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# Bridging the gap between gas and liquid chromatography

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ABSTRACT

The rapid and complete baseline separation of both volatile ($C_5$ to $C_{16}$ alkanes in gasoline or terpenes in plant extracts) and non-volatile ($>C_{20}$ alkanes) organic compounds was achieved by combining (1) low-density fluid chromatography (LDFC) using carbon dioxide at elevated temperature (>90 °C) and low pressure (1500 psi) designed to increase the retention of the most volatile compounds and (2) high-vacuum technology ($<10^{-4}$ Torr) in order to preserve the maximum efficiency of short analytical columns (3.0 mm × 150 mm packed with 1.8 μm fully porous HSS-SB-$C_{18}$ particles) when used in LDFC. The volatile compounds are eluted first under isobaric conditions (1500 psi) in less than a minute followed by a linear gradient of the column back pressure (from 1500 to 3500 psi in 5 min) for the elution of the non-volatile compounds up to $C_{40}$. The experimental results demonstrate that LDFC performed with short 3.0 mm i.d. columns packed with sub-2 μm particles and placed under adiabatic conditions enables the analysts to deliver a single, fast, and high-resolution separation of both volatile and non-volatile compounds.

## 1. Introduction

The high-resolution analysis of volatile compounds present in complex mixtures is successfully carried out by head-space gas chromatography (GC) [1] while the heaviest fractions containing high-molecular-weight compounds are usually analyzed by high-performance liquid chromatography [2] or capillary gradient SFC [3]. The analyst is then constrained to operate different types of instruments and to dispose of a wide spectrum of mobile and stationary phases. This makes the complete analysis of these mixtures relatively complex, costly, and time-consuming. In order to develop more efficient and user-friendly separation methods, the concept of unified chromatography initially relayed by Giddings [4] half a century ago has slowly emerged into the academic and industrial laboratories with the development of commercial supercritical fluid chromatographs since 1982 [5]. It was hoped that supercritical fluids (or low-density fluids in general) could enable the analytes to bridge the gap between GC and LC analysis and potentially merge them into a single, faster, more efficient, and more versatile separation technique [5].

Supercritical fluid chromatography (SFC) is *a priori* particularly attractive because (1) the viscosity of supercritical fluids (mostly carbon dioxide) is one order of magnitude smaller than that of liquid water, (2) carbon dioxide is an environment-friendly solvent, and (3) the elution strength of supercritical fluids can be easily tuned by independently adjusting its temperature, pressure, and the content of organic solvents [6–8]. Consequently, high-molecular-weight compounds can be easily separated at ambient/moderate temperatures (30–60 °C) by applying either high back column pressures (>2000 psi) or high contents of organic solvent up to 40% in volume [9,10]. In such conditions, the density of the mobile phase is large and the separation mechanism is similar to that encountered in LC [11,12] so that retention decreases with increasing temperature. Interestingly, the density of carbon dioxide can also be decreased to such low values [11] that a reversal in the retention behavior can be observed: retention factors increase with increasing temperature due to the reduction of the intensity of the analyte/fluid interactions. This property of low-density fluids is used to increase the retention of the most volatile compounds: pure carbon dioxide, elevated temperatures >90 °C, and low column back pressure around 1500 psi can be applied.

At the same time, when using 2.1–4.6 mm i.d. columns, the advantage of low-density fluid chromatography (LDFC) can become problematic if the column is not perfectly insulated from the external thermal environment. This is due to the significant amount of heat exchanged between the column and its surroundings as the mobile phase is decompressing [13–19]. In the case of LDFC, the eluent is expanding and cooling during decompression which induces radial density gradients across the column diameter. Capillary columns are not affected by the thermal effects because the volume of mobile phase is extremely small. The impact of thermal effects on chromatographic performance has been observed in SFC

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for 2.1–4.6 mm i.d. columns [20] and it has been deeply understood from the simulation of chromatograms under LDFC conditions [21–23]. Peaks are severely distorted, therefore, it is extremely challenging to detect and separate efficiently volatile compounds due to the dramatic loss in column performance: for instance, standard SFC methods cannot provide satisfactory separation of volatile alkanes from $C_5$ (pentane) to $C_{16}$ because these compounds are too weakly retained and/or they are often co-eluted with the sample solvent [3]. Complex, long, and tedious two-dimensional SFC–GC or GC–GC separation techniques are then required [3]. Therefore, it is relevant to find and apply new strategies that combat against the nefarious effects of thermal phenomena that may affect the efficiency of 2.1–4.6 mm SFC columns. Vacuum ovens that were shown to provide effective solutions to these problems in vHPLC [24,25] and SFC [26,27] could then be tested for the analysis of both volatile and non-volatile compounds in real-life sample mixtures.

In this work, it is proposed to separate completely organic volatile compounds ($C_5$ to $C_{16}$ alkanes, terpenes) in a very short time (<1 min) by one-dimensional LDFC using a 3.0 mm × 150 mm analytical column packed with 2.0 μm High Strength Silica (HSS) Stable Bond (SB) $C_{18}$ fully porous particles. LSFC is used by applying high temperatures (>90 °C) and low column back pressures (1500 psi). Additionally, the peak shape of the retained volatiles is preserved by fully insulating the column in a 3.0 cm × 25 cm housing chamber at air pressures lower than $10^{-4}$ Torr using a turbomolecular pump [24–26]. The performance of one-dimensional LDFC combined with high-vacuum technologies is compared to that of LDFC under atmospheric pressure and to that of the standard configuration of a SFC system. Finally, it is shown how both volatile and non-volatile compounds can be analyzed and fully separated in a short and single LDFC run by combining isobaric and back pressure gradient elution modes at high temperatures. Direct applications are given for real sample mixtures such as plant extracts, gasoline, and paraffin wax.

## 2. Experimental

### 2.1. Chemicals

The mobile phase used was industrial carbon dioxide (99.8% pure) purchased from Airgas (Worcester, MA, USA). Carbon disulfide was used as the sample solvent: it was HPLC grade from Fisher Scientific (Fair Lawn, NJ, USA). Four sample mixtures were injected: (1) A terpene mixture was prepared by dissolving limonene (10 μL), $\alpha$-humulene (10 μL), and $\beta$-caryophyllene (10 μL) in 1 mL of carbon disulfide; (2) A *n*-alkanes mixture was prepared by dissolving 10 μL of pentane, hexanes, heptane, 2,2,4-trimethylpentane, octane, decane, undecane, dodecane, tetradecane, hexadecane, and 9 mg of octadecane and eisodecane in 1 mL carbon disulfide; (3) A $C_5$–$C_{20}$ + paraffin wax (melting point between 57 and 67 °C, from $C_{20}$ to $C_{40}$) mixture was prepared by dissolving 25 mg of the paraffin wax in the sample mixture 2); and (4) Gasoline (GULF 87) was directly collected from a local gas station in Milford, MA, USA. Each commercial compound was purchased from Sigma–Aldrich (Suwannee, GA, USA) with a minimum purity of 99%.

### 2.2. Instrument and materials

The ACQUITY UPC$^2$ system (Waters, Milford, USA) was used to record the concentration profiles of these analytes. All the parts of the instrument are those of the standard configuration of the ACQUITY UPC$^2$ system, except for the 8 μL UV–Vis detection cell which was bypassed and replaced with a flame ionization detector (FID: $H_2 + O_2$) for detection. A metal tee was used to split the flow of carbon dioxide leaving the column outlet into a 50 μm i.d. 50 cm long capillary (connecting the tee to the FID detector) and a 180 μm × 40 cm PEEK tube (connecting the tee to the ABPR unit). The system includes a binary pump with solvent selection valves and a two-valves injection system. A 1 μL loop was used with injection volume varying between 0.2 μL and 1 μL. The volume of the eluent mixer is 50 μL. The ACQUITY UPC$^2$ system is controlled by the Empower software 3.0 (Waters, Milford, USA).

The required technical details regarding the materials used or the assembling of the home-made vacuum housing were already reported in previous works [24–26]. Briefly, the vacuum chamber (25 cm × 6 cm i.d.) may be pumped by a nominal 60 L/s turbomolecular pump (model TMH 071P, 90 000 rpm) from Pfeiffer Vacuum (Asslar, Germany). The turbomolecular pump is used to generate pressures as low as $2.0 \times 10^{-6}$ Torr in about 12 h. No one vacuum gauge technology is optimal across the full range of air pressure investigated ($5 \times 10^{-6}$ Torr to 750 Torr). An inverted magnetron gauge (AIM-C, less than $10^{-3}$ Torr) was used to check the air pressure in the housing chamber and the achievement of a high vacuum (<$10^{-4}$ Torr). The gauge was controlled by an Active Gauge Controller. The gauge and the controller were obtained from Edwards High Vacuum (Crawley, UK).

In order to minimize heat losses by radiation, the external surface area of the chromatographic column and most of the inner wall of the vacuum housing were covered with a thin and low emissivity aluminum tape. Aluminum-coated polyester tape was selected and purchased from 3M (Saint Paul, MN, USA). It is generally reported by users that the emissivity coefficient of aluminum-coated surface is about $\epsilon_{Al} = 0.04$. For the sake of comparison, the emissivity of machined and polished stainless steel 316 at 297 K is about $\epsilon_{SS} = 0.28$.

### 2.3. Columns

A 3.0 × 150 mm column packed with 1.8 μm fully porous HSS-SB-$C_{18}$ particles from Waters (Milford, MA, USA) was used in this work.

### 2.4. Chromatographic experiments

The chromatographic conditions are described for each sample mixture (terpenes, *n*-alkanes, and paraffin wax).

#### 2.4.1. Volatile terpenes

Two different sets of experiment were carried out:

1. Pure carbon dioxide was pumped in a liquid state at 12 °C at a flow rate of 2.4 mL/min and at a steady state pressure of 4323 psi in presence of the chromatographic column. The inlet temperature of carbon dioxide was elevated at a temperature of 107 °C with the mobile phase active preheater. The 8.0 mm o.d. chromatographic column is encapsulated inside the 6 cm i.d. housing chamber, which is placed horizontally under still-air conditions at ambient temperature ($T = 24$ °C). The ABPR pressure was set at 1500 psi. 0.2 μL of the sample mixture was injected. The housing air pressure was either 1 atm (vented) or $9.0 \times 10^{-6}$ Torr (high vacuum).
2. Carbon dioxide was pumped in a liquid state at 12 °C at a flow rate of 3.0 mL/min and at a steady state pressure of 5500 psi in presence of the chromatographic column. The inlet temperature of carbon dioxide was elevated at a temperature of 90 °C with the mobile phase active preheater. The 8.0 mm o.d. chromatographic column is either embedded inside the 6 cm i.d. housing chamber, which is placed horizontally under still-air conditions at ambient temperature ($T = 24$ °C), or in the conventional oven compartment of the ACQUITY UPC$^2$ instrument. The housing air