



# Fingerprints of flower absolutes using supercritical fluid chromatography hyphenated with high resolution mass spectrometry



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## ABSTRACT

Supercritical fluid chromatography hyphenated with high resolution mass spectrometry (SFC-HRMS) was developed for fingerprint analysis of different flower absolutes commonly used in cosmetics field, especially in perfumes. Supercritical fluid chromatography-atmospheric pressure photoionization-high resolution mass spectrometry (SFC-APPI-HRMS) technique was employed to identify the components of the fingerprint. The samples were separated with a porous graphitic carbon (PGC) Hypercarb™ column (100 mm × 2.1 mm, 3 μm) by gradient elution using supercritical CO<sub>2</sub> and ethanol (0.0–20.0 min (2–30% B), 20.0–25.0 min (30% B), 25.0–26.0 min (30–2% B) and 26.0–30.0 min (2% B)) as mobile phase at a flow rate of 1.5 mL/min. In order to compare the SFC fingerprints between five different flower absolutes: *Jasminum grandiflorum* absolutes, *Jasminum sambac* absolutes, *Narcissus jonquilla* absolutes, *Narcissus poeticus* absolutes, *Lavandula angustifolia* absolutes from different suppliers and batches, the chemometric procedure including principal component analysis (PCA) was applied to classify the samples according to their genus and their species. Consistent results were obtained to show that samples could be successfully discriminated.

## 1. Introduction

Flower absolutes are obtained by double step liquid extraction. Usually first step employs apolar solvents such as petroleum ether or hexane [1]. After filtration and solvent evaporation, a concrete is obtained. The resulted concrete is then dissolved in ethanol and heated. After cooling, fats are precipitated. Finally, the solution is filtered and ethanol is fully removed to get the absolute.

To identify and quantify volatile compounds in flower absolute, gas chromatography hyphenated with mass spectrometry (GC–MS) is the dedicated techniques since several decades [2,3]. Nevertheless, semi- or non-volatile compounds are improperly analyzed by GC–MS due to low volatility and degradation at high temperature injection. Thus new techniques are required need to be developed in order to get the largest chemical fingerprints of the flower absolute including compounds of various polarities. In particular, it is admitted that an absolute approximately contains between 40 and 60% of non-volatile compounds. For example, one publication related to HPLC analysis was describing the chemical fingerprints of *Jasminum grandiflorum* extracts [4] but none on absolutes.

Moreover, Scientific Committee on Consumer Safety (SCCS) emitted an opinion on fragrance allergens in cosmetic products in 2012 [5]. A

new list of 82 allergens was published with 24 natural extracts including *Jasminum grandiflorum* absolute which is highly used in cosmetics field including perfumes. Due to the lack of standardized samples and markers of absolute in complex matrices, these natural extracts were removed from the new list.

Supercritical fluids (SF) have densities and dissolving capacities similar to liquids, but lower viscosities and better diffusion properties similar to gases. Accordingly, SF used as mobile phases in chromatography should act both as substance carriers like the mobile phases in GC and also dissolve these substances like the solvents in liquid chromatography (HPLC). This chromatographic variant is known as supercritical fluid chromatography (SFC) [6]. CO<sub>2</sub> is commonly used as fluid because of several advantageous properties [7] such as low cost, low toxicity, low reactivity.... SFC using CO<sub>2</sub> is very well suited for the analysis of apolar or mid-polar compounds soluble in organic solvents such as constituents existing in flower absolutes [8] as SF CO<sub>2</sub> is bearing the same elution strength and solvation power than heptane.

Furthermore, the SFC can be efficiently hyphenated with mass spectrometry (MS) using various ionization sources among which Electropray ionization (ESI) [9], Atmospheric Pressure Chemical Ionization Source (APCI) [10], Atmospheric Pressure Photoionization (APPI) [11]. For transferring compounds from SFC to the ion source, an

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additional solvent could be introduced using a make-up pump in order to avoid the precipitation of analytes when depressurizing the mobile phase [12]. The SFC appears more and more as a complementary technique to conventional GC and HPLC.

In collaboration with Laboratoires Rosier-Davenne, SFC-HRMS has been implemented for the analysis of flower absolutes in order to access to the largest chemical fingerprints and to identify reliable markers for their detection in complexes matrices, such as cosmetic products.

## 2. Materials and methods

### 2.1. Samples

Seventeen flower absolutes were kindly provided by different suppliers. Six *Jasminum grandiflorum* absolutes from ROBERTET (batch R22483), from QUIMDIS, from ROSIER DAVENNE (batch 287BW402), from LMR (batch INDE 3270), from LMR (batch INDE 00100264), from ALBERT VIEILLE (batch EGYPT 5483), four *Jasminum sambac* absolutes from ROBERTET (batch R22532), from CHARABOT (batch 5494), from ROSIER DAVENNE (batch 344RD302), from LMR (batch 00100340), two *Narcissus jonquilla* absolutes from IFF (batch 5671), from LMR (batch 00100336), two *Narcissus poeticus* absolutes from IFF (batch 5670), from LMR (batch 00140138), and three *Lavandula angustifolia* absolutes from ROBERTET (batch R22321, batch 569600121416), LMR).

Ethanol (EtOH) was Ethanol Absolute for HPLC from Fisher Chemical. CO<sub>2</sub> was procured from AIR LIQUIDE (Purity CO<sub>2</sub> ≥ 99.7%, Impurity H<sub>2</sub>O ≤ 200 ppm v/v). Methanol (MeOH) was LC-grade Methanol from J.T. Baker.

### 2.2. Instrumentation and SFC–QTOF–MS conditions

SFC experiments were performed on an Agilent 1260 Infinity Analytical System consisted of an SFC binary pump, a degasser, an SFC autosampler with 5 µL loop, an Aurora SFC Fusion™ A5 module, a thermostatted column compartment. A quadrupole time-of-flight (Q-TOF) mass spectrometer (Q-TOF LC/MS 6540 series, Agilent Technologies) hyphenated with electrospray ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI) or Atmospheric Pressure Photoionization (APPI). APPI was used for high resolution measurements ( $R > 20,000$  at  $m/z$  922). The final operating source conditions for MS scan in APPI mode were as follows: the fragmentor voltage at 150 V, the capillary at 2000 V for positive mode and 1300 V for negative mode, the skimmer at 45 V, nitrogen was used as the drying (300 °C, 5 L/min) and nebulizing gas (55 psi). Before analysis internal calibration was carried out using ESI tuning mix (Agilent Technologies). The lock masses used for analysis were  $m/z$  121.0508 (purine) and  $m/z$  922.0097 (Hexakis (1H, 1H, 3H-perfluoropropoxy) phosphazene). Instrument control and data collection were carried out using MassHunter Workstation software (B06.01).

### 2.3. Chromatographic conditions

For column screening, eleven different stationary phases were tested: Torus Acquity UPC<sup>2</sup> 1-AA, Torus Acquity UPC<sup>2</sup> 2-PIC, Torus Acquity UPC<sup>2</sup> DEA (all 150 × 2.1 mm, 1.7 µm) and Acquity UPC<sup>2</sup> BEH 2-EP (2-Ethylpyridine) (100 × 2.1 mm, 1.7 µm). The Hypercarb™ (100 × 2.1 mm, 3 µm) was purchased from Thermo Fisher Scientific (Courtaboeuf, France). The Luna NH2 (150 × 2.0 mm, 3 µm) was purchased from Phenomenex (Le Pecq, France). The four last columns (Zorbax RX-Silica (Si) RRHT, Zorbax SB-Cyano (CN) RRHT, both 100 × 2.1 mm, 1.8 µm, ZORBAX Eclipse Plus C18 - Rapid Resolution HT 150 × 2.1 mm, 1.8 µm and Pursuit 3 pentafluorophenyl (PFP) 150 × 2 mm, 3 µm) were purchased from Agilent Technologies (Massy, France).

Gradient mobile phase system consisting of CO<sub>2</sub> (A) and ethanol (B)

was applied at a flow rate of 1.5 mL/min. Run time of 30 min with: 0.0–20.0 min (2–30% B), 20.0–25.0 min (30% B), 25.0–26.0 min (30–2% B) and 26.0–30.0 min (2% B) was used. The column temperature was kept at 30 °C and the back-pressure was fixed at 150 bar. The injection volume was set as 1 µL. The final chromatographic separation was achieved on a Hypercarb™ column 100 mm × 2.1 mm, 3 µm (Thermo Fisher Scientific). For hyphenation with HRMS, LC-grade methanol was used as make-up solvent at a flow rate of 0.2 mL/min.

Samples were prepared at a concentration of 33 mg/mL in ethanol.

### 2.4. Statistical analysis

All absolute samples were chemically profiled under the above mentioned chromatographic and mass spectrometric conditions. The chromatographic fingerprints of 17 batches of absolute samples carried out in triplicate were analyzed automatically using Mass Profiler Professional (MPP\_GENESPRING 12.0) software from Agilent Technologies. Qualitative data ( $m/z$  and retention time) obtained from chromatographic peaks of each injection were analyzed by principal component analysis (PCA) to obtain products positioning.

## 3. Results & discussion

In order to develop the SFC method, all relevant parameters were evaluated based on peak shapes and retention times obtained for selected compounds of jasmine absolute.

### 3.1. Column screening

The selection of suitable stationary phases plays a key role in the development of a SFC method. A column classification of stationary phases depending on a five-dimensional classification based on retention data for 111 solutes was published by West et al. [13]. Nevertheless, this classification cannot be easily employed for retention prediction. Therefore eleven different phases were selected according to different positions in the classification related to different retention mechanisms [13] and tested using isocratic solvent program. Fig. 1 displays the extraction ion chromatogram of jasmone (C<sub>11</sub>H<sub>16</sub>O, [M + H]<sup>+</sup> at  $m/z$  165.1279) for four representative stationary phases. Partial retention of the compound was only observed for PFP and Hypercarb stationary phases contrary to the other 9 stationary phases.

The same behavior was observed for other characteristic compound, such as (Z)-methyl epi-jasmonate (C<sub>13</sub>H<sub>20</sub>O<sub>3</sub>, [M + H]<sup>+</sup> at  $m/z$  225.1485) (Fig. 2).

As shown in Table 1, experiments with Hypercarb™ column gave the best results in terms of retention time, peak shape including symmetry and width, and finally sensitivity with the highest area. Therefore this particular stationary phase was selected for further method development.

### 3.2. Co-solvent choice and gradient mode

A generic gradient SFC system: run time of 30 min with a gradient elution: 0.0–20.0 min (2–30% co-solvent), 20.0–25.0 min (30% co-solvent), 25.0–26.0 min (30–2% co-solvent) and 26.0–30.0 min (2% co-solvent) was used for following experiments in order to obtain satisfactory chromatography and to optimize chromatographic resolution between compounds with a wide range of chemical properties present into flower absolutes.

SFC is more commonly applied to the separation of nonpolar and relatively hydrophobic compounds due to the low polarity of SF CO<sub>2</sub> [14]. A small amount of organic solvent, such as methanol (MeOH), ethanol (EtOH) and acetonitrile (ACN), is often added to the mobile phase to modify the chromatographic retention, selectivity, peak shape [15] and sample solubility. Methanol was not selected due to its environmental and human toxicity [16]. In order to show the influence of

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