FLOWERS ABSOLUTE FINGERPRINT WITH SFC-HRMS NON TARGETED METHOD

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ABSTRACT

Cosmetics and perfumes industries are very late for using supercritical fluids compared with pharmaceutical industry, for example. The protection and the environmental respect became a major subject of concern nowadays. Furthermore with the increasing interest of the professionals for the natural raw materials with the complexity of their compositions, Supercritical Fluid Chromatography (SFC) seems to be imperative to us as green chemistry tool. Hyphenation is very easy with Mass Spectrometry (MS) using various ionization sources (ESI, APPI, and APCI) and various ionization modes (+,-). First step consists in stationary phase screening (Si, C18, Hypercarb…) using gradient mode (methanol, ethanol, isopropanol as modifier and various final percentage of modifier). Hypercarb was selected as the most retentive stationary phase for this type of analysis. Then chromatographic resolution has been improved by checking the effect of column temperature, CO_2 back pressure. Indeed volatile compounds of flower absolutes are already well known and described in the literature using Gas Chromatography (GC) coupled with MS but non-volatile fraction remains incompletely described. Two data processing were realized, one graphically using the EIC (Extracted-Ion Chromatogram) to obtain a chromatographic profile from compounds and a second statistical treatment to have type PCA's (Principal Component Analysis) representation.

INTRODUCTION

In the first stage of the process the plant material is extracted with an organic solvent, usually hexane or toluene [1]. This extract is then subjected to vacuum distillation to remove the solvent which can then be recycled. The aromatic material obtained after this first process is known as a concrete or resinoid, depending if the extract is waxy or resinous. Concretes and resinoids are used in a wide range of industries such as perfumes and cosmetic products. The second stage in this process involves using ethanol (alcohol) to separate the aromatic compounds from pigments and waxes, which are usually present in the extracted material if it is derived from a flower or herb. Many of these waxes have little aromatic value and make the oil difficult to use due to their insolubility, although these waxes are useful in skin care products. After being chilled, the waxes and non-odiferous materials become separated and are removed, the remainder is filtered, and finally the alcohol is recovered using vacuum distillation.

The volatile samples are usually analyzed by GC-MS techniques [2,3]. The aim of this study was to find a complementary analytical tool allowing us to identify volatile and non-volatile compounds at the same time. In that context, supercritical fluid chromatography (SFC)

seemed to be a perfect candidate. Supercritical fluids are obtained by increasing the pressure and temperature of a pure compound beyond the critical points. SF shows physical-chemical properties between a gas and a liquid and can interestingly replace hexane or heptane as eluent for chromatography.

MATERIALS AND METHODS

Samples

Seventeen flower absolutes were kindly provided by different suppliers. Six *Jasminum grandiflorum* absolutes, four *Jasminum sambac* absolutes, two *Narcissus jonquilla*, two *Narcissus poeticus* absolutes and three *Lavandula angustifolia* absolutes. Ethanol (EtOH) was Ethanol Absolute for HPLC from Fisher Chemical. CO_2 was procured from AIR LIQUIDE (Purity $CO_2 \geq 99.7$ %, Impurity $H_2O \leq 200$ ppm v/v). Methanol (MeOH) was LC-grade Methanol from J.T. Baker.

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 Photoionization (APPI) and Atmospheric Pressure Chemical Ionization (APPI) were used for high resolution measurement (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).

Chromatographic conditions

Gradient mobile phase system consisting of CO_2 (A) and ethanol (B) was applied at a flow rate of 1.5 mL/min. Run time of 30 min with a gradient elution: 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.

Statistical analysis

All absolute samples were chemically profiled under the above analytical conditions. The fingerprints of 17 batches of absolute samples carried out in triplicate were investigated using Mass Profiler Professional (MPP_GENESPRING 12.0) software from Agilent Technologies. Classification analysis by principal component analysis (PCA) was achieved using qualification data (*m/z* and retention time) from chromatographic peaks of each injection.

RESULTS

Chromatographic Profiling

In order to generate the more exhaustive chromatographic profiling for each absolute, extraction ion chromatogram under the optimal extraction and chromatographic conditions were generated. More than 1000 *m/z* values were calculated based on common chemical families present in natural extracts (hydrocarbon, alcohol, ketone, amine, amide…) using Excel software with 9 to 24 carbons and 16 to 50 hydrogens; Oxygen or nitrogen were added to the formula to access oxidized or nitrogenized derivate. Finally proton was added or removed to calculate $[M+H]^+$ or $[M-H]^-$ and sodium was added to calculate $[M+Na]^+$. All the generated *m/z* values were introduced in Mass Hunter using EIC function to obtain specific profiles for each plant genus as shown in Fig.1.

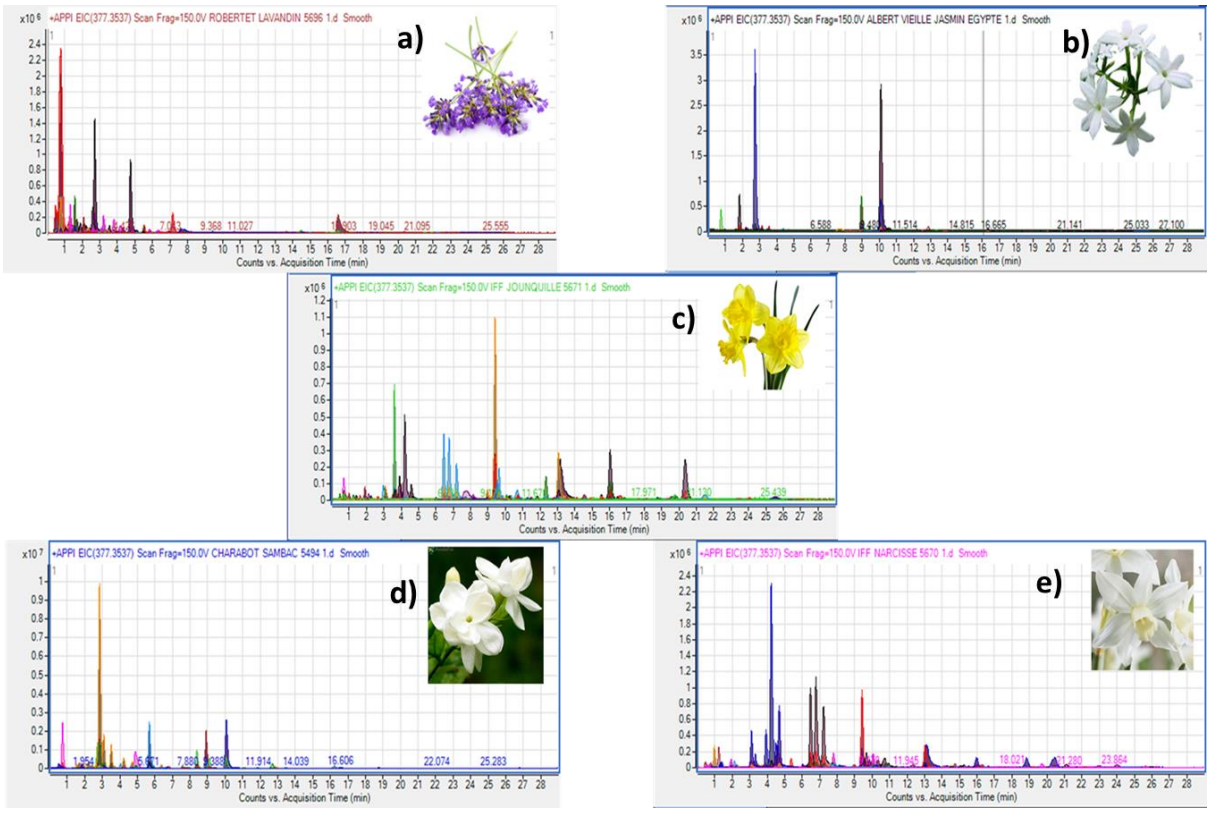

Figure 1. Chromatogram using Extraction Ion Chromatogram (EIC) function for all calculated *m/z* values. Run time of 30 min with a gradient elution: 0.0–20.0 min (2-30% EtOH), 20.0–25.0 min (30% EtOH), 25.0–26.0 min (30-2% EtOH) and 26.0–30.0 min (2% EtOH) was used. The column temperature was kept at 30 °C, a flow rate of 1.5 mL/min. and the back-pressure was fixed at 150 bar : (a) *Lavandula angustifolia* absolute; (b) *Jasminum grandiflorum* absolute; (c) *Narcissus jonquilla* absolute; (d) *Jasminum sambac* absolute; (e) *Narcissus poeticus* absolute

The discrimination between each genus was easily accessible just looking at chromatographic profiles. Moreover several peaks appeared specific to each species inside the same genus and allowed us make the distinction between *Jasminum grandiflorum* and *jasminum sambac* absolutes for example as shown in green on Fig.2.

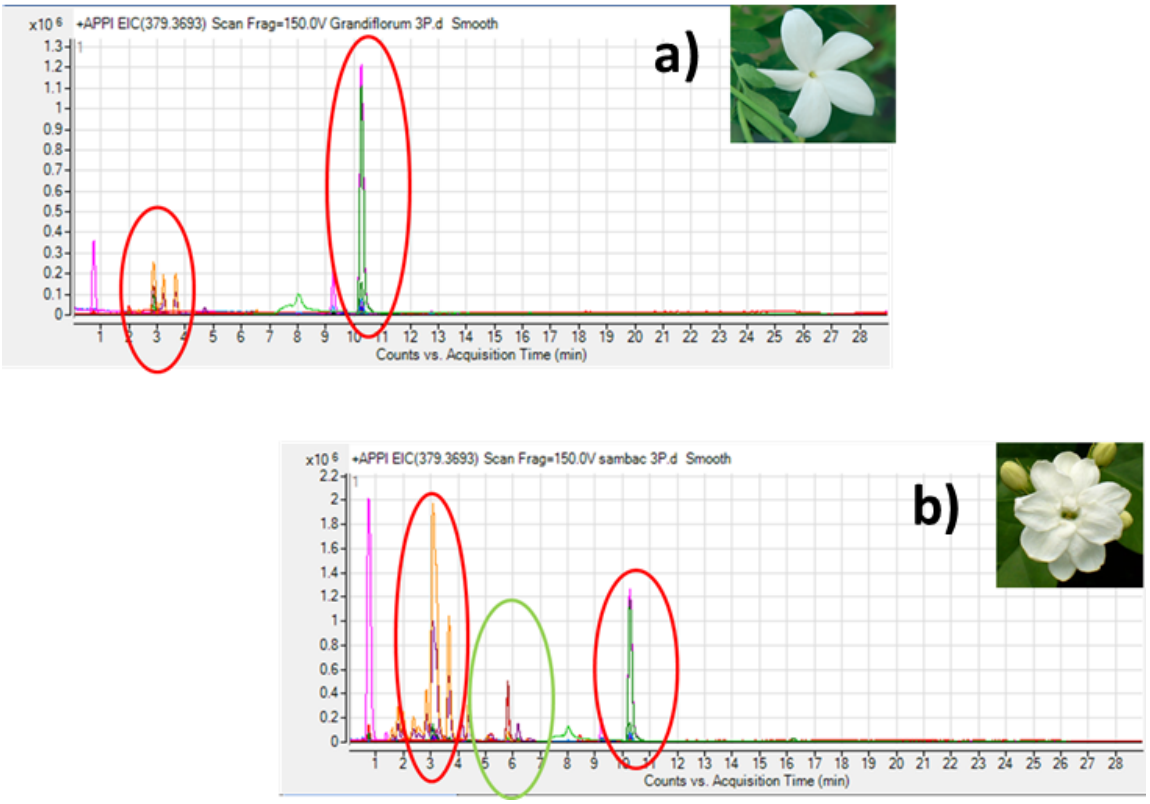

Figure 2. Chromatogram using Extraction Ion Chromatogram (EIC) function for all calculated *m/z* values. Run time of 30 min with a gradient elution: 0.0–20.0 min (2-30% EtOH), 20.0–25.0 min (30% EtOH), 25.0–26.0 min (30-2% EtOH) and 26.0–30.0 min (2% EtOH) was used. The column temperature was kept at 30 °C, a flow rate of 1.5 mL/min. and the back-pressure was fixed at 150 bar : (a) *Jasminum grandiflorum* absolute; (b) *Jasminum sambac* absolute

Statistical analysis

PCA, a commonly used unsupervised chemometric pattern recognition method, can provide classification and clustering of the samples [4]. From EIC chromatograms and using Mass Profiler Pro software from Agilent Technologies, it was possible to perform a multivariate analysis. In our case the PCA was used to discriminate the genus and the species of each plant according their chemical composition. Triplicates of each sample were employed showing that our method is reproducible (Fig. 3).

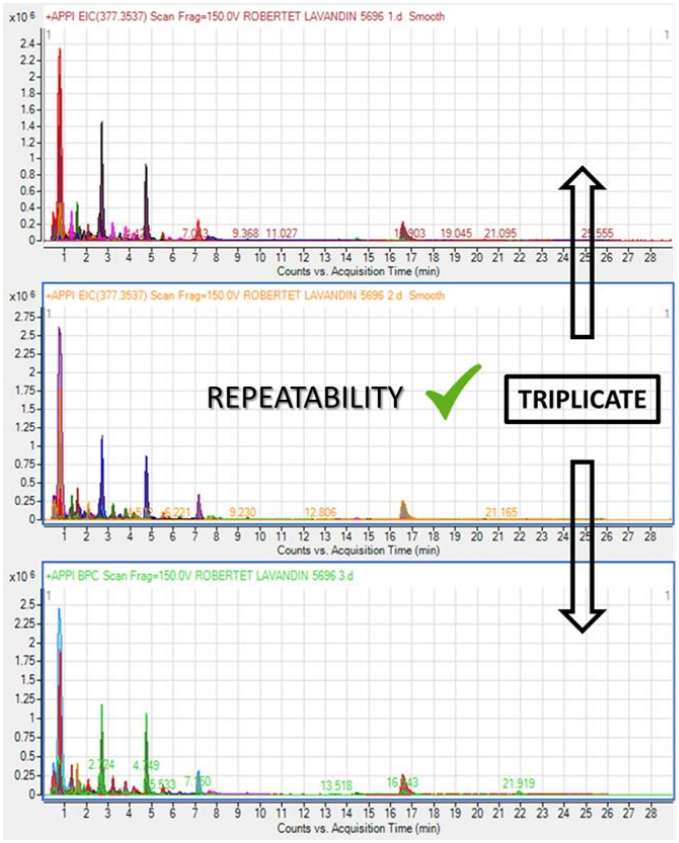

Figure 3. Chromatogram using Extraction Ion Chromatogram (EIC) function for all calculated *m/z* values. Run time of 30 min with a gradient elution: 0.0–20.0 min (2-30% EtOH), 20.0–25.0 min (30% EtOH), 25.0–26.0 min (30-2% EtOH) and 26.0–30.0 min (2% EtOH) was used. The column temperature was kept at 30 °C, a flow rate of 1.5 mL/min. and the back-pressure was fixed at 150 bar : three incetion of *Lavandula angustifolia* absolute

Regarding 3D PCA analysis Fig. 4., it was possible to discriminate species of the same genus for example *jasminum grandiflorum* from *jasminum sambac.*

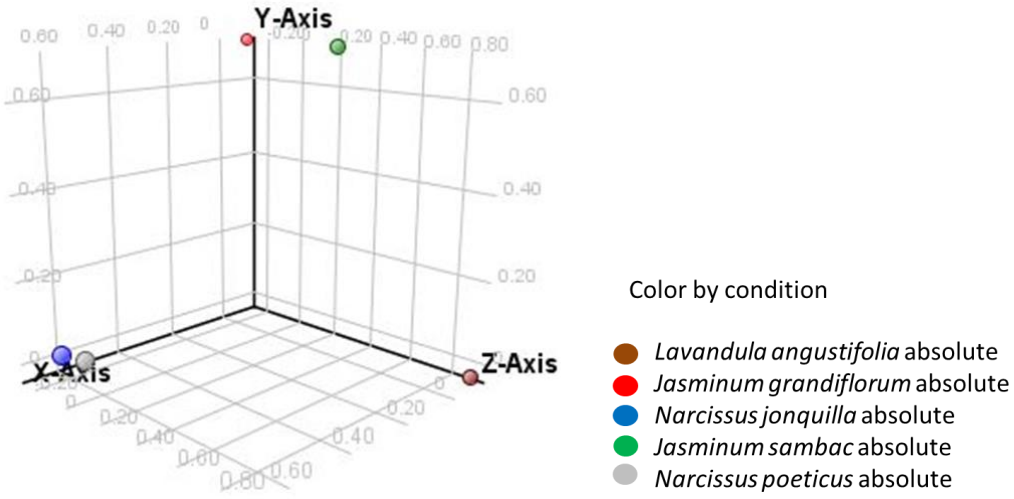

Figure 4. Principal components analysis (PCA) of all injection by genus (5 dots). In grey: *Narcissus poeticus* absolute, blue: *Narcissus jonquilla* absolute, green : *Jasminum sambac* absolute, red : *Jasminum grandiflorum* absolute and brown : *Lavandula angustifolia* absolute. SFC-APPI-HRMS analysis gradient elution: 0.0–20.0 min (2-30% ethanol), 20.0–25.0 min (30% ethanol), 25.0–26.0 min (30-2% ethanol) and 26.0–30.0 min (2% ethanol), Hypercarb^{TM} stationary phase.

Considering different genus together, *jasminum* species were far from *narcissus* species or *lavandula* species, so the discrimination was efficient.

CONCLUSION

A green and rapid method, based on SFC-HRMS was established for analysis and discrimination between different flower absolutes samples and/or flowers from the same families with different genus or species, combining simultaneous chromatographic profile study and statistical confirmation tools.

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