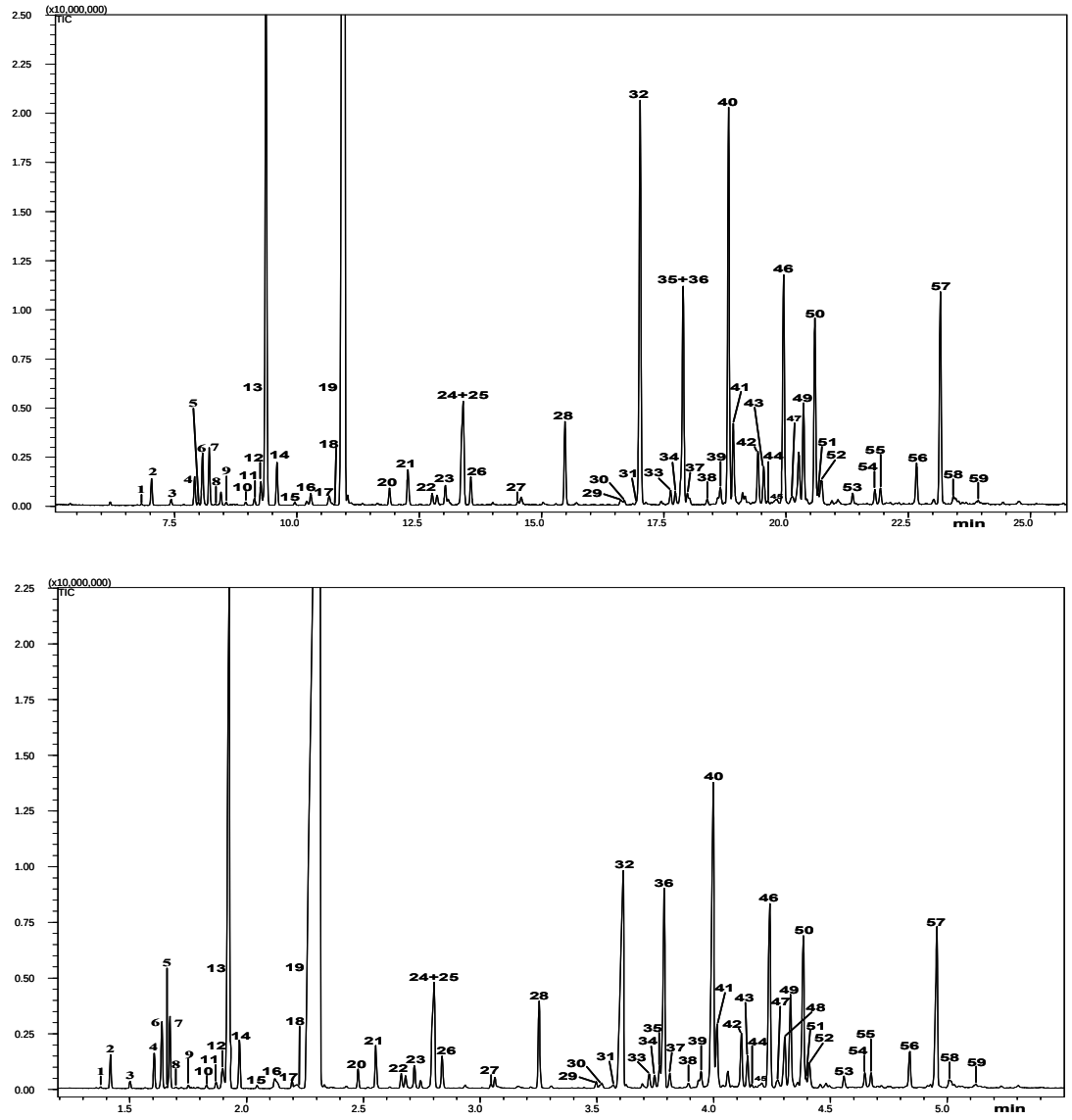

Figure 2: Fast GC-MS chromatogram relative to basil essential oil. Refer to Table 1 for peak identification.

fast" and under 1 min as "ultra fast". In terms of efficiency, and considering ideal analytical conditions, the conventional column should generate about 4,000 *N*/m, while the micro-bore capillary should generate approx. 10,000 *N*/m. Column efficiency should be slightly higher for the 0.25 mm I.D. column: 120,000 *N* vs. 100,000 *N*. In Figure 1 and 2, it can be observed that peak resolution was altogether similar in both applications: 59 peaks were identified in the rapid experiment using the same previously-applied search procedure (Table 1). The suitability of use of the LRI-filtered search was demonstrated by the general good agreement between the conventional and fast GC linear retention index values.

Occasionally, in fast GC applications, variations in compound chromatogram positions can occur. In this respect, a slight difference in selectivity was observed in the rapid experiment, *viz.*, peak 5 eluted between peaks 6 and 7; all other compounds were characterized by the same elution order. The proper re-construction of a GC peak profile requires at least 10 data points per peak. The rapid-scanning mass spectrometer employed in the present research was operated at a 20 Hz data acquisition frequency which was more-than-sufficient for fast GC-MS requirements: peak widths ranged from 900 to 1500 ms across the chromatogram. A possible negative aspect of quadrupole mass scanning is related to peak skewing, *viz.*, the inconsistency of mass spectra across a peak. The direct consequence of this

negative effect is that a single acquired spectrum may present different ion abundances with respect to the averaged mass spectrum present in the MS library. In fast GC-MS applications, rapid analyte bands are introduced into the ion source and the data acquisition rate should be high enough to guarantee that analyte concentration changes that occur therein should not generate substantial relative ion intensity differences across a peak. In this respect, the effects of mass spectral skewing were also evaluated for 5 basil oil volatiles (peaks 2, 21, 28, 42, 56) considering mass ratios for two abundant ions in each acquired spectrum. For example, mass ratios for *m/z* 93 and 77 were considered for α-pinene (peak 2) in thirteen acquired spectra. In general, the effects of peak skewing were found to be negligible, as only slight mass ratio variations were observed across each of the five peaks.

The GC-MS method described in the present contribution has proved to be a rapid and effective screening tool in the determination of volatiles contained in a mediumly-complex essential oil. The option of a fast response for matrices of such wide production is certainly a valuable one. The use of the full scan mode enabled an evaluation on the possible presence (or absence) of co-eluting interferences. Moreover, the analytical benefits of full spectral information are provided for all sample components. As for all monodimensional methods, it can fail when the number of sample compounds greatly exceeds the system peak capacity.

Experimental

Samples and standard compounds: The basil essential oil was of industrial origin. No information on the essential oil extraction process was provided. The essential oil was stored at +4°C and diluted 1:100 in *n*-hexane prior to GC analysis. A C_7-C_{30} linear alkane series was provided by Supelco (Milan, Italy).

Instrumentation: All analyses were carried out on a Shimadzu GCMS QP2010 gas chromatograph-quadrupole mass spectrometer, equipped with an AOC20i autoinjector and split/splitless injector (280°C) (Shimadzu, Milan, Italy).

Conventional GC-MS

Column: SLB-5ms [silphenylene polymer virtually equivalent in polarity to poly(5% diphenyl/95% methyl siloxane)], 30 m x 0.25 mm I.D., 0.25 μm film thickness (Supelco, Bellefonte, PA, USA); initial helium head pressure: 30.1 kPa; constant linear velocity: 30 cm/sec; temperature program: from 50°C to 300°C at 6°C/min; injection volume and mode: 1 μL, split (40:1); MS conditions: acquisition mode: scan; scan speed: 3333 amu/s; mass range: 40-400 m/z.

Fast GC-MS column: SLB-5ms 10 m x 0.1 mm I.D., 0.1 μm film thickness (Supelco); initial helium head pressure: 302 kPa; constant linear velocity: 45 cm/sec; temperature program: from 50°C to 300°C at 27°C/min; injection volume and mode: 1 μL, split (100:1); MS conditions: acquisition mode: scan; scan speed: 10,000 amu/s; mass range: 40-400 m/z.

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