Nat. Volatiles & Essent. Oils, 2015; 2(2): 1-15

NVEO
Natural Volatiles & Essential Oils

REVIEW

Advanced preparative techniques for the collection of pure components from essential oils

Danilo Sciarrone[1], Sebastiano Pantò[1], Francesco Cacciola[2], Rosaria Costa[1], Paola Dugo[1, 3, 4] and Luigi Mondello[1 ,3, 4*]

[1] Scienze del Farmaco e Prodotti per la Salute Department, University of Messina, Viale Annunziata, 98168 Messina, Italy
[2] Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute Department, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy
[3] University Campus Bio-Medico of Rome, Via Álvaro del Portillo 21, 00128 Roma, Italy
[4] Chromaleont s.r.l. c/o Scienze del Farmaco e Prodotti per la Salute Department, University of Messina, viale Annunziata, 98168 Messina, Italy

*Corresponding author. Email: lmondello@unime.it

Abstract

Preparative gas chromatography (prep-GC), as opposed to analytical capillary GC, is an analytical technique that allows the separation and isolation of natural products, specifically volatile pure components from complex matrices. Over the years, different approaches were used for this purpose, using both mono- and multidimensional systems coupled with different types of collection systems. In this paper, some of the most relevant results obtained in the isolation of components from essential oils by prep-GC, are reviewed. Furthermore, the main limitations of prep-GC arising from its daily use and the possible solutions for overcoming drawbacks, are discussed.

Keywords: Preparative gas chromatography (prep-GC), isolation, multidimensional preparative gas chromatography, Deans switch

Introduction

When Mikhail Tswett, in 1906, gave birth to chromatography, he didn't mean to develop an analytical methodology, instead he wanted to isolate specific pigments from plant material. Similarly, all the early applications of gas chromatography were directed toward preparative purposes, in other words, collection of chromatographed components. During the years, liquid chromatography demonstrated higher suitability to preparative approaches rather than GC; the latter, on the other hand, developed successfully in the field of separation science. Nevertheless, prep-GC wasn't left apart and many applications of the last decades related to various topics demonstrate the vitality of a technique, which is still relevant and irreplaceable. Various are the fields of application of prep-GC: isolation of compounds for flavour and fragrance manufacturing (Sciarrone et al., 2012; Ledauphin et al., 2004; Marriott, Eyres, & Dufour, 2009; Mason, Johnson, & Hamming, 1966); environmental chemistry (Meinert et al., 2010; Sansone, Popp, & Rust, 1997); pharmaceuticals (Codina, Ryan, Joyce, & Richards, 2010); biology and toxicology (Needham et al., 1982; Smith, Reynolds, Downie, Patel, & Rennie, 1998; Waskell, 1979); radio-carbon analysis (Currie, Eglinton, Benner, & Pearson, 1997; Ball, Xu, McNichol, & Aluwihare, 2012). As already stated, prep-GC is a convenient tool for isolation of pure components or specific sample fractions. This can have more than one practical implication: production of raw material, through collection of the isolated component and exploitation in chemical formulations; sub-trace level analysis, due to concentration of the target analyte by means of repetitive injections; iii) enhancement of the identification process, through elimination of impurities and

further elucidation with different (i.e. spectroscopic) means. Whatever the purpose of the prep-GC analysis, it is a matter of fact that this technique suffers from limits, which make its exploitation quite challenging. Such difficulties derive from the optimization of the analytical parameters with peculiar reference to the instrumental equipment. It is not an easy task to obtain an acceptable amount of a pure component in one single analysis. In order to isolate single components, sometimes present at low level in complex matrices, it is necessary to inject sample volumes which are definitely higher than those conventionally used in analytical gas chromatography. This would cause a column overloading in case of capillary columns. It is for this reason that wide-bore columns (0.5-0.7 mm I.D.) have been traditionally utilized in prep-GC, thanks to their higher sample capacity with respect to micro-bore GC columns (0.1-0.25 mm I.D.). Unfortunately, the use of columns with wide diameter can lead to severe loss in efficiency and resolution, causing coelutions and collection of impure fractions. In order to face the issue of sample loading, attention has been paid also to the injection systems: automated samplers with microprocessors, cool injection systems, gas booster sample injection devices, multi-port valves and expanded vaporizers are some examples of customized approaches (Hua-Li, Feng-Qing, Wei-Hua, & Zhi-Ning, 2013). The collection systems are critical as well to the success of a prep-GC process. Also at this level, prep-GC systems show some weakness: inefficient collections are often due to condensation phenomena occurring in the connection device between the detector outlet and the collector. Many trapping devices have been designed to improve prep-GC performance: cold-traps are basically used, with either solid CO_2 or liquid nitrogen, depending upon the boiling point of analytes to be trapped. In addition, thermal gradient traps, electrostatic precipitation, Volmar collectors and potassium bromide traps have been used (Hua-Li et al., 2013). Many of the drawbacks so far described have been successfully overcome through the application of multidimensional gas chromatography (MDGC) to prep-GC (Eyres, Urban, Morrison, Dufour, & Marriott, 2008; Eyres, Urban, Morrison, & Marriott, 2008; Rühle et al., 2009; Schomburg, Kötter, Stoffels, & Reissig, 1984; Rijks, & Rijkes, 1990; Sciarrone et al., 2012; Sciarrone et al., 2013; Sciarrone, Pantò, Tranchida, Dugo, & Mondello, 2014). Great part of prep-MDGC applications focused on the isolation and collection of pure components from essential oils. In particular, the present work gives an overview on a multidimensional GC system developed by Sciarrone et al. in 2012, and originally applied to the isolation of the sesquiterpenoid carotol from carrot seed oil. The system consisted of three hyphenated gas chromatographs, equipped with three Deans-switches and a CO_2 cool trap. Successively, the same prep-MDGC system was applied to the isolation and identification of a misidentified compound from wampee essential oil. NMR, FTIR, and MS were also used for sure identification of the collected compound (Sciarrone et al., 2013). A further improvement of the prep-MDGC system was reported by Sciarrone et al., 2014, through the addition of a liquid chromatographic fourth dimension, which served as a preseparation step for the successful collection of two sesquiterpenoids from vetiver essential oil. Considerations about the prep-MDGC system's troubleshooting, advantages and disadvantages, and positive outcomes from the applications, are here given, along with a description of the instrumental apparatus.

Materials and Methods

Samples, Chemicals and Sample Preparation

Carrot seed essential oil was provided by Supelco/Sigma-Aldrich (Bellefonte, PA, USA). *Clausena lansium* Skeels essential oil was obtained by hydrodistillation of 150 g of fresh leaves, collected in January 2012 from plants grown in Messina (Italy). Mandarin essential oil was provided by a local industry (Capua, Reggio Calabria, Italy). Vetiver essential oil (Haitian) was provided by Firmenich SA (Genève, Switzerland).

n-Hexane (GC grade), *n*-hexane (LC grade), *n*-octane, *n*-nonane, *n*-decane, *n*-tetradecane (C14), *n*-hexadecane (C16), deuterated chloroform and *tert*-butyl methyl ether, were supplied by Supelco/Sigma-Aldrich. Spirogalbanone was provided by L'Oreal (Aulnay-sous-Bois, France).

2-Hexenal, 4-hexe*n*-1-ol, hexanethiol, camphene, methyl octanoate, γ-terpinene, linalool, camphor, γ-heptalactone, 2-decanone, decanal, linalyl acetate, δ-nonalactone, caryophyllene, caryophyllene oxide and α-bisabolol, were all supplied by Supelco/Sigma-Aldrich. Five different concentrations of standard solutions were prepared (10, 50, 100, 250 and 500 μg/mL), each added with 100 μg/mL of *n*-nonane, as internal standard.

Two stock solutions, containing respectively 300 mg of C14 and C16 (Sol. A) and 300 mg of caryophyllene oxide and spirogalbanone (Sol. B), were prepared each in 5 mL of hexane. Vetiver essential oil was diluted 1:5 (*v/v*) in hexane prior to the injection.

Apparatus and Operational Conditions

Isolation of carotol

A Shimadzu (Kyoto, Japan) *GC-2010* gas chromatograph was used in all GC-FID analyses. The column was an SLB-5ms 30 m × 0.25 mm i.d. × 0.25 μm d_f (Supelco, Milan, Italy); temperature program, 50−280°C at 5.0°C/min; split/splitless injector (250°C); injection mode, split, 1:100 ratio; injection volume, 1.0 μL; inlet pressure, 99.5 kPa, carrier gas, He; constant gas linear velocity, 30.0 cm/s. Detector (300 °C) gases: H_2, 40 mL/min; air, 400 mL/min; makeup (N_2), 40 mL/min; sampling rate, 10 Hz. Data were handled through the use of *GCSolution* software (Shimadzu).

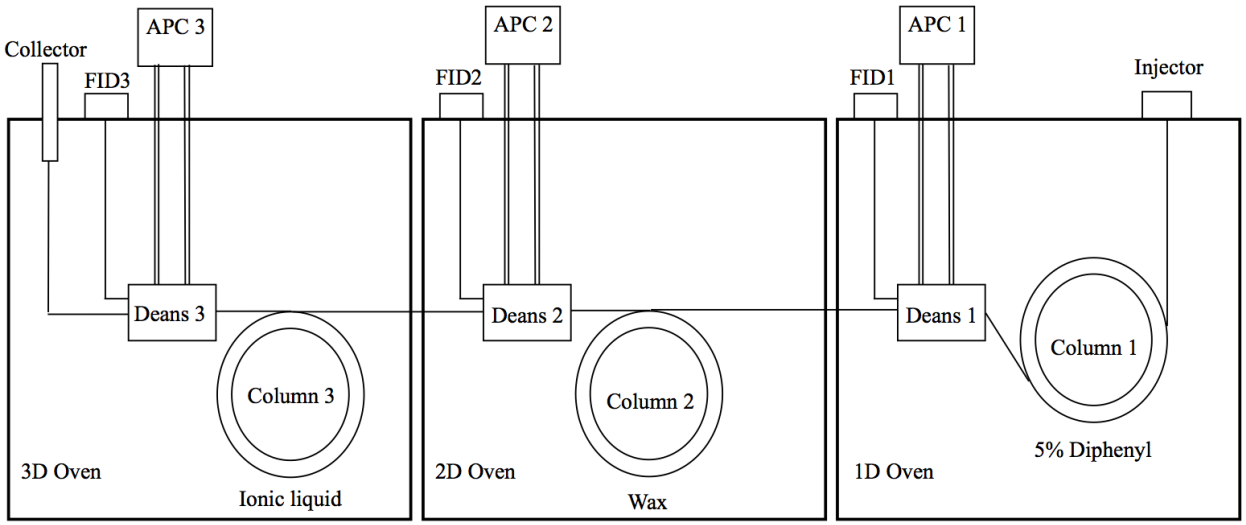
Samples were analyzed by GC/MS (EI) on a *GCMS-QP2010 Plus* system (Shimadzu). GC conditions, in terms of column type, injection mode, and temperature were the same as for the GC-FID analysis, apart from the inlet pressure (30.6 kPa) and temperature program: 50−280°C at 3.0°C/min. MS scan conditions: ion source temperature, 200°C; interface temperature, 250°C; EI energy, 70 eV; scan range, 40−400 m/z. Data handling was by *GCMSsolution* software (Shimadzu); identification was supported by the use of a GC/MS mass spectral database equipped with linear retention indices (*FFNSC 2.0*, Shimadzu Europe).

For one-dimensional prep-GC analyses, Shimadzu GC2010 equipped with an Equity-5 column, 30 m × 0.53 mm i.d. × 5 μm d_f (Supelco), combined with a 1 m uncoated column segment of the same i.d. (at the head of the analytical column), was used; temperature program: 150−280°C (14 min) at 5°C/min; split/splitless injector (260°C); injection volume, 0.5, 1.0, 2.0, 3.0 μL; injection mode, splitless (1 min), then split 1:10; carrier gas, He; inlet pressure, 80 kPa, in the constant pressure mode (initial linear velocity, 22 cm/s). The Deans switch system pressure was 65 kPa (constant). The uncoated columns connected to the FID and collection system were both 1.0 m × 0.18 mm i.d. Detector (280°C) gases H_2, 40 mL/min; air, 400 mL/min; sampling rate, 5 Hz.

Multidimensional prep-GC analyses were carried out by means of a prep-MDGC instrument (see Figure 1), consisting of three GC systems (defined as GC1 GC2, and GC3), equipped with three Deans switch transfer devices, namely, DS1 (between first and second column), DS2 (between the second and third column), and DS3 (between the third column and the collection system). The MDGC switching elements, located inside the ovens, were connected to three advanced pressure control systems (APC1, 2, and 3), which supplied carrier gas (He) at constant pressure. GC1 was equipped with a split/splitless injector and a flame ionization detector (FID1). Column 1 was the same as for one-dimensional prep-GC. The operational conditions were as follows: constant inlet pressure 140 kPa (initial linear velocity 17 cm/s); splitless mode (280°C) for 1 min, then 1:10 (gas carrier He); injected volume 3.0 μL. Temperature program: 150−280°C (hold 14 min) at 5°C/min. The

FID1 (300°C) was connected via a 0.4 m × 0.25 mm i.d. stainless steel uncoated column to the DS1. The APC1 constant pressure was 125 kPa. GC2 was equipped with a split/splitless injector (not used) and a flame ionization detector (FID2). The transfer line between GC1 and GC2 was maintained at 240°C. The secondary column was a Supelcowax-10, 30 m × 0.53 mm i.d. × 2 µm d_f (Supelco) directly connected to the DS1 through the heated transfer line between GC1 and GC2. Temperature program: 150°C (hold 20 min); 150−240°C at 5°C/min. DS2 was used at the end of the secondary column to direct the effluent either to the FID2 (280°C) or to the third column. APC2 constant pressure was 95 kPa (initial gas linear velocity, 35 cm/s). The branch of uncoated column to connect FID2 to the transfer system was 0.5 m × 0.25 mm i.d. GC3 was equipped with a split/splitless injector (not used) and a flame ionization detector (FID3). The transfer line between GC2 and GC3 was maintained at 240°C. The third column was a custom-made SLB-IL59 30 m × 0.53 mm i.d. × 0.85 µm d_f column (Supelco) directly connected to the DS2 through the heated transfer line between GC2 and GC3. Temperature program: 150°C (hold 40 min); 150−240°C at 5°C/min. DS3 was used at the end of the third column to direct the effluent either to FID3 (300°C) or to the collection system (300°C). Connections were made via two 0.5 m × 0.32 mm i.d. stainless steel uncoated columns. APC3 constant pressure was 35 kPa (initial gas linear velocity, 70 cm/s). Detector gases and sampling rate conditions were the same as applied in the one-dimensional experiment. Data were collected by the *MDGCsolution* software (Shimadzu).

Figure 1. Triple Deans-switch multidimensional prep-GC system scheme. *Reprinted with permission from D. Sciarrone, S. Pantò, C. Ragonese, P.Q. Tranchida, P. Dugo, and L. Mondello,* Increasing the isolated quantities and purities of volatile compounds by using triple Deans-switch multidimensional preparative gas chromatographic system with an apolar-wax-ionic liquid stationary-phase combination*, Analytical Chemistry, 84: 7092-7098. Copyright 2012 American Chemical Society.*

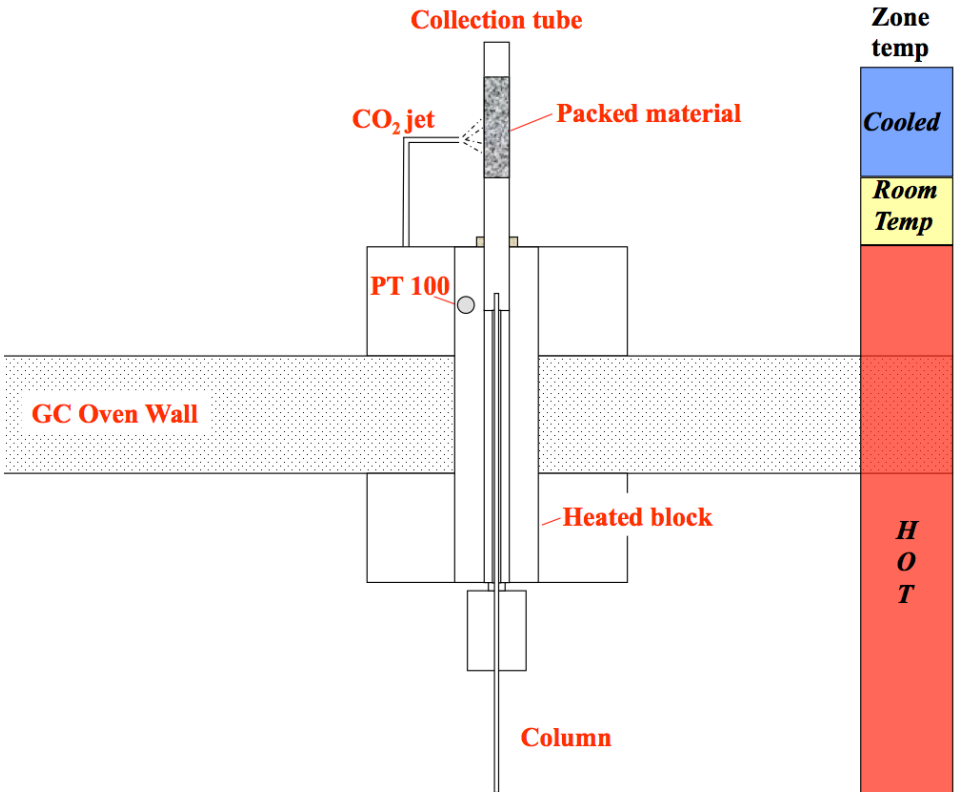


Collection system

The simple and low-cost lab- constructed collection system was formed of a heated aluminum block (11 cm height × 3 cm wide × 1.5 cm deep), equipped with a PT-100 temperature sensor, and was located through the GC oven roof (Figure 2). The block was characterized by a 0.5 cm diameter hole, which enabled the introduction of a GC liner and of the collection glass tube. A 90 mm × 0.75 mm i.d. deactivated liner was located inside the lower part of the block, while a 80 mm × 3.5 mm i.d. glass tube was positioned above the liner. About 25% of the glass tube was located inside the heated zone, while the remaining part was situated

outside the block at room temperature, with or without packing material (depending on the specific needs). The liner and the collection tube were sealed and held in position by using two nuts of appropriate dimensions; the lower was used to connect the column by using a ferrule for FID detection, while the second upper one contained a holed rubber septum. The last 5 mm of the uncoated column protruded inside the glass tube.

Figure 2. Scheme of the collection device installed in the prep-MDGC system. *Reprinted with permission from D. Sciarrone, S. Pantò, C. Ragonese, P.Q. Tranchida, P. Dugo, and L. Mondello,* Increasing the isolated quantities and purities of volatile compounds by using triple Deans-switch multidimensional preparative gas chromatographic system with an apolar-wax-ionic liquid stationary-phase combination*, Analytical Chemistry, 84: 7092-7098. Copyright 2012 American Chemical Society.*



As an option, a CO_2 cold jet stream, through a 1/8 in. tube, was directed to the empty or packed (10% SP-2100 on 80/100 Supelcoport) collection vessel to improve the collection of highly volatile components. The cold jet was switched on 1 min before and turned off 0.5 min after collection. The upper part of the glass tube was cooled down to −60°C when using CO_2, a temperature measured by means of an external PT-100 sensor. After analyte isolation, the collection vessel was removed immediately and flushed four times (in a 1.5 mL vial) with 250 µL of a n-hexane solution, spiked with 100 µg/mL of n-nonane, used as internal standard. The solution containing both the internal standard and the collected volatile was then analyzed by GC/MS and by GC-FID for qualitative and quantitative purposes, respectively. Finally, recovery was extrapolated from a calibration curve, accounting for dilution related to flushing (1 mL of internal standard solution).

For recovery measurement, five-point calibration curves were constructed (n = 3), namely, 10, 50, 100, 250, and 500 µg/mL, adding n-nonane as internal standard at a fixed concentration of 100 µg mL^{-1} (regression coefficients > 0.9985). The concentration levels were selected in order to cover a wide concentration range for the isolated components, diluted in ≈1 mL of hexane when flushed from the collection tube. Several representative compounds for a variety of chemical groups were calibrated. To measure the volume of the

collected solution, the vial was weighed before and after the process (after drying the vial for 30 min at room temperature).

Wampee essential oil

GC analyses

GC-FID conditions were the same as above reported, with the exception of: oven program rate: 3.0°C/min; injector temperature and volume: 280°C and 0.2 μL; FID hydrogen flow rate: 50.0 mL/min.

GC-MS conditions were the same as reported in section isolation of carotol.

The prep-MDGC system was the same as described above. The collection device was the same as for carotol experiment, as well as the recovery measurement procedure (Sciarrone et al., 2012). In this last case, caryophyllene was chosen as representative compound of sesquiterpenes.

NMR analysis

^{1}H, $^{13}C\{^{1}H\}$ NMR spectra were run on a Varian 500 spectrometer (operated at 499.74 and 125.73 Mz, respectively, for the mentioned nuclei), controlled by a *VNMRJ* (*2.2MI version*) software package. In order to attain a profound insight on the molecular structure, beyond the standard ^{1}H and $^{13}C\{^{1}H\}$-APT 1D spectra, 2D ^{1}H–^{1}H homo-nuclear TOCSY and NOESY, along with ^{1}H–^{13}C heteronuclear g-HSQCAD and g-HMBC experiments, were achieved. These data were all processed and analyzed by the *Mestrenova* software package (Mestrelab Research) and the reported chemical shifts at 273 K, are referenced to the solvent (^{1}H, δ = 7.26 ppm; ^{13}C, δ = 77.16, triplet). The low temperature is necessary to prevent decomposition occurring in chloroform. For elucidation of NMR parameters, readers are referred to Sciarrone et al., 2013.

GC-FTIR analysis

A Shimadzu *GC2010* gas chromatograph, equipped with an AOC- 20i series autoinjector, was coupled to a *Bruker Vortex 80 FT-IR* system (Bruker Italia, Milan, Italy), by means of a heated transfer line (250°C). An MCT detector was used, cooled by liquid nitrogen, and operated at a scan velocity of 320 kHz and 4 cm^{-1} resolution. The software *Opus 7.0*, with 3D and chromatography options, was used to acquire the FT-IR data (Bruker). Two 0.5 m × 0.25 mm ID uncoated columns were used to: connect the analytical column to a capillary GC-FTIR interface with a solid gold light-pipe (250°C), and from the latter to an FID (280°C), again inside the GC oven. Two heated transfer lines passed through the GC side wall. The GC method and column were the same as used in the GC-FID analysis, apart from the injection volume, which was 1 μL in the splitless mode.

Vetiver essential oil

LC analyses

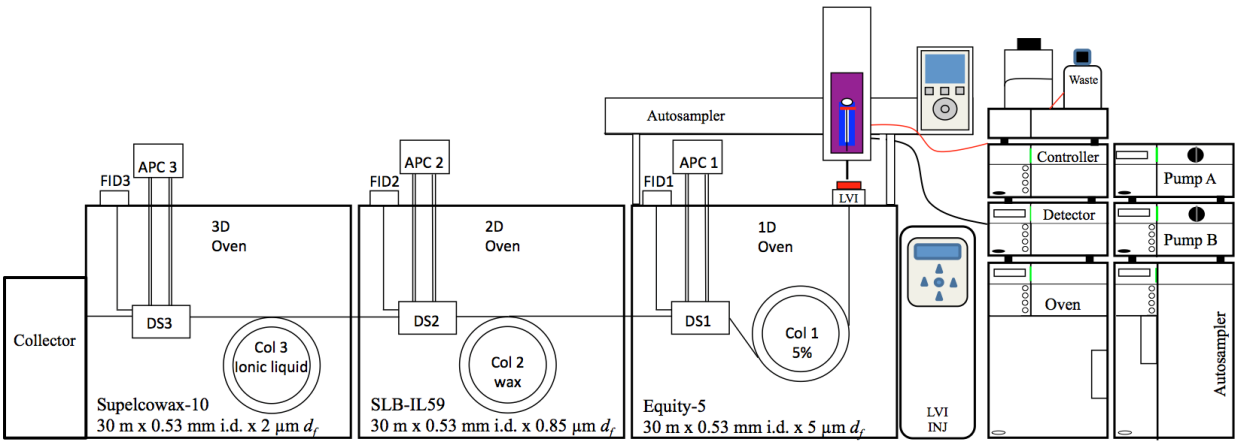
The LC preseparation of vetiver oil was performed by using an LC system (Shimadzu, Kyoto, Japan), equipped with a Model CBM-20A communication bus module, two Model LC-20AD dual-plunger parallel-flow pumps, a Model DGU-20A online degasser, a Model SPD- 20A UV detector, a Model CTO-20A column oven, and a Model SIL-20AC autosampler. Five microliters and 50 μL of a vetiver oil solution were injected into a 250 mm × 4.6 mm ID × 5 μm dp SupelcoSil LC-Si column (Supelco/Sigma-Aldrich, Milan, Italy), operated under the following gradient conditions: flow rate was 1 mL/min (reduced to 0.35 mL/min during the transfer step): from 0 to 6 min, the LC effluent was directed to waste; from 6 min to 10 min (1400 μL−100% hexane); and from 14 min to 18 min (1400 μL−100% MTBE) the LC effluent containing the hydrocarbon and oxygenated fractions, respectively, were directed to the first GC. Data were acquired by the *LCsolution* software (Shimadzu).

The LC-GC transfer device consisted of a dual-side-port 25-µL syringe (CTC Analytics AG, Zwingen, Switzerland), controlled by means of a Shimadzu Model AOC- 5000 autosampler. Chromatography band transfer was achieved, in the stop-flow mode. The lower part of the syringe was connected, via two transfer lines, to the LC detector exit and to waste. A Teflon plug was located at the end of the syringe plunger; the latter was characterized by a lower OD, with respect to the barrel ID, thus enabling mobile phase flow inside the syringe. In the waste mode, the plug was located below both lines and the effluent was directed to waste. In the cut position, the plug was positioned between the upper and lower line, and the effluent flowed to the first GC. For more details on the syringe interface, the reader is referred to Sciarrone et al., 2014.

Prep-GC analyses

The configuration of the prep-MDGC system was the same as for previous experiments, with some modifications (see figure 3). GC1 was equipped with an Optic 3 (ATAS GL International, Eindhoven, The Netherlands) large volume injector (LVI) and a flame ionization detector (FID1). The LVI temperature program and flow rate were optimized for each chemical class. LVI conditions for the hydrocarbon fraction: during the transfer step (4 min) and for the first 0.75 min of the analysis time, the split mode was used (total flow rate was 230 mL/min, at 45°C), followed by a 1 min splitless period; afterward, the split mode was applied (126 mL/min), heating the injector to 300°C at a rate of 15°C/s. LVI conditions for the oxygenated fraction: during the transfer step (4 min) and for the first 0.50 min of the analysis time, the split mode was used (total flow rate was 332 mL/min at 35 °C), followed by a 1 min splitless period; afterward, the split mode was applied (126 mL/min), heating the injector to 300°C at a rate of 15°C/s. Column 1 was an Equity-5, 30 m × 0.53 mm ID × 5.0 µm d_f, preceded by a 1 m segment of uncoated precolumn, with the same ID. Helium was the carrier gas, having the following pressure conditions: 80 kPa for 0.75 and 0.50 min, for hydrocarbon and oxygenated compounds, respectively; then to 140 kPa at a rate of 400 kPa/min, with the pressure remaining constant afterward (initial gas linear velocity ≈ 22 cm/s). Oven temperature program: 45°C for 1.75 min (35°C for 1.50 min in the case of the oxygenated compounds), to 300°C at a rate of 15°C/min. APC1 pressure: 27.5 kPa for 0.75 min (0.50 min in the case of the oxygenated compounds); then to 125 kPa at a rate of 400 kPa/min. Transfer line between GC1 and GC2 was maintained at 280 °C. The FID1 (330°C) was connected via a 0.25 m × 0.18 mm ID stainless steel uncoated column to the TD1.

Figure 3. Scheme of the LC-GC-GC-GC preparative system. *Reprinted with permission from D. Sciarrone, S. Pantò, P.Q. Tranchida, P. Dugo, and L. Mondello,* Rapid isolation of high solute amounts using an online four-dimensional preparative system: normal phase-liquid chromatography coupled to methyl siloxane-ionic liquid-wax phase gas chromatography, *Analytical Chemistry, 86: 4295-4301. Copyright 2014 American Chemical Society.*



The second column was a custom-made ionic liquid one (SLB-IL59) of the following dimensions: 30 m × 0.53 mm ID × 0.85 µm d_f (Supelco). Oven temperature program for the hydrocarbon fraction: from 50 °C to 100 °C (20.21 min), at a rate of 5 °C/min, then to 240 °C, at a rate of 5 °C/min. Oven temperature program for the oxygenated fraction: from 50 °C to 150°C (23min), at a rate of 5°C/min,then to 240°C,at a rate of 5 °C/min. APC2 pressure: 7.8 kPa for 0.75 min (0.50 min in the case of the oxygenated compounds) to 95 kPa at 400 kPa/min. The transfer line between GC2 and GC3 was maintained at 240°C. The uncoated column used to connect the TD2 to the FID2 was of the same dimensions as that reported for the FID1.

The third column was a Supelcowax-10, 30 m × 0.53 mm ID × 2.0 µm d_f. Oven temperature program for the hydrocarbon fraction: from 50°C to 110°C (16.96 min) at a rate of 2°C/min, and then to 240°C at a rate of 5°C/min. Oven temperature program for the oxygenated fraction: from 50°C to 150°C (11.65 min), at a rate of 2°C/min, then to 240°C, at a rate of 5°C/min. APC3 was maintained off during the LC-GC transfer step, then to 35 kPa at 400 kPa/min. The connection of the FID3 with the TD3 was realized by means of a 1 m × 0.32 mm ID uncoated column. Detector gases for FID1, FID2, and FID3 (330°C) were as follows: H_2, 50.0 mL/min; air, 400 mL/min; makeup (N_2), 40.0 mL/min (sampling rate = 5 Hz). Data were collected by the *MDGCsolution* software (Shimadzu). The collection system was a dedicated preparative *Brechbühler Prep9000 station* (Brechbühler AG, Switzerland), connected to the Deans switch system (TD3) by means of a flexible heated transfer line (280°C) containing a 1.4 m branch of uncoated column. The system was equipped with a ten-position carousel with Carbotrap C (40 mesh) adsorption tubes. The collection system was linked to a vacuum circuit, isolated by a solenoid valve. During the collection process, the valve was opened, with the effects of the vacuum enabling a more rapid and effective analyte accumulation; additionally, the condensation of high boiling components, at the conjunction point between the transfer-line end and the adsorption tube, is avoided. During normal operation (no collection), the solenoid valve was closed. A scheme of the LC-GC-GC-GC system is reported in Figure 3.

GC analyses

A Shimadzu GC2010 equipped with an SLB-5ms, 30 m × 0.25 mm ID × 0.25 µm d_f, was used. Temperature program: from 100°C to 300°C, at a rate of 5.0°C/min. Injection (280 °C) volume was 0.2 µL, in split mode (1:100 ratio); carrier gas, He (constant gas linear velocity: 30.0 cm/s). A GCMS-QP2010 Ultra was used for GC-MS analyses, same conditions as for GC-FID analyses, except: inlet pressure, that was 30.6 kPa; ion source temperature, 200 °C; interface temperature, 250 °C; mass scan range, 40−400 m/z.

For calculation of LC-GC transfer recovery, normalized peak areas were used. The reference peak area was determined through the splitless injection of 1.0 µL of the standard solutions (A and B) into GC1. The amounts of components introduced onto the column were ∼60 µg for each solution. Effectively collected amounts were measured by means of two five-point calibration curves by choosing caryophyllene as representative of sesquiterpenes and spirogalbanone for oxygenated sesquiterpenes.

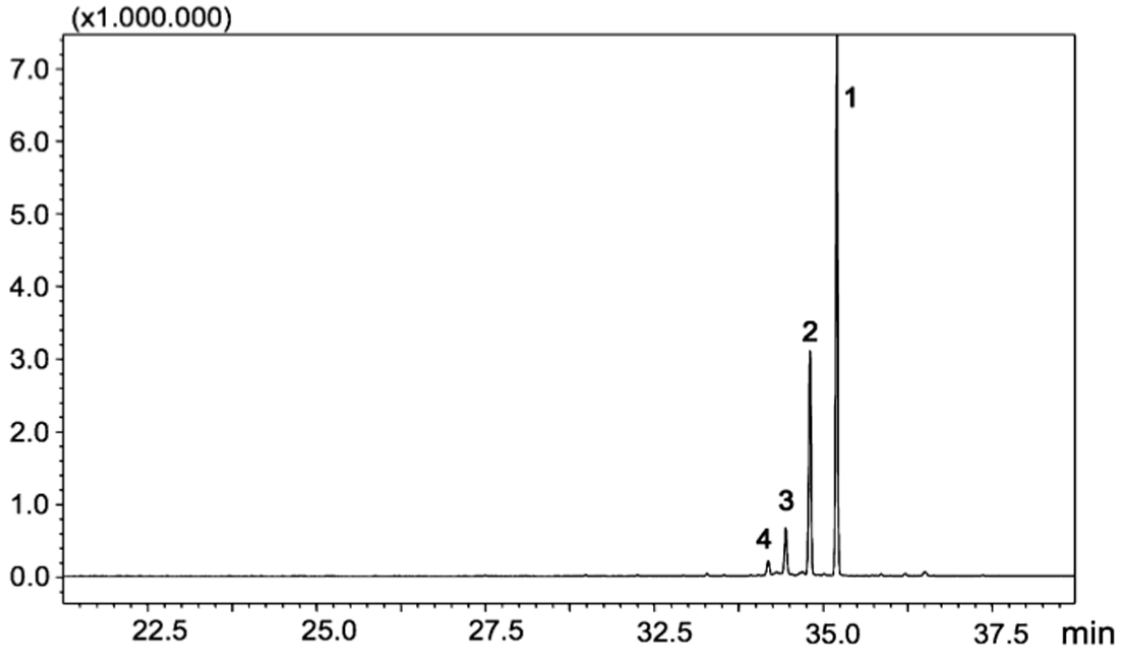
Results and Discussion

Isolation of Carotol

The first application of the prep-MDGC system here described consisted of the isolation of a characteristic compound from carrot seed oil, namely carotol (Sciarrone et al., 2012). Preliminarily, during method development, different collection conditions were evaluated. It was demonstrated that recovery values were dependent on boiling point of analytes, use of packed or empty collection tubes, use of cold CO2. These parameters were tested on a series of standard compounds, which are natural constituents of carrot seed

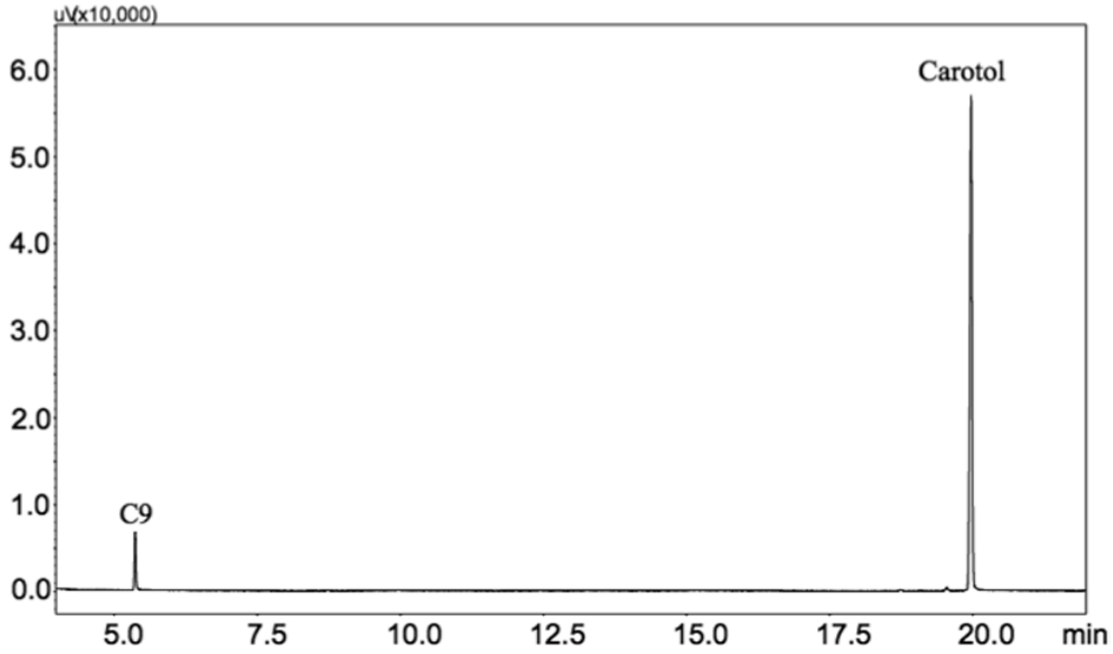
oil. Successively, a one-dimensional prep-GC analysis was carried out, with the purpose of collecting the compound carotol, which was present at a 30% level in the essential oil. Briefly, in the single dimension prep-GC configuration, a megabore 5% diphenyl column was used, with a low injection volume (1 μL of essential oil) and a fast oven temperature program. After collection, the fraction was separately injected in a GC-MS system, the resulting chromatogram being shown in Figure 4.

Figure 4. GC-MS chromatogram of the fraction collected by means of one-dimensional prep-GC. Peak identification: (1) carotol; (2) caryophyllene oxide; (3) and (4) unknowns. *Reprinted with permission from D. Sciarrone, S. Pantò, C. Ragonese, P.Q. Tranchida, P. Dugo, and L. Mondello,* Increasing the isolated quantities and purities of volatile compounds by using triple Deans-switch multidimensional preparative gas chromatographic system with an apolar-wax-ionic liquid stationary-phase combination*, Analytical Chemistry, 84: 7092-7098. Copyright 2012 American Chemical Society.*



As can be seen, not only carotol was present in the isolated fraction: other three peaks were detected, namely, caryophyllene oxide and other two unknown substances. The three peaks accounted for about 25% of the total fraction. Such a situation was definitely improved through the injection of 3 μL of essential oil into the multidimensional system. As depicted in figure 1, the prep-MDGC configuration involved the use of three columns, having different selectivities and dimensions. Specifically, a wide-bore column was used in the first GC oven, for its sample capacity, so to accommodate a higher amount of essential oil. Parameters such as gas flow rates and pressures were tuned in order to optimize resolution, purity and collection yield of carotol. The results obtained from this analytical procedure are shown in figure 5, where a GC-FID chromatogram relative to the collected fraction is depicted. In this case, the fraction isolated contained 99.6% pure carotol, identified by means of GC-MS with a spectral similarity of 99%. From recovery measurement, considering the density of the essential oil and volume injection, it came out that for the collection of about 2 mg of pure carotol, at least three prep-MDGC cycles were necessary. Indeed, 2.22 mg of pure carotol were successfully collected.

Figure 5. GC-FID chromatogram of carotol, collected by means of the prep-MDGC instrument. *Reprinted with permission from D. Sciarrone, S. Pantò, C. Ragonese, P.Q. Tranchida, P. Dugo, and L. Mondello,* Increasing the isolated quantities and purities of volatile compounds by using triple Deans-switch multidimensional preparative gas chromatographic system with an apolar-wax-ionic liquid stationary-phase combination*, Analytical Chemistry, 84: 7092-7098. Copyright 2012 American Chemical Society.*



Structure Elucidation of a Wampee Essential Oil Compound

Once developed, the prep-MDGC system here described has been applied to the isolation and identification, by means of spectroscopic techniques, of a compound from *Clausena lansium* essential oil. Preliminarily, the hydrodistilled essential oil was investigated by means of conventional GC-FID and GC-MS. One of the last eluting peaks, in the sesquiterpene region of the chromatogram, couldn't be assigned, although accounting for about 10% of the whole sample. The GC-MS library matching procedure returned as best candidate, with a quite low similarity score (84%), the compound α-sinensal, a sesquiterpene previously found in the leaves of this plant. In order to clarify and possibly confirm what reported in literature, the compound in question was isolated by exploiting the prep-MDGC system. The relative chromatograms are shown in figure 6. The use of three different dimensions of selectivity was essential for purification of the analyte to be isolated. As can be seen in figure 6, the efficiency of the first dimension separation was low, for obvious reasons of sample overloading. Also, in consideration of the complexity of the matrix and the region of the chromatogram to be cut, which was crowded of peaks, this first fraction presented numerous coelutions. The two successive steps of separation, through the use of stationary phases of different selectivity, allowed to obtain a final collection of a 99.1% pure unknown compound. About 2 mg of analyte were recovered after a 13 hours period. Successively, the isolated fraction was subjected to further investigation by means of NMR, GC-FTIR and GC-MS techniques. All the three spectroscopic methodologies confirmed that the unknown analyte under investigation was (2E, 6E)-2-methyl-6-(4-methylcyclohex-3-enylidene)hept-2-enal.The correspondent NMR spectrum is shown in figure 7. A further GC-MS analysis of the newly identified component and of pure α-sinensal highlighted differences upon the ion fragment abundances.

Figure 6. Prep-MDGC stand-by (upper trace) and cut (lower trace) chromatograms, derived from the analysis of *Clausena lansium* Skeels essential oil, relative to the first (upper chromatogram), second (middle chromatogram) and third GC dimensions. "Reprinted from Analytica Chimica Acta, 785, D. Sciarrone, S. Pantò, A. Rotondo, L. Tedone, P.Q. Tranchida, P. Dugo, & L. Mondello, Rapid collection and identification of a novel component from *Clausena lansium* Skeels leaves by means of three-dimensional preparative gas chromatography and nuclear magnetic resonance/infrared/mass spectrometric analysis, pages 119-125, Copyright (2013), with permission from Elsevier".

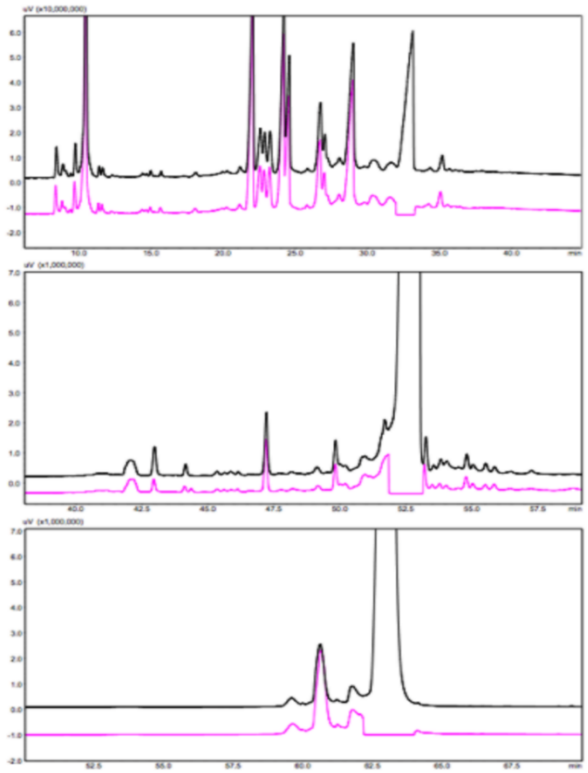
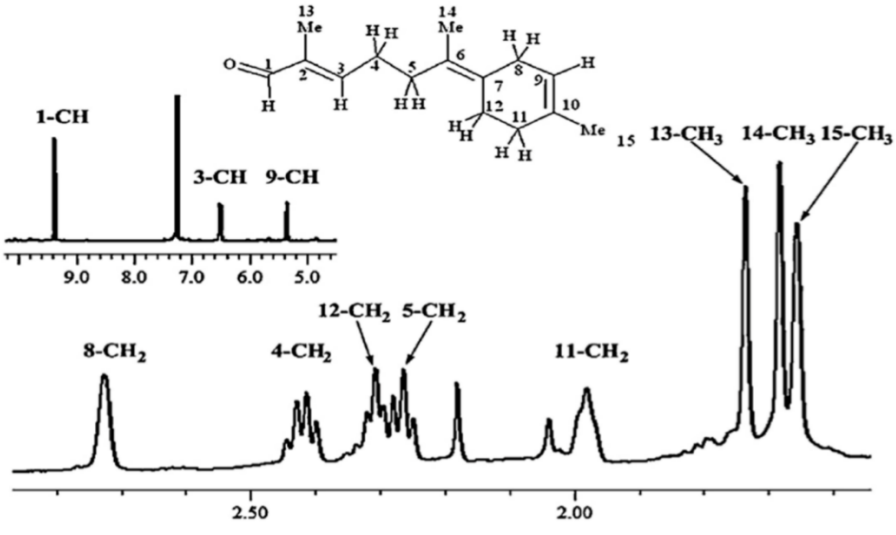


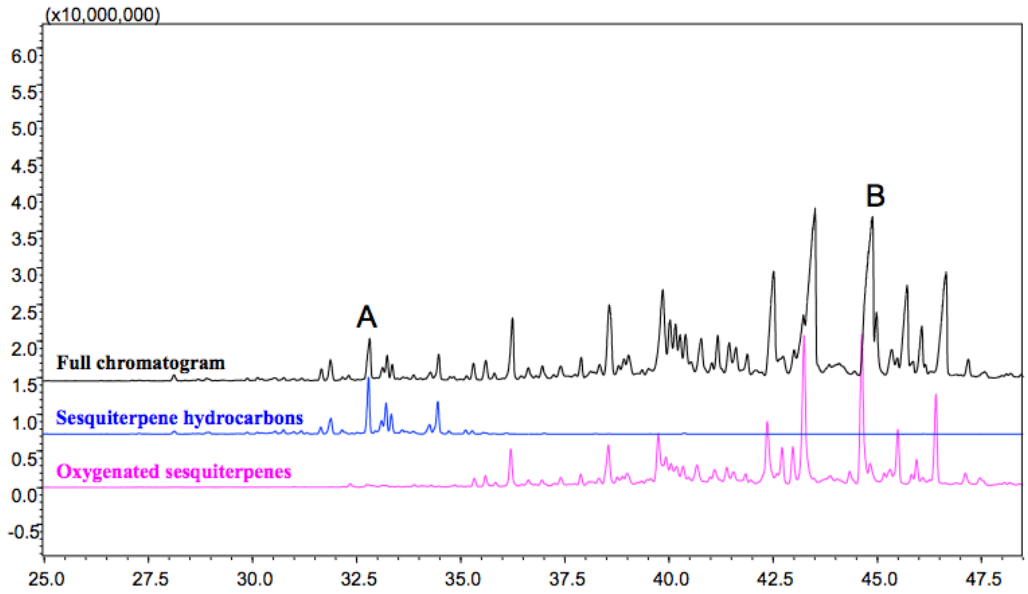
Figure 7. ^{1}H spectrum of the unknown compound with the complete assignment, isolated from wampee essential oil. "Reprinted from Analytica Chimica Acta, 785, D. Sciarrone, S. Pantò, A. Rotondo, L. Tedone, P.Q. Tranchida, P. Dugo, & L. Mondello, Rapid collection and identification of a novel component from *Clausena lansium* Skeels leaves by means of three-dimensional preparative gas chromatography and nuclear magnetic resonance/infrared/mass spectrometric analysis, pages 119-125, Copyright (2013), with permission from Elsevier"



Isolation of Sesquiterpenes from Vetiver Essential Oil

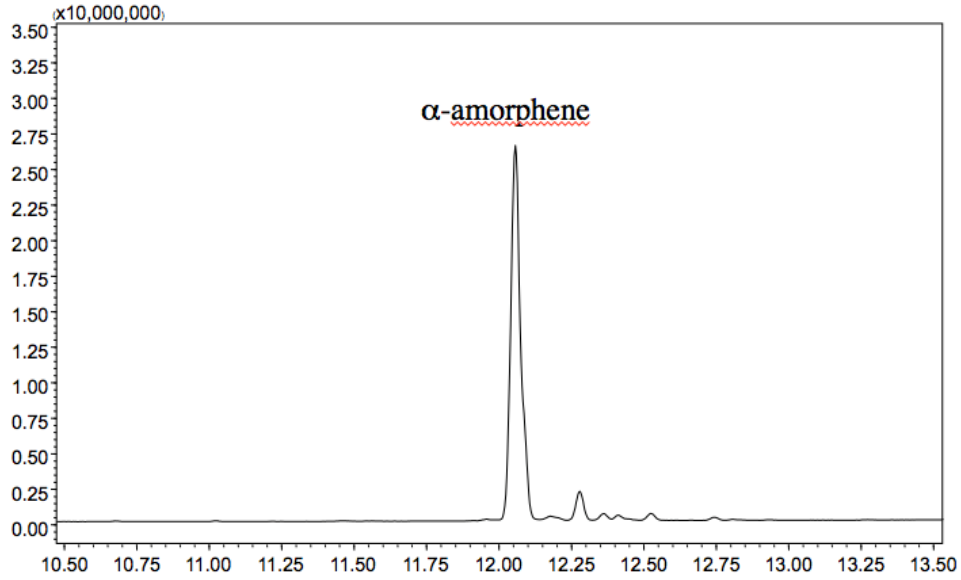
An additional dimension of separation, consisting of a liquid chromatograph, was hyphenated to the prep-MDGC apparatus and applied to the isolation and purification of two sesquiterpenoids from vetiver essential oil. The extra LC dimension served as a pre-fractionation step of the various chemical groups present in vetiver essential oil. The goal of this application was basically to develop a preparative methodology for recovery of pure compounds present at a <10% level. Different parameters, such as LC flow, pressure and temperature of the GC1 injector, split flow and vent time, were tuned to optimize the LC-GC transfer. Standard solutions were used for this part of method development. For a detailed description of troubleshooting related to this issue, the reader is referred to Sciarrone et al., 2014. After optimization, the final conditions chosen for transferring the sesquiterpene fraction from LC to GC1 dimension were: initial injector temperature and pressure, 45°C and 80 KPa; LC transfer flow rate, 350 μL/min with a split flow at 230 mL/min. Once occurred the LC transfer into the GC system, a pressure and temperature program was applied to the GC injector. Slight modifications to these conditions were applied to the transfer of the oxygenated sesquiterpene fraction. After optimization, the real sample of vetiver essential oil was subjected to the LC-GC-GC-GC preparative analysis. Figure 8 shows an expansion of the monodimensional GC-MS chromatogram, along with the two traces relative to the fractions isolated by means of the prep-MDGC system.

Figure 8. GC-MS chromatogram of vetiver essential oil (peak A, α–amorphene; peak B, β-vetivone), and GC-MS chromatograms of the pre-separated LC hydrocarbon (middle trace) and oxygenated sesquiterpene fractions (lower trace) obtained on an SLB-5ms 30 m × 0.25 mm ID × 0.25 μm d_f. *Reprinted with permission from D. Sciarrone, S. Pantò, P.Q. Tranchida, P. Dugo, and L. Mondello,* Rapid isolation of high solute amounts using an online four-dimensional preparative system: normal phase-liquid chromatography coupled to methyl siloxane-ionic liquid-wax phase gas chromatography, *Analytical Chemistry, 86: 4295-4301. Copyright 2014 American Chemical Society.*



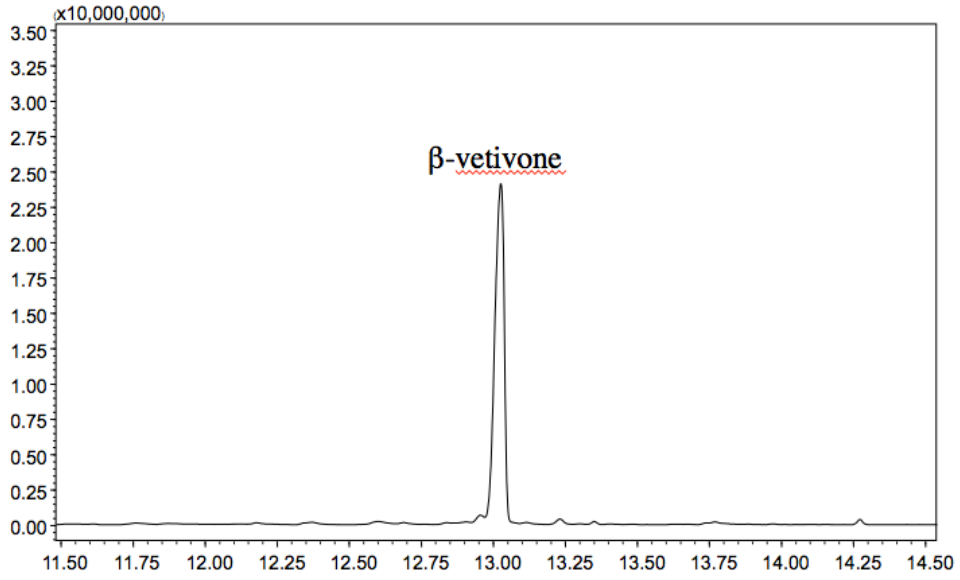
As can be seen, vetiver essential oil is a highly complex matrix, characterized by the presence of an abundant sesquiterpene fraction eluting in the last part of the chromatogram. The LC primary step of separation resulted to be essential for various reasons: i) reduction of the matrix complexity, through a rough separation of target fractions; ii) reduction of GC column overloading; iii) decrease of the amount of high boiling point compounds entering the GC system. For the isolation of 1 mg ca. of α-amorphene, seven prep-MDGC analyses were necessary, each lasting for about 80 min. The purity of this compound was assessed as 90%, through GC-FID and GC-MS analyses (see Figure 9).

Figure 9. GC-MS chromatogram of the compound α-amorphene isolated by means of the LC-GC-GC-GC system. *Reprinted with permission from D. Sciarrone, S. Pantò, P.Q. Tranchida, P. Dugo, and L. Mondello,* Rapid isolation of high solute amounts using an online four-dimensional preparative system: normal phase-liquid chromatography coupled to methyl siloxane-ionic liquid-wax phase gas chromatography, *Analytical Chemistry, 86: 4295-4301. Copyright 2014 American Chemical Society.*



The same analytical approach was applied to the isolation of another sesquiterpenoid present at higher amount in vetiver essential oil: β-vetivone. In this case, a lower number of prep-MDGC cycles was required (two prep-cycles) to obtain 1 mg of about 94% purity (see Figure 10).

Figure 10. GC-MS chromatogram of β-vetivone isolated by means of the prep-MDGC system. *Reprinted with permission from D. Sciarrone, S. Pantò, P.Q. Tranchida, P. Dugo, and L. Mondello,* Rapid isolation of high solute amounts using an online four-dimensional preparative system: normal phase-liquid chromatography coupled to methyl siloxane-ionic liquid-wax phase gas chromatography, *Analytical Chemistry, 86: 4295-4301. Copyright 2014 American Chemical Society.*



In conclusion, the use of a tridimensional prep-GC system has been proven to be an effective approach for the collection of pure components in the 10-30% range, with high purity degree. Moreover, an additional LC dimension was of great support in the collection of low concentrated components, in a reasonably short time of collection. Benefits from the prep-LC-3DGC system were a reduction of GC contamination from dirty samples (non volatile components) and collection of multiple components in one single run. The versatility of the system (operated both in an on-line/off-line fashion) makes it an interesting alternative for the production of pure standard compounds.

REFERENCES

Codina, A., Ryan, R.W., Joyce, R., & Richards, D.S. (2010). Identification of multiple impurities in a pharmaceutical matrix using preparative gas chromatography and computer-assisted structure elucidation. *Analytical Chemistry, 82*, 9127-9133.

Currie, L.A., Eglinton, T.I., Benner, B.A., & Pearson, A. (1997). Radiocarbon "dating" of individual chemical compounds in atmospheric aerosol: first results comparing direct isotopic and multivariate statistical apportionment of specific polycyclic aromatic hydrocarbons. *Nuclear instruments and methods in physics research section B, 123*, 475-486.

Eyres, G.T., Urban, S., Morrison, P.D., Dufour, J.-P., & Marriott, P.J. (2008). Method for small-molecule discovery based on microscale-preparative multidimensional gas chromatography isolation with nuclear magnetic resonance spectroscopy. *Analytical Chemistry, 80*, 6293-6299.

Eyres, G.T., Urban, S., Morrison, P.D., & Marriott, P.J. (2008). Application of microscale-preparative multidimensional gas chromatography with nuclear magnetic resonance spectroscopy for identification of pure methylnaphthalenes from crude oils. *Journal of chromatography A, 1215 (1-2)*, 168-176.

Huan-Li, Z., Feng-Qing, Y., Wei-Hua, H., & Zhi-Ning, X. (2013). Preparative gas chromatography and its applications. *Journal of Chromatographic Science*, *51,* 704-715.

Ledauphin, J., Saint-Clair, J.-F., Lablanquie, O., Guichard, H., Founier, N., Guichard, E., & Barillier, D. (2004). Identification of trace volatile compounds in freshly distilled calvados and cognac using preparative separations coupled with gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry, 52*, 5124-5134.

Marriott, P.J., Eyres, G.T., & Dufour, J.P. (2009). Emerging opportunities for flavor analysis through hyphenated gas chromatography. *Journal of Agricultural and Food Chemistry, 57*, 9962-9971.

Mason, M.E., Johnson, B., & Hamming, M. (1966). Flavor components of roasted peanuts. Some low molecular weight pyrazines and pyrrole. *Journal of Agricultural and Food Chemistry, 14*, 454-460.

Meinert, C., Schymanski, E., Küster, E., Kühne, R., Schüürmann, G., & Brack, W. (2010). Application of preparative capillary GC (pcGC), automated structure generation and mutagenicity prediction to improve effect-directed analysis of genotoxicants in a contaminated ground water. *Environmental Science and Pollution Research, 17*, 885-897.

Needham, L.L., Hill, R.H. Jr., Orti, D.L., Patterson, D.G., Kimbrough, R.D., Groce, D.F., & Liddle, J.A. (1982). Investigation of hyperkeratotic activity of polybrominated biphenyls in firemaster FF-1. *Journal of Toxicology and Environmental Health Part A, 9*, 877-887.

Rijks, J.P.E.M., & Rijks, J.A. (1990). Programmed cold sample introduction and multidimensional preparative capillary gas chromatography. Part I: introduction, design and operation of a new mass flow controlled multidimensional GC system. *Journal of High Resolution Chromatography, 13*, 261-266.

Rühle, C., Eyres, G.T., Urban, S., Dufour, J.-P., Morrison, P.D., & Marriott, P.J. (2009). Multiple component isolation in preparative multidimensional gas chromatography with characterization by mass spectrometry and nuclear magnetic resonance spectroscopy. *Journal of Chromatography A, 1216*, 5740-5747.

Sansone, F.J., Popp, B.N., & Rust, T.M. (1996). Stable carbon isotopic analysis of low-level methane in water and gas. *Analytical Chemistry, 69*, 40-44.

Schomburg, G., Kötter, H., Stoffels, D., & Reissig, W. (1984). Automated instrumentation for multidimensional preparative scale GC (PSGC). *Chromatographia, 19*, 382-390.

Sciarrone, D., Pantò, S., Ragonese, C., Tranchida, P.Q., Dugo, P., & Mondello, L. (2012). Increasing the isolated quantities and purities of volatile compounds by using a triple Deans-switch multidimensional preparative gas chromatographic system with an apolar-wax-ionic liquid stationary-phase combination. *Analytical Chemistry, 84*, 7092-7098.

Sciarrone, D., Pantò, S., Rotondo, A., Tedone, L., Tranchida, P.Q., Dugo, P., & Mondello, L. (2013). Rapid collection and identification of a novel component from *Clausena lansium* Skeels leaves by means of three-dimensional preparative gas chromatography and nuclear magnetic resonance/infrared/mass spectrometric analysis. *Analytica Chimica Acta, 785*, 119-125.

Sciarrone, D., Pantò, S., Tranchida, P.Q., Dugo, P., & Mondello, L. (2014). Rapid isolation of high solute amounts using an online four-dimensional preparative system: normal phase-liquid chromatography coupled to methyl siloxane-ionic liquid-wax phase gas chromatography. *Analytical Chemistry, 86*, 4295-4301.

Waskell, L. (1979). Lack of mutagenicity of two possible metabolites of halothane. *Anesthesiology*, *50 (1),* 9-12.