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Technical Report

Off-line LC–GC×GC–MS: A Powerful Approach for Highly Detailed Analysis of Essential Oils

Enhanced resolution and sensitivity in essential oil analysis

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Abstract:

The present contribution is focused on the off-line combination of high performance liquid chromatography and comprehensive two-dimensional gas chromatography–quadrupole mass spectrometry (GC×GC–quadMS), and its application to the detailed qualitative analysis of orange essential oil. Specifically, a silica column was exploited for the separation of the essential oil constituents in two groups, namely hydrocarbon and oxygenated compounds. After, each HPLC-fraction was reduced in volume, and then subjected to cryogenically-modulated GC×GC–quadMS analysis.

Keywords: comprehensive two-dimensional gas chromatography, quadrupole mass spectrometry, LC–GC

1. Introduction

All essential oils are attained through the application of hydro distillation, steam or dry distillation, or a mechanical process at ambient temperature (*e.g.*, cold-pressed Citrus oils). Essential oils are mixtures composed mainly of volatile constituents, and are characterized by high economical importance, and are employed in a series of industrial products, from foods, cosmetics and cigarettes, to pharmaceuticals, insect repellents and perfumes. In general, the volatile fraction of essential oils is composed of mono- and sesquiterpene hydrocarbons, along with oxygenated derivatives, and aliphatic aldehydes, alcohols, and esters. The technique of choice, for the qualitative analysis of the volatile fraction of essential oils is, with no doubt, GC–MS. Identification is usually performed through automatic MS-database matching, with the support of linear retention index (LRI) information.

Apparently, a conventional GC capillary (*e.g.*, 30 m L. × 0.25 mm I.D. × 0.25 µm d_f), combined with a low-resolution single-quad or time-of-flight MS system, is a sufficient tool for the full, or better, near-to-full elucidation of essential oil volatiles.

Also, the use of classical MDGC is a good choice for the high-resolution analysis of target analytes. If the complete untargeted separation of a complex sample (≥200 constituents) is desired, then a comprehensive MDGC (GC×GC) method is the best choice. GC×GC separations are performed on a sequence of two columns, with a transfer system (modulator) located somewhere between them. The function of the modulator, (usually) cryogenic, is to "cut", and re-inject, chromatographic bands from the first onto the second dimension.

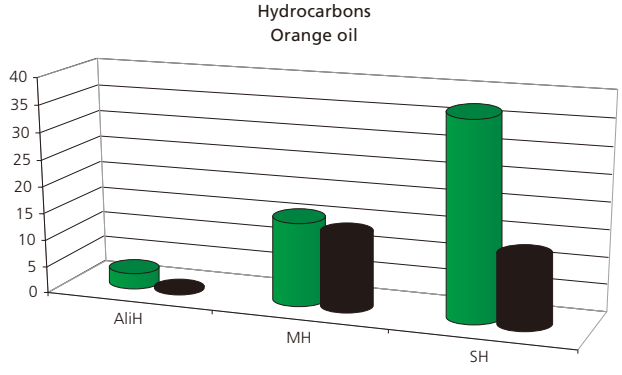

Fig. 1 Graph illustrating the number of orange oil hydrocarbons, identified in the GC×GC–quadMS and GC–quadMS experiments. The first columns refer to the GC×GC–quadMS analyses.

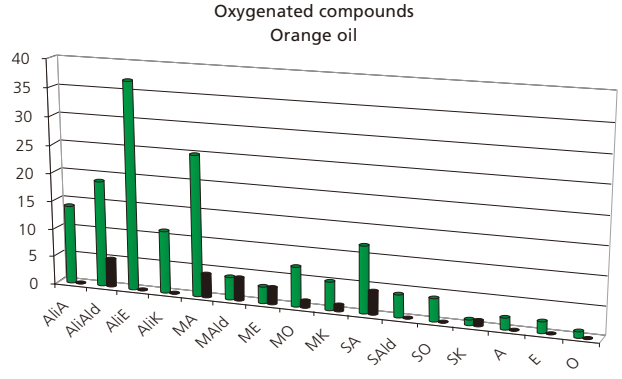

Fig. 2 Graph illustrating the number of orange oil oxygenated compounds, identified in the GC×GC–quadMS and GC–quadMS experiments. The first columns refer to the GC×GC–quadMS analyses.

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The main advantages of GC×GC, over one-dimensional GC, are: (I) enhanced separation power; (II) increased selectivity; (III) higher sensitivity due to band compression; (IV) formation of patterns of homologous compounds. One of the main problems that can be encountered in the analysis of essential oils is the predominance of a single, or a couple of compounds, over all the others, that could overload the modulator. The present work is related to the concept of using LC–GC in the analysis of essential oils. Specifically, the first dimension was exploited to separate the essential oil in two fractions, namely hydrocarbons and oxygenated compounds. The two fractions were collected, reduced in volume, and injected off-line in a GC×GC–quadMS instrument.

2. Experimental

2-1. LC Pre-separation

Analyses were performed by using an LC×GC system (Shimadzu) consisting of:

(1) An LC system, equipped with a CBM-20A communication bus module, two LC-30AD dual-plunger parallel-flow pumps, a DGU-20A degassing unit, an SPD-M20A photodiode array detector, a CTO-20A column oven, and an SIL-30AC autosampler. Data were acquired by the LCsolution software.

(2) An AOC-5000 auto injector equipped with a dedicated dual side-port syringe, employed as a transfer device (not used in the present investigation). LC fractions were collected by disconnecting the transfer line (linking the outlet of LC detector to the syringe), from the syringe side.

2-2. LC Conditions

A 100 mm L. × 3 mm I.D. × 5 µm d_p silica column (SUPELCOSIL LC-Si, Supelco, Milan, Italy) was operated under the following gradient conditions (flow: 0.35 mL/min): 0–4.5 min (100% hexane); from 4.5 to 6.0 min 100% MTBE (until the end of the analysis). Injection volume: 20 µL.

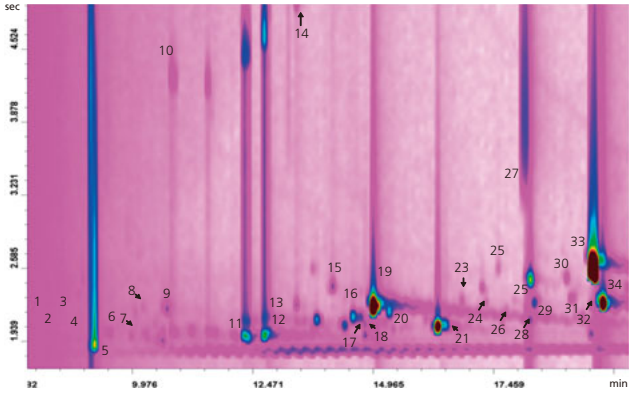

Fig. 3 Chromatogram expansion A, relative to the GC×GC–quadMS analysis of oxygenated compounds in Orange oil.

2-3. LC Fractions

Hydrocarbons were collected from 1.5 to 3 min (525 µL), while the oxygenated compounds were collected from 7.3 to 14 min (2345 µL); Prior to GC×GC–quadMS injection, the fractions were reduced to a volume of 100 µL (under a gentle stream of nitrogen).

2-4. GC×GC–quadMS Analysis

All GC×GC–quadMS applications were carried out on a GC×GC–MS system, consisting of a GC-2010 gas chromatograph, and a GCMS-QP2010 Ultra quadrupole mass spectrometer (Shimadzu). The primary column, an SLB-5ms 30 m L. × 0.25 mm I.D. × 0.25 µm d_f column (Supelco), was connected to an uncoated capillary segment (1.5 m L. × 0.18 mm I.D., used to create a double-loop), by using an SGE SilTite mini-union (SGE, Ringwood, Victoria, Australia). The uncoated capillary was then connected to a segment of Supelcowax-10 (100% polyethylene glycol) 1.0 m L. × 0.10 mm I.D. × 0.10 µm d_f column (Supelco), by using another union (SGE). Modulation was carried out every 5 s, by using a loop-type modulator (under license from Zoex Corporation, Houston, TX, USA). The duration of the hot pulse (400 °C) was 400 ms. GC conditions: temperature program was 50–250 °C at 3 °C/min. Carrier gas, helium, was supplied at an initial pressure of 173.5 kPa (constant linear velocity). Injection temperature: 250 °C.

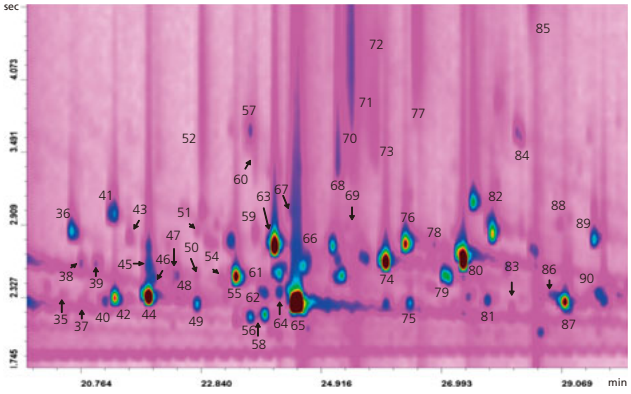

Fig. 4 Chromatogram expansion B, relative to the GC×GC–quadMS analysis of oxygenated compounds in Orange oil.

Injection mode and volume for monoterpene hydrocarbons: split (1:150), 0.4 µL.

Injection mode and volume for sesquiterpene hydrocarbons: split (1:20), 1.0 µL.

Injection mode and volume for oxygenated compounds: split (1:20), 1.0 µL.

2-5. MS Parameters

The sample was analyzed in the full scan mode using a mass range of 40–360 *m/z*; spectra generation frequency: 33 Hz; interface and ion source temperatures were 250 °C and 200 °C, respectively. MS ionization mode: electron ionization. Data were collected by the GCMSsolution software; bidimensional visualization was carried out by using the ChromSquare v. 2.0 software.

2-6. GC–quadMS Analysis

All GC–quadMS applications were carried out on a GCMS-QP2010 system, consisting of a GC-2010 gas chromatograph, and a GCMS-QP2010 Ultra quadrupole mass spectrometer. Column: SLB-5ms 30 m L. × 0.25 mm I.D. × 0.25 µm d_f. GC oven temperature program: 50–250 °C at 3 °C/min. Carrier gas, He, was supplied at an initial pressure of 26.7 kPa (constant linear velocity). Injection temperature: 250 °C. Injection mode and volume: split (1:50), 0.5 µL.

2-7. MS Parameters

The sample was analyzed in the full scan mode using a mass range of 40–360 *m/z*; spectra generation frequency: 2 Hz; interface and ion source temperatures were 250 °C and 200 °C, respectively. MS ionization mode: electron ionization.

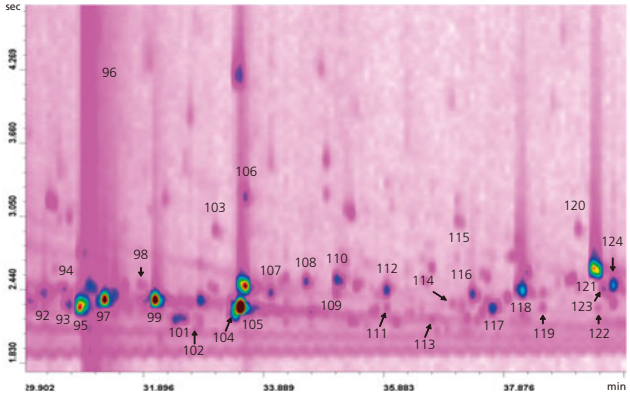

Fig. 5 Chromatogram expansion C, relative to the GC×GC–quadMS analysis of oxygenated compounds in Orange oil.

3. Results and Discussion

3-1. GC–quadMS Analyses

Two cold-pressed samples of orange essential oil (herein defined Orange I/II) were subjected each to three sequential qualitative GC–qMS experiments, using a conventional apolar column. The total number of orange oil analytes identified were 50. Peak assignment was performed through the use of MS database spectral matching and LRI data (comparison between the experimental and MS database values).

The levels of identification herein arbitrarily applied (in all experiments) were three, namely (I) "reliably": MS database similarity equal to, or above 90%, and experimental LRI value within a ±5 LRI unit window, with respect to the database value; (II) "presumably": either MS database similarity ≥90%, or experimental LRI value within a ±5 LRI unit window; a "presumably" identified compound cannot be characterized by a similarity match <80%, or an experimental LRI value outside a ±10 LRI unit range; (III) "tentatively": MS database similarity above 75% and experimental LRI value within a ±15 LRI unit range, compared to the database value. Considering the orange oil compounds, all were reliably identified, apart from nine (presumably identified) for which similarity matches were below 90%.

All 50 analytes have been widely reported in orange oil [1, 2], belonging to the following chemical groups: (14) monoterpene and (13) sesquiterpene hydrocarbons (MH–SH), (8) monoterpene and sesquiterpene alcohols (MA–SA), (9) aliphatic and monoterpene aldehydes (AliAld-MAld), (2) monoterpene and sesquiterpene ketones (MK–SK), (3) monoterpene esters (ME), and a monoterpene oxide (MO). The most abundant compound in orange oil is limonene, with percentages easily excessing 90% [1, 2].

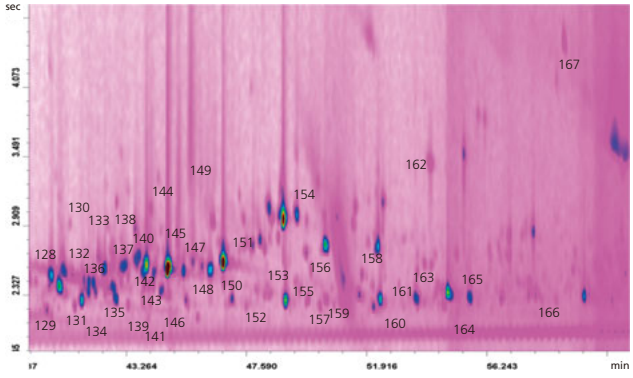

Fig. 6 Chromatogram expansion D, relative to the GC×GC–quadMS analysis of oxygenated compounds in orange oil.

3-2. Orange Oil Hydrocarbons

The data reported in the present sub-section are represented in the graph shown in Fig. 1. Altogether 56 hydrocarbons were given a name in the GC×GC–quadMS experiments (*vs.* 27 in the GC–quadMS applications), and to the best of the authors' knowledge eighteen have never been reported previously in this sample-type. Considering the analysis of MH, a total number of 16 analytes was identified, of which fourteen reliably, and two tentatively (β-phellandrene, (Z)-β-ocimene).

The reason for the low similarity value observed (82% in both cases) can be related to the fact that both volatiles elute on the tail of limonene, and the second-dimension column did not resolve such an interference. Proceeding on to the SH, 37 solutes were identified, of which 29 reliably, seven presumably and one tentatively. Strangely a single SH, namely α-selinene (compound 44), was identified only through GC–quadMS analyses. At the moment, we can only hypothesize co-elution both in the first and second dimension. Specifically,

α-selinene partially overlapped with valencene, a compound present in higher amounts, on the right-hand shoulder of the peak. With regards to AliH, three were assigned, all at the first level of identification. Five hydrocarbons were found in only one of the two samples.

3-3. Orange Oil Oxygenated Compounds

In the case of the oxygenated compounds, the analytical power of the proposed approach is fully demonstrated. The data described in the present sub-section are represented in the graph shown in Fig. 2. Altogether 162 oxygenated compounds were given a name in the GC×GC–quadMS experiments (*vs.* 23 in the GC–quadMS applications); a bidimensional chromatogram relative to Orange I, highlighting the complexity of the oxygenated fraction, is illustrated in four expansions reported in Figs. 3–6.

Eight oxygenated compounds were found in only one of the two samples: ethyl hexanoate, isobutyl isovalerate, *n*-butyl crotonate and geranyl butyrate were identified in Orange I, while nonylol, δ-terpineol, linalyl propionate and biphenyl (a xenobiotic component) were identified only in Orange II. As can be observed, many compounds remained unidentified (they did not reach the minimum identification level), with approx. 300 compounds appearing on the 2D plane. The reasons for the cases of non-assignment can be related to: (I) the low intensity of many signals; (II) the lack of the correct spectrum in the MS database.

Many of the chemical classes found were entirely absent in the GC–quadMS experiments, such as AliA (14 compounds), AliE (37 compounds), AliK (11 compounds), SAld (4 compounds) and SO (4 compounds). The number of analytes identified was much higher for other chemical groups: AliAld (19 *vs.* 5), MA (25 *vs.* 4), MO (7 *vs.* 1), MK (5 *vs.* 1), and SA (12 *vs.* 4). An equal number of solutes, for a specific class, was found only in three cases: MAld (4), ME (3), and SK (1). To the best of the authors' knowledge 91 of the oxygenated compounds identified, have never been reported previously in orange oil. Of such constituents, 25, 47, and 19 analytes were reliably, presumably, and tentatively identified, respectively.

In conclusion, the off-line LC–GC×GC–quadMS approach enabled the identification of a total number of 219 analytes, against the 50 solutes assigned by using GC–quadMS. Considering identification level I, 128 and 41 analytes were identified using GC×GC–quadMS and GC–quadMS, respectively. Among the 128 compounds, to the best of the authors' knowledge 38 have never been reported previously.

4. Conclusion

Finally, the off-line LC–GC×GC–quadMS method herein proposed can be considered as a very powerful tool for the profound analysis of essential oils and, hopefully, has opened a new analytical door. In fact, to the best of the authors' knowledge, no other single study has reported the identification (or detection) of so many orange oil analytes (in particular, the oxygenated compounds). It can be anticipated, with no doubt, that such detailed results can be attained for several types of essential oils. Additionally, the LC + GC×GC–MS combination, in an off- or on-line manner, is potentially of great interest also for other sample-types.

References

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