IMPACT OF INJECTION SOLVENTS ON EFFICIENCY IN SUPERCRITICAL FLUID CHROMATOGRAPHY

Victor Abrahamsson*, and Margareta Sandahl

Department of Chemistry
Lund University
P.O. Box 124, SE-221 00, Lund, Sweden

Email: Victor.Abrahamsson@chem.lu.se

Abstract. The fundamental knowledge of injection solvents' impact on the chromatographic efficiency in high-performance liquid chromatography (HPLC) is well understood, but has been left rather uninvestigated within the domain of supercritical fluid chromatography (SFC). In this work we present a study between injection solvent properties relationship to chromatographic efficiency. Seven different analytes of various characteristics were diluted with 16 different solvents and injected onto a 2-ethylpyridine column, a bare-silica column and a C18 column. Exploratory data analysis was applied by utilizing a principle components analysis and calculations of correlation coefficients. It is suggested that injection solvents affect the chromatography by polar interactions and hydrogen interactions with the stationary phase. Furthermore it is suggested that the plate number is dependent on the solubility of the injection solvent, which is associated with vapor pressure, boiling point and the molar density. The surface tension and the viscosity were found to be of little influence on the plate number.

Keywords : Supercritical fluid chromatography, injection solvent, chromatographic efficiency

1. Introduction

Supercritical fluid chromatography (SFC) has experienced a rapid development in instrumentation recently. Much of the regained attention is due to its versatility in chiral separations but also because of its ability to replace normal-phase liquid chromatographic methods that involves toxic and non-environmentally friendly organic solvents [1]. According to the principles of green analytical chemistry, organic solvents should be avoided. In this context, the three R's consisting of reduce, replace and recycle are often referred to as a way to minimizing organic solvent waste [2].

Therefore, an excellent alternative approach is SFC, which due to the high diffusion rates and low viscosity provides quick column equilibration and low backpressure thus rapid analysis. Furthermore, the mobile phases used in SFC are almost exclusively CO_2 and a co-solvent *i.e.* methanol or ethanol. It should however be noted that the mobile phase is usually modified with 5 up to 25% co-solvent and the SFC is rarely operated under conditions where the mobile phase becomes supercritical, but rather a gas-expanded liquid [3].

Even though the technique has existed for many years and is readily used, relatively little is known about the fundamentals of *i.e.* retention mechanisms are known compared to high-performance liquid chromatography (HPLC). One of the considerations which is well-known and important to take into account in order to maintain high chromatographic efficiency is the choice of injection solvent when using HPLC. Good practice involves diluting the sample in a solvent mixture that has less elution strength than the mobile phase, although maintaining miscibility. Running reversed-phase HPLC using a non-polar column and injecting a sample diluted in a very non-polar solvent will cause displacement of the sample from the stationary phase as it enters the column. As a consequence retention times will shift and broader peaks will be obtained. Preparing a sample in a weaker solvent will instead create a focusing effect and higher plate numbers will be achieved. More narrow peaks will result in better resolution and attaining better limits of detection [4].

Applying these theories from HPLC to SFC is not completely straightforward. One important factor is solubility, which is not easily predicted in supercritical carbon dioxide ($scCO_2$), further complicated by the addition of co-solvents. The elution power in SFC is to a large extent governed by the density of the mobile phase. Even though low backpressures are maintained over the column due to the low viscosity, minor changes in density imposed by either the backpressure regulator (BPR) or temperature may severely affect the chromatography. Thus changes in density over the column due to the pressure drop will also affect the chromatography [3].

Taking these factors into consideration combined with adsorption effects from injected samples makes the overall picture more complex. Previous studies have shown that water or methanol will adsorb to accessible silanol groups of the stationary phase, which will remain over subsequent injections until sufficient $scCO_2$ has passed through to wash it away. The long-term affects will only remain if very little co-solvent (<2%) is added into the mobile phase, but any coverage of silanol groups will affect the chromatography in terms of retention times and chromatographic efficiency, namely plate number (N) [5]. There is only one recent study available in the literature, which states that increased injection volumes of methanol caused peak broadening due to local change in polarity thus the elution strength. However, the same authors also stated that the polarity of the injection solvent had minor influence on the plate number [6]. Therefore there is a need in further studying injection solvent properties to derive which interactions are important.

The aim of this study was to investigate which properties of injection solvents affect the chromatographic efficiency in SFC. Several analytes of various characteristics diluted in 16 different solvents were injected onto a 2-ethylpyridene (2-EP), a bare-silica and a C18 column. By using exploratory data analysis *i.e.* calculating correlation coefficients and utilizing principal components analysis (PCA), each of the properties were evaluated based on the plate number. To our best of knowledge this is the first time a study focuses on distinguishing which interactions between solute – injection solvent and injection solvent – stationary phase that affect the chromatography in SFC. Not only is this interesting in order to achieve the separation possible but also on a fundamental level of understanding retention mechanisms in SFC.

2. Materials and methods

2.1 Chemicals

Ethanol (99.7%, Solveco, Rosenberg, Sweden) was used as a co-solvent in SFE. Methanol of LC-grade (>99.9%, Honeywell Burdick & Jackson, Seezle, Germany) was used as a co-solvent in SFC. Ultrapure CO_2 was provided by Air Products (Amsterdam, Netherlands) and used for both SFE and SFC. The dissolution solvents were of analytical grade or higher; 2-propanol, acetone, acetontrile, heptane (Honeywell Burdick & Jackson, Seezle, Germany), Chloroform, methyl tert-butyl ether, toluene (Merck, Darmstadt, Germany), Cyclohexane (Acros organics, Pittsburg, PA), Cyclopentane, diethyl ether, hexane, pentane, (Fluka, Buchs, Switzerland), dichloromethane and tetrahydrofuran (Fischer Scientific, Pittsburg, PA).

A standard mixture was prepared by dissolving 300 mg L^{-1} of diclofenac sodium salt, naproxen, fluoranthene, progesterone, sulfanilamide (Sigma-Aldrich, St Louis, MO), caffeine and uracil (Merck, Darmstadt, Germany) in hot ethanol. The solution was subsequently diluted 10 times in each of the 16 solvents, generating 16 solutions containing 30 mg L^{-1} of each analyte**.**

2.2 The supercritical fluid chromatographic system

A Thar Investigator semi-preparative SFC (Pittsburgh, PA) was used for separating the analytes of interest, consisting of a cooled fluid delivery module with a 6 co-solvent switching valve, a modified Spark Holland Alias autosampler with a 48-vial plate, an analytical-2-prep oven with a 10 column switching valve, an automated backpressure regulator and a Waters 2998 photodiode array detector (Milford, MA). The fluid delivery module was cooled by a Neslab RTE7 cooling bath controlled by a Digital One thermoregulator. ChromScope (version 1.10, Waters) was used to control the instrument and subsequently analyze the chromatograms.

Three different types of columns were used in the experiments, a SunFire C18 (4.6 x 250 mm, 5 µm particle size, 100 Å pore size, Waters), a Viridis SFC silica 2-ethylpyridine (4.6 x 250 mm, 5 µm particle size, 100 Å pore size, Waters) and a Viridis SFC silica (4.6 x 150 mm, 5 µm particle size, 100 Å pore size, Waters).

2.3 Chromatographic conditions

The conditions for all experiments were kept isocratic throughout the separation. The flowrate for all experiments was 5 mL min^{-1} and the mobilephase consisted of solely carbon dioxide and methanol. Separation using the C18 column was carried out with 5% modifier, backpressure was 100 bar, temperature was 40 °C. Separation using the 2-EP column was carried out with 13% modifier, backpressure was 120 bar, temperature was 40 °C. Separation using the silica column was carried out with 10% modifier, backpressure was 140 bar, temperature was 40 °C. The injection volume was 10 µL.

2.4 Data analysis

All statistical data processing was performed using MATLAB R2012b including the statistical toolbox (MathWorks Inc., Natick, MA, USA). Principal components analysis was performed using data standardized by variance. The chosen properties of interest were boiling point, density, vapor pressure, viscosity, surface tension, eluent strength, dielectric constant, dipole moment, hydrogen donating and accepting capabilities. The eluent strength ($\varepsilon°$) in this work is referred to the measure of solvents adsorption energy to bare-silica. A PCA was chosen due to the multivariate nature of the data such as properties being highly correlated with each other and with intermolecular interactions.

3. Results and discussions

The plate numbers attained through injection of seven different analytes (Figure 1) of various properties diluted in 16 different injection solvents were injected onto three different columns. By PCA it was observed that polar solvents were associated with higher plate numbers when injected onto a C18 column, while the opposite effect was observed on the 2-ethylpyridine (2-EP) and the bare-silica column (Figure 2). The only analyte, which does not possess any possibilities for hydrogen interactions, is the fluoranthene. The chromatographic efficiency for fluoranthene was not associated with any variation in plate number based on hydrogen donor or acceptor capabilities of the injection solvents (Figure 3). This suggests that the injection solvent with capabilities of hydrogen interaction block polar sites of the stationary phase. If a non-polar stationary phase like the C18 is utilized any secondary retention mechanisms are usually undesirable and therefore blocking accessible silanol groups is beneficial. However, while using polar columns an injection solvent with good capabilities of hydrogen interactions might locally increase the elution strength. These interactions are closely related to the eluent strength which is a measurement of adsorption energies of a solvent onto bare-silica. Furthermore, hydrogen interactions kept a negative correlation coefficient in relation to the number of plates even at injection volumes of 50 µL onto the 2-EP column besides for fluoranthene that remained unaffected. This suggests that hydrogen interactions between injection solvent and the CO_2 do not play an important role in solubility of the injection plug.

The dielectric constant and the dipole moment of the injection solvents were similarly associated with the chromatographic efficiency for the different analytes as were observed with the hydrogen interactions. Further strengthening that the injection solvents interactions with the stationary phase play an important role in the chromatography.

Somewhat surprisingly the viscosity and the surface tension was not well correlated with attained plate numbers. The loadings of the PCA also show that they are less influential. In HPLC viscosities that are higher for injection solvents than the mobile phase will cause a resistance in solvation thus creating distorted peaks [4]. The observed phenomenon is most likely explained by the high diffusivities and the low viscosity of the mobile phase, which readily penetrates the injection plug and dissolves it.

The PCA and an factor analysis (data not shown) provides similar results and suggests that because of orthogonal projection that properties associated with polar compounds can be factored into one component whilst physical properties such as vapor pressure and boiling point can be factored into another. The Peng – Robinson equation of state states that the solubility is determined by factors such as vapor pressure, molar volume and the critical temperature of the solute. The solute in this scenario is of course the injection solvent. More thorough discussions about solubility models are however described by G. Brunner [7] and T. Clifford [8]. Hence some part of the chromatographic efficiency is determined by the solubility of the injection plug into the mobile phase. Our findings are in agreement with the existing theory regarding solubility, indicating that solvents with high vapor pressure and low boiling point facilities higher plate numbers by more easily dissolving into the mobile phase. Lower molar densities were also associated with higher plate numbers.

These effects became much more apparent when increasing the injection volume from 10 µL to 30 or 50 µL (Figure 3).

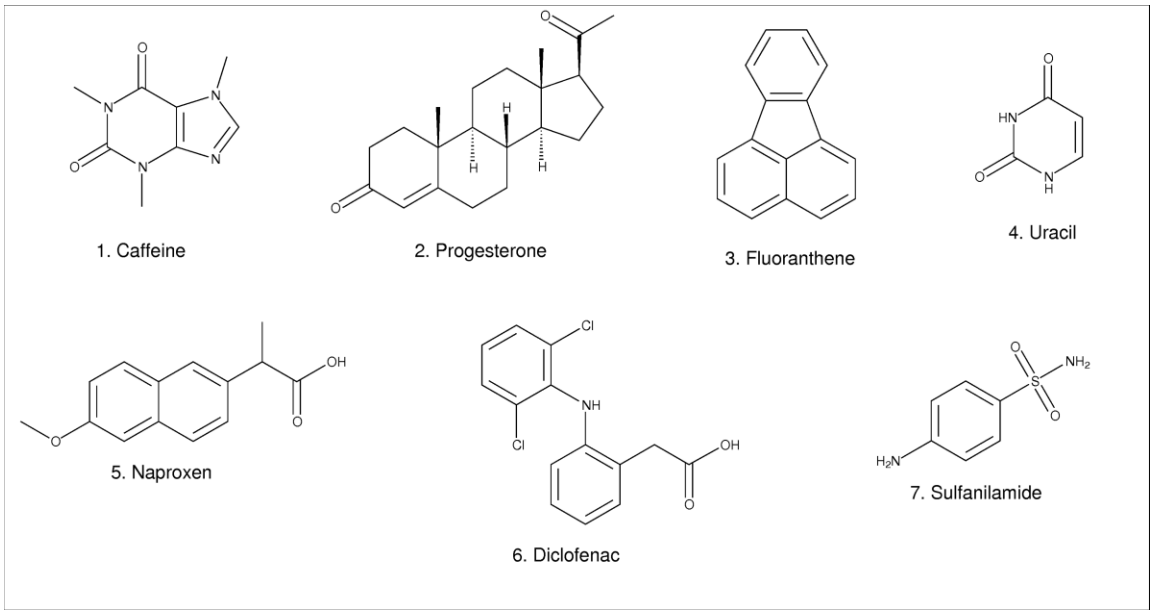

Figure 1. Analytes dissolved in different injection solvents and injected onto the different columns. The analytes are presented in the same order as they eluted on the 2-EP column.

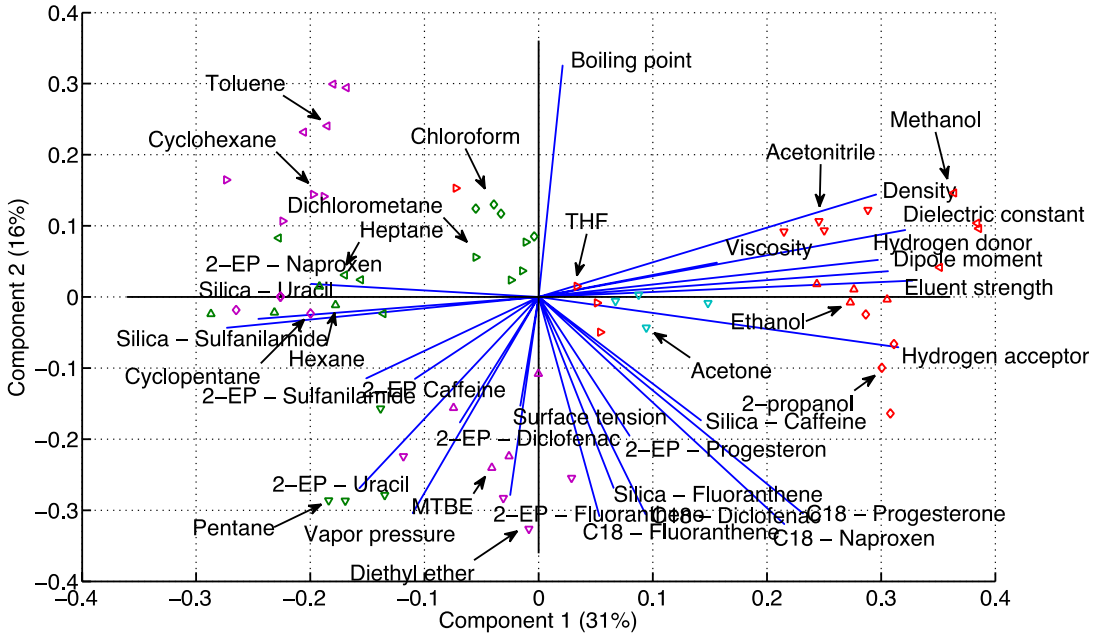

Figure 2. Principle components analysis of seven different analytes diluted in 16 different solvents injected onto three different columns. The data was standardized by variance.

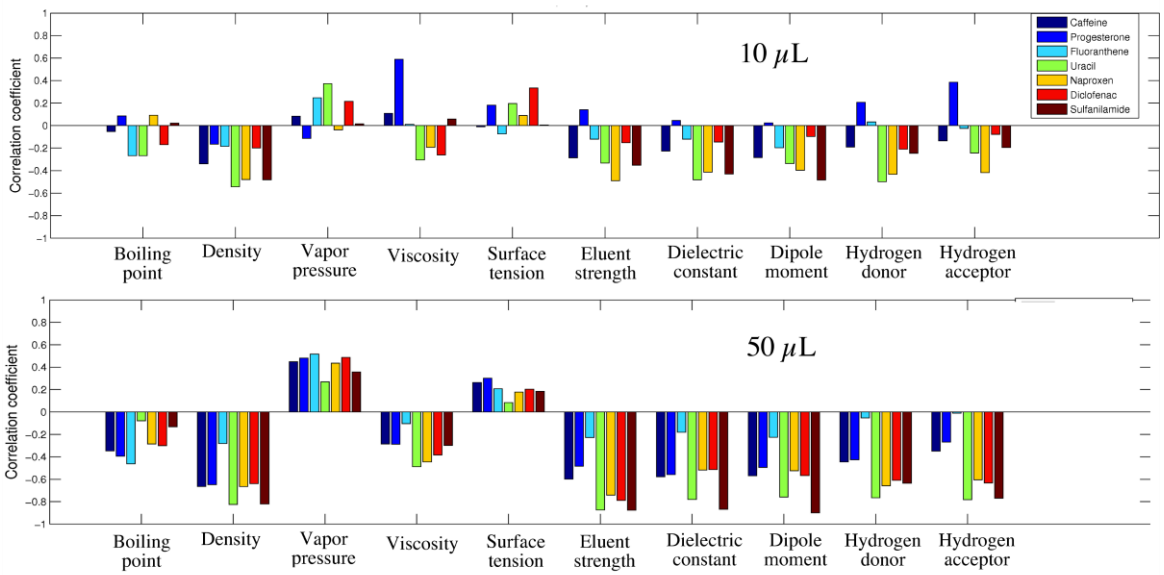

Figure 3. Barplot of the calculated correlation coefficients between each solvent property and plate number. Injections were performed on a 2-EP column and the injection volume was 10 µL (top) compared to 50 µL (bottom)

4. Conclusions

The choice of injection solvent plays an important role in optimizing the chromatographic efficiency in SFC. This is especially true if larger volumes are injected onto the system. Solubility of the injection plug and injection solvent interactions with the stationary phase may be the two primary types of interactions that affect the plate number. The solvation of the injection plug in SFC is suggested to be associated primarily with the vapor pressure, the boiling point and also molar density.

Injection solvents of that are polar or capable of hydrogen interactions may cover accessible polar groups on C18 columns, thus generating higher plate numbers due to masking of secondary retention mechanisms even if the column is end-capped. However, on polar columns due to increased elution strength and local displacement of the sample lower plate numbers are achieved using polar solvents.

The surface tension and the viscosity of the injection solvent were found to be less associated with the chromatographic efficiency due to the high diffusivities and low viscosity of the mobile phase.

Acknowledgements

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