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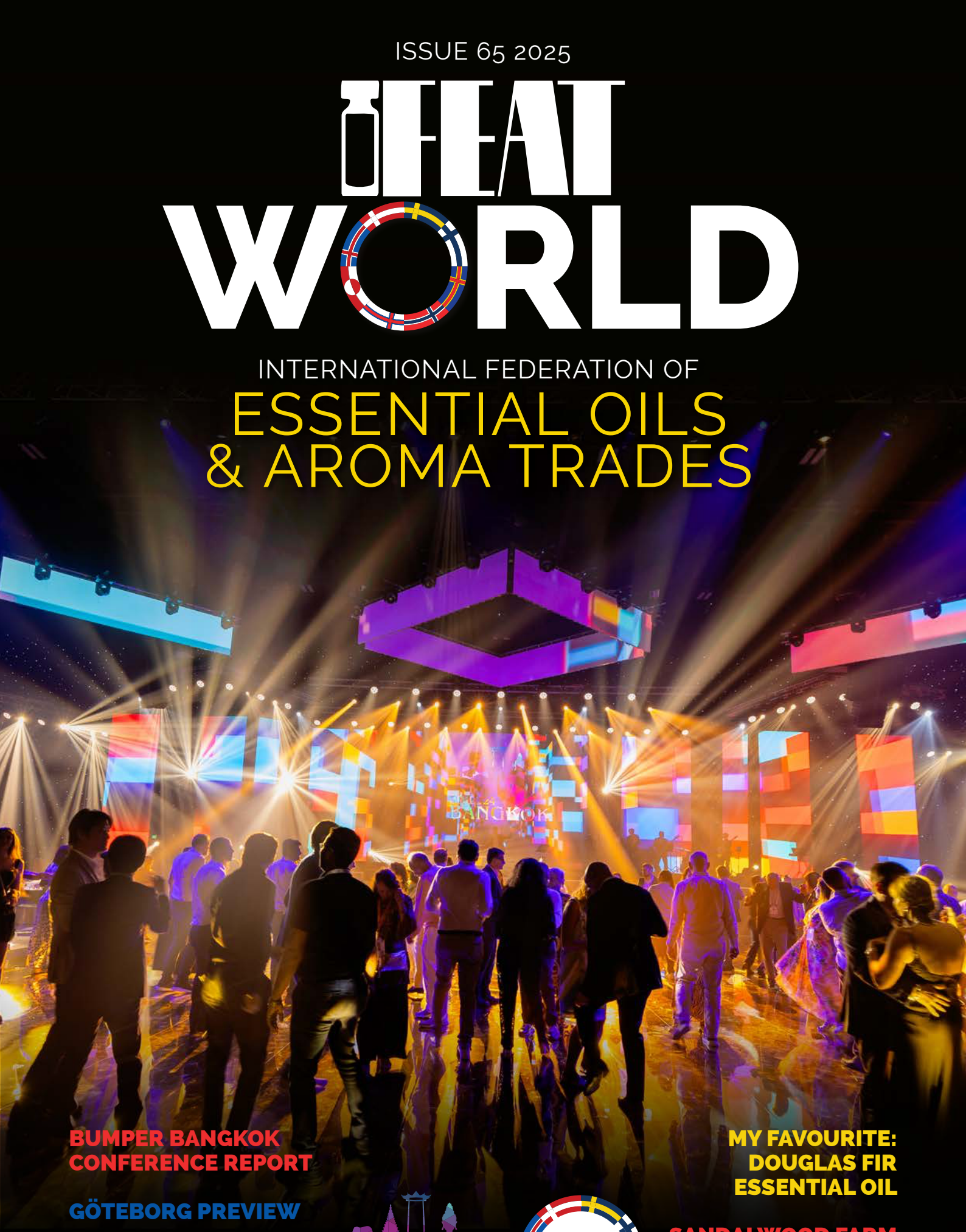

BUMPER BANGKOK CONFERENCE REPORT

GÖTEBORG PREVIEW

GREEN TECHNIQUES FOR ESSENTIAL OIL ANALYSIS

IFEAT 2024 BANGKOK

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Green Techniques and Materials for Essential Oil Analysis

Chromaleont S.r.l. at the Messina Institute of Technology (MeIT), Italy, report on their work to develop alternative and sustainable methods for analysis of volatile and non-volatile essential oil components with reduced waste and costs.

FIGURE 1. Chromaleont's laboratory at the Messina Institute of Technology (MeIT), University of Messina, Italy.

Introduction

Increasing interest and awareness in ensuring greenness and sustainability have been determining the trends in analytical chemistry in recent years. Thus, the implementation of appropriate and environmentally friendly methods for routine analysis is becoming increasingly mandatory in all sectors, accordingly.

The concept of green analytical chemistry (GAC), described for the first time in 1998 by Anastas & Warner[1], focuses on reducing or eliminating the negative impact of analytical methods to protect the environment and ensure operator safety. Recently, 12 fundamental ethics of GAC were proposed by Galuszka et al.[2] as essential guidelines for creating greener analytical methods. The key goals to be achieved in greening analytical methods are: (i) elimination or reduction of the use of chemical substances (solvents, reagents, preservatives, additives for pH adjustment and others); (ii) minimisation of energy consumption; (iii) proper management of analytical waste; and (iv) increased safety for the operator.

An important issue related to the implementation of GAC principles is the need to properly offset and reconcile the greenness of the method with its possible effectiveness. To this end, the greenness criteria should also be complemented by analytical efficiency expressed in terms of validation criteria such as accuracy, precision, sensitivity, etc., as well as practical and economic considerations, e.g. cost of analysis, sample throughput, and overall simplicity of method.

All these considerations have a huge impact on all the areas of scientific research in which analytical chemistry is involved, including the global essential oil and aroma field. The typical and unique composition of essential oils contemplates very complex mixtures that require consistent, reliable, and accurate analyses to verify safety and genuineness and to reveal authenticity fraud.

The research group of Chromaleont S.r.l. has engaged in essential oil analysis over many years and is now targeting its efforts to improve the sustainability of analytical methods based on gas chromatography (GC) and supercritical fluid chromatography (SFC).

FIGURE 2. Analysis of citrus essential oils volatile and non-volatile fractions.

Analysis of the volatile fraction

Emphasis has been recently placed on the development of GC methods using flame ionisation detection (FID) or mass spectrometry (MS) using more sustainable alternatives to helium carrier gases for the analysis of volatile substances. These include monoterpene and sesquiterpene hydrocarbons, oxygenated derivatives, and aliphatic oxygenated compounds, which make up the volatile fraction in citrus essential oils (EOs).

Recent global challenges, helium shortage or its slow supply have led to increasing costs per analysis and pushed the research towards alternative solutions such as hydrogen (H_2) and nitrogen (N_2) gases for GC-MS and GC-FID analyses, respectively. Both carrier gases meet the eco-sustainability principles, given that they can be produced in-lab using dedicated generators (ready-to-use) while minimising environmental, social, and economic impact.

Due to its high diffusivity, H_2 is known to have the highest optimal linear velocity; thus, it is the carrier gas of choice for many applications as it allows for faster analyses with no reduction in resolution[3]. Moreover, under optimum gas velocity conditions, both He and H_2 gases provide similar separation efficiencies measured in terms of height equivalent to one theoretical plate (HETP). As a result, H_2-based GC analysis can be performed in a shorter time, while generating the same sort of chromatographic separation.

Figure 3 shows the separation of volatile constituents of lemon (*Citrus Limon L.*) EO, attained using a 5% phenyl equivalent column (SLB-5ms, L × I.D. 30 m × 0.25 mm, df 0.25 µm, Merck KGaA, Darmstadt, Germany). Consistent GC-MS profile was obtained using H_2 as the carrier gas, although a significant increase in the gas linear velocity (60 cm s^{-1} for H_2 vs. 30 cm s^{-1} for He) was applied under the same temperature program (50 °C to 200 °C at 3 °C min^{-1}). A total of 50 compounds, including monoterpene, sesquiterpene, and oxygenated derivatives were eluted in about 37 min, which is faster than the analysis times typically required when using He as carrier gas (> 45 min)[4].

The method developed was fast, efficient, and highly reproducible, and accuracy was determined by analysis of certified standard mixture of terpene components (Merck KGaA, Darmstadt, Germany).

Identification was carried out using a dedicated mass spectral database (FFNSC version 4.0 GC/MS library, Shimadzu), combining mass spectra similarity (≥ 85%) and linear retention index (LRI) values (±10 units tolerance window). Given the high repeatability of retention times obtained on apolar columns, an excellent agreement was observed within experimental and library LRI values, with Δ≤5 for most components. Noticeably, absolute correspondence between experimental and reference mass spectra was also obtained, meaning that carrier gas switching to H_2 does not necessitate adjusting or modifying the database.

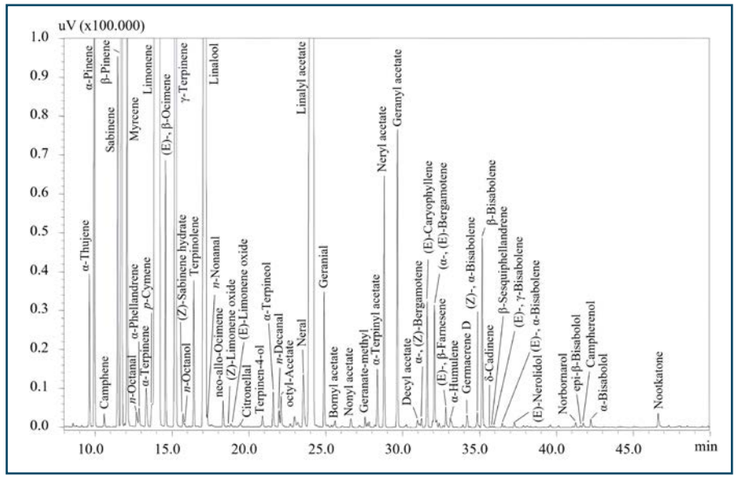

FIGURE 4. GC-FID chromatogram of bergamot essential oil (cold-pressed peels) obtained using N_2 as carrier gas (injection: 0.5 µL, split 1:10).

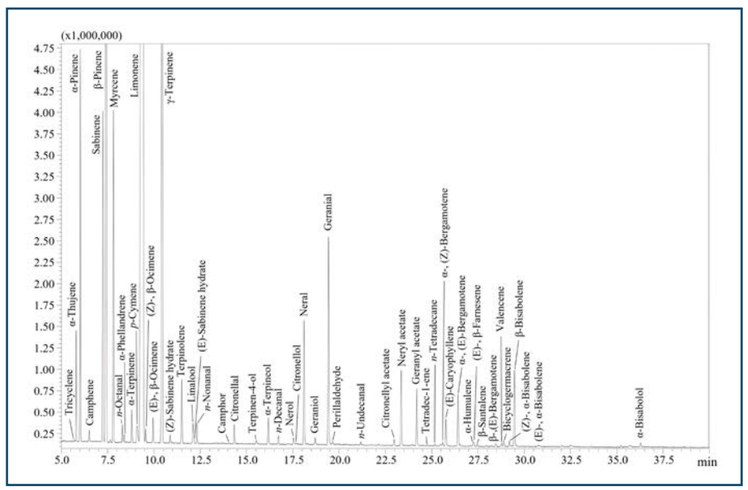

FIGURE 3. GC-MS chromatogram of lemon essential oil (cold-pressed peels) obtained using H_2 as carrier gas (injection volume: 0.5 µL, split 1:10).

Most GC-FID applications employ helium as a carrier gas due to its inertness, thermal stability, and predictable behaviour. Nitrogen is often overlooked as an alternative to helium as it is seen as a slow gas. With a low optimal linear velocity of around 10 cm s^{-1}, analysis times will be increased if the analyst wants to maintain optimal performance[5]. In addition, N_2 has a much steeper Golay curve than He and H_2 gases, thus the separation efficiency decreases significantly as the flow rate increases. Nevertheless, there are key elements that indicate N_2 as a suitable and effective carrier gas for conventional and simple-routine GC-FID analyses, such as being readily available, cost-effective, chemically inert, and safe.

Figure 4 illustrates the GC-FID chromatogram of bergamot (*Citrus bergamia*) EO obtained using N_2 as carrier gas at a constant linear velocity of 20 cm s^{-1}. Although a higher-than-

optimum linear velocity was employed, a satisfactory chromatographic performance was attained. A total of 55 components were separated in about 47 min, which is comparable to the analysis times typically required when using He as carrier gas (> 45 min)[6].

Identification was carried out using a dedicated mass spectral database (FFNSC 4 GC/MS library), combining mass spectra (similarity ≥ 85%) and linear retention index (LRI) values (±10 units tolerance window) calculated using a C_7-C_{30} alkane standard mixture. Noticeably, absolute correspondence between experimental and reference data was obtained, meaning that carrier gas switching to H_2 does not necessitate adjusting or modifying the database.

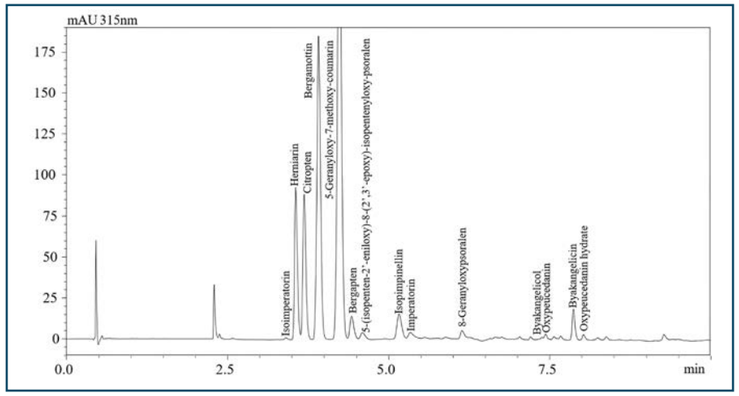

FIGURE 5. SFC-PDA chromatogram (detection at 315 nm) of lime essential oil (cold-pressed peels) obtained using bioethanol as mobile phase co-solvent (injection: 1 µL).

Experimental

Instrumentation: GCMS-QP2020 NX equipped with a split-splitless injector and an AOC-20i autosampler. GCMS solution software ver. 4.41; Nexis GC-2030 high-performance capillary gas chromatograph equipped with a split-splitless injector, an AOC-20i autosampler and an FID detector. LabSolution software ver. 5.93 with Automatic Adjustment Retention Time (AART) algorithm (Shimadzu, Duisburg, Germany).

Column: SLB-5ms, L × I.D. 30 m × 0.25 mm, df 0.25 µm (Merck KGaA, Darmstadt, Germany).

Standard material: C7-C30 Saturated Alkanes (1000 µg mL^{-1} each component); Terpene Mix A certified reference material (2000 µg/mL each component in methanol); Terpene Mix B certified reference material (2000 µg/mL each component in methanol) (Merck KGaA, Darmstadt, Germany).

Column temperature: 50 °C to 200 °C at 3 °C min^{-1}.

Analysis of the non-volatile fraction

Cold-pressed citrus EOs (namely lemon, bergamot, lime, grapefruit, mandarin, sweet and bitter orange) obtained from the peel of citrus fruits are widespread ingredients in food, cosmetic, and pharmaceutical industries. The study of the non-volatile fraction of cold-pressed citrus EOs is a valid tool to exploit their quality and authenticity, and it also represents an important means of product control.

Oxygen heterocyclic compounds (OHCs), i.e. coumarins (Cs), furocoumarins (FCs), and polymethoxyflavones (PMFs) account for 10–20% of the non-volatile fraction of citrus essences and exert several biological effects on human health[7]. Furocoumarins exhibit strong photoactivity in combination with UVA radiation and have long been investigated for their phototoxicity. For these reasons, the maximum amount of FCs in cosmetics and other products is subject to official regulation, though the Opinions in this regard are still numerous and contrasting[8-10]. Moreover, rising demand from industry, along with difficult harvest and processing, have created an avenue for fraudsters to put on the market counterfeit as well as substandard EO products, boosting the importance of accurate assessment of their authenticity.

Traditionally, reversed-phase liquid chromatography (RP-HPLC) has been the gold-standard technique for OHC analysis, delivering optimal results in terms of accuracy, repeatability, limit of detection, and quantification[7]. On the other hand, supercritical fluid chromatography (SFC) is re-emerging as a greener alternative technique, since the reduced organic solvent consumption and waste have favourable fallout in terms of toxicity, costs, and environmental impact[11]. In addition, the low mobile phase viscosity and the higher diffusion coefficients allow for faster or more efficient separations to be attained, with shorter re-equilibration times[12].

Figure 5 shows the separation of OHC constituents of lime EO (*Citrus aurantifolia*) attained on a 15 cm × 2.1 mm column based on fused-core particle technology, which allowed to obtain high speed and high efficiencies at moderate backpressures (Ascentis® Express HILIC, Merck KGaA, Darmstadt, Germany). The mobile phase consisted of CO_2 (solvent A) and bioethanol (solvent B), at 1.0 mL min^{-1}.

For method optimisation, a standard mixture containing 16 furocoumarin components was employed (Merck KGaA, Darmstadt, Germany). A total of 37 compounds were separated in less than 10 min, under a linear gradient going to 10% B in 6 min and detected by photodiode-array (PDA).

Afterwards, the method's greenness was assessed by the Analytical GREEnness calculator, a comprehensive, flexible, and straightforward assessment approach that provides an easily interpretable and informative result (open-source and downloadable from **https://mostwiedzy.pl/AGREE**). The assessment criteria are taken from the 12 principles of green analytical chemistry (SIGNIFICANCE) and are transformed into a unified 0–1 scale.

The resulting pictogram in Figure 6 gave a final score of 0.8, calculated based on the SIGNIFICANCE principles and the performance of the analytical procedure in each criterion. Significant advantages of the SFC-PDA method over RP-HPLC-PDA[13] consisted in the volume of analytical waste generated per analysis (point 7, viz. 10 mL vs 20 mL), the use of reagents obtained from renewable sources (point 10, viz. bioethanol from corn), the use of toxic reagents (point 11, viz. ethanol vs methanol and tetrahydrofuran).

FIGURE 6. AGREE graphics obtained for RP-HPLC-PDA (left) and SFC-PDA (right) methods for the analysis of oxygen heterocyclic compounds in citrus EOs.

Experimental

Instrumentation: Nexera-UC system with PDA detector (Shimadzu, Duisburg, Germany).

Ascentis® Express HILIC (2.7 µm, 90 Å) HPLC Column, 2.7 µm particle size, L × I.D. 15 cm × 2.1 mm (Merck KGaA, Darmstadt, Germany).

Standard material: Furocoumarin Mix (16 components in acetonitrile, certified reference material) (Merck KGaA, Darmstadt, Germany).

Solvents: Ethanol (gradient grade for liquid chromatography) (Merck KGaA, Darmstadt, Germany).

Gradient: 0 min, 2% B; 1 min, 3% B; 4 min, 3% B; 6 min, 10% B at 1.0 mL min-1.

Conclusions

One of the most important challenges to the future of chemical analysis is to reach a compromise between the increasing quality of the results and the improving environmental friendliness.

For the analysis of citrus essential oils, the use of H_2 and N_2 as carrier gases for terpene and terpenoid profiling was demonstrated to be a valid alternative for GC-MS and GC-FID analyses. A neat gain in terms of costs per analysis was obtained, confirming that both gases represent a valid, sustainable, and environmentally friendly alternative to helium. As the price of helium is set to continue to rise for the foreseeable future, the results here show that nitrogen and hydrogen can be used as convenient alternative carrier gases without loss of performance.

On the other hand, switching the separation technique for the non-volatile EO fraction from RP-HPLC to SFC afforded comparable performance while meeting the demands for greener analytical methods, as demonstrated by quantitative greenness evaluation metrics.

MORE INFO AT:
https://www.chromaleont.it/essential-oils-analysis

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