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The off-line combination of high performance liquid chromatography and comprehensive two-dimensional gas chromatography–mass spectrometry: A powerful approach for highly detailed essential oil analysis

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ABSTRACT

The present contribution is focused on the off-line combination of high performance liquid chromatography (HPLC) and comprehensive two-dimensional gas chromatography–quadrupole mass spectrometry (GC × GC–quadMS), and its application to the detailed qualitative analysis of essential oils. 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. The volatiles were separated on a normal-phase GC × GC column set, and identified through database matching and linear retention index information. The concentrated HPLC fractions gave origin to unexpectably crowded chromatograms, due to two fundamental GC × GC characteristics, namely the enhanced separation power and sensitivity. The results attained were particularly stimulating with regards to the oxygenated compounds, namely those constituents which contribute most to the essential oil aroma, and are of more use for the evaluation of quality and genuineness. Two genuine *Citrus* essential oils, bergamot and sweet orange, were subjected to analysis, and compared to applications carried out with a GC–quadMS instrument.

1. Introduction

Essential oils are mixtures composed mainly of volatile constituents, are characterized by high economical importance, and are employed in a series of industrial products, from foods, cigarettes and cosmetics, to pharmaceuticals, perfumes and insect repellents. 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). Such extraction processes are applied to the plant, or to parts of it [1,2].

In general, the volatile fraction of essential oils is composed of mono- and sesquiterpene hydrocarbons, along with oxygenated derivatives (aldehydes, ketones, alcohols, etc.), 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, gas chromatography combined with mass spectrometry (GC–MS). Identification is usually performed through automatic MS-database matching, with the support of linear retention index (LRI) information [2,3]. Apparently, a conventional GC capillary (*e.g.*, 30 m × 0.25 mm ID × 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.

Heart-cutting or classical multidimensional GC (MDGC) methods enable the transfer of selected chromatography bands, from a first to a second column, of a different selectivity. Classical MDGC methods have been widely employed in essential oil analysis, in particular in the analysis of enantiomers, by using an achiral–chiral combination of stationary phases [4,5].

In general, 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. In GC × GC, a transfer device, in most instances a cryogenic modulator, has the function of cutting, and transferring chromatography bands from the first dimension, onto a second analytical

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column; the transfer process (defined as modulation) is performed in a continuous and sequential manner. 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 [6]. GC × GC has been often employed in the analysis of essential oils, with the first application (spearmint and peppermint) appearing in 2000 [7]; in the same year, Marriott et al. highlighted the highly complex nature of vetiver oil [8].

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. For example, in a comparative study (GC–MS and GC × GC) on nine samples of lavender oil, the authors reported the predominance of oxygenated compounds, such as linalool and linalyl acetate [9]. In a GC × GC–FID investigation on lemon essential oil, the authors reported that the monoterpene hydrocarbon limonene, severely overloaded the modulator [10]. Though such studies do demonstrate the usefulness of GC × GC in the analysis of essential oils, they also highlight the fact that attention must be devoted to the sample amount reaching the column. If one injects more, to detect a higher number of compounds, then column (in both dimensions) and modulator overloading is the price to pay.

A further MD method, with a demonstrated effectiveness for the analysis of essential oils, is on-line LC–GC [11]. HPLC is very useful for the performance of polarity-based separations (*e.g.*, hydrocarbons and oxygenated constituents); after, simplified fractions can be subjected to GC analysis, injecting large sample volumes, enabling the detailed analysis of essential oil profiles.

The present research 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. The off-line method enabled the highly detailed qualitative analysis of two essential oils, chosen as test samples: sweet orange and bergamot oil.

2. Experimental

2.1. Samples and sample preparation

A C_7–C_{30} *n*-alkane series was kindly provided by Sigma–Aldrich (Milan, Italy), for the calculation of LRI values.

Genuine cold-pressed samples of sweet orange (2) and bergamot (2) oil were provided by the industry "Simone Gatto", located in S. Pier Niceto, Sicily, Italy.

Prior to LC analyses the oils were diluted 1:2 (*v*/*v*) in hexane. Prior to direct GC–quadMS analyses the oils were diluted 1:101 in hexane (*v*/*v*).

2.2. LC pre-separation

LC pre-separations were performed on the sweet orange and bergamot oils by using an LC × GC system (Shimadzu, Kyoto, Japan) 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 online degasser, an SPD-M20A photodiode array detector, a CTO-20A column oven, and an SIL-30AC autosampler. Data were acquired by the LC solution software (Shimadzu).
(2) An AOC-5000 auto injector equipped with a dedicated dual side-port syringe, employed as 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.

2.2.1. LC conditions

A 100 mm × 3 mm ID × 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.

2.2.2. LC fractions

Hydrocarbons were collected from 1.5 to 3 min (525 μL); sweet orange oil oxygenated compounds were collected from 7.3 to 14 min (2345 μL); bergamot oil oxygenated compounds were collected from 7.5 to 13 min (1925 μL).

Prior to GC × GC–quadMS injection, the fractions were reduced to a volume of 100 μL (under a gentle stream of nitrogen).

2.3. GC × GC–quadMS analysis

All GC × GC–quadMS applications were carried out on a GC × GC–MS system, consisting of a GC2010 gas chromatograph, and a QP2010 Ultra quadrupole mass spectrometer (Shimadzu).

The primary column, an SLB-5ms 30 m × 0.25 mm ID × 0.25 μm d_f column (Supelco), was connected to an uncoated capillary segment (1.5 m × 0.18 mm ID, 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 × 0.10 mm ID × 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.

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.

2.3.1. MS parameters

The sample was analyzed in the full scan mode using a mas 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 GCMS Solution software (Shimadzu); bidimensional visualization was carried out by using the ChromSquare v.1.6 software (Shimadzu Europe, Duisburg, Germany).

2.4. GC–quadMS analysis

All GC–quadMS applications were carried out on a GCMS-QP2010 system, consisting of a GC2010 gas chromatograph, and a QP2010 Plus quadrupole mass spectrometer (Shimadzu).

Column: SLB-5ms 30 m × 0.25 mm ID × 0.25 μm d_f. GC oven temperature program: 50–250 °C at 3 °C/min. Carrier gas, helium, 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.