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Chemometric evaluation of the combined effect of temperature, pressure, and co-solvent fractions on the chiral separation of basic pharmaceuticals using actual vs set operational conditions

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ABSTRACT

The need to determine the *actual* operational conditions, instead of merely using the set operational conditions, was investigated for in packed supercritical fluid chromatography (SFC) by design of experiments (DoE) using a most important type of compounds, pharmaceutical basics, as models. The actual values of temperature, pressure, and methanol levels were recorded and calculated from external sensors, while the responses in the DoE were the retention factors and selectivity. A Kromasil CelluCoat column was used as the stationary phase, carbon dioxide containing varying methanol contents as the mobile phase, and the six racemates of alprenolol, atenolol, metoprolol, propranolol, clenbuterol, and mianserin were selected as model solutes. For the retention modeling, the most important term was the methanol fraction followed by the temperature and pressure. Significant differences ($p < 0.05$) between most of the coefficients in the retention models were observed when comparing models from *set* and *actual* conditions. The selectivity was much less affected by operational changes, and therefore was not severely affected by difference between *set* and *actual* conditions. The temperature differences were usually small, maximum ±1.4 °C, whereas the pressure differences were larger, typically approximately +10.5 bar. The set and actual fractions of methanol also differed, usually by ±0.4 percentage points. A cautious conclusion is that the primary reason for the discrepancy between the models is a mismatch between the *set* and *actual* methanol fractions. This mismatch is more serious in retention models at low methanol fractions. The study demonstrates that the *actual* conditions should almost always be preferred.

1. Introduction

The inherent property that makes supercritical fluid chromatography (SFC) much more complex than liquid chromatography (LC) is the compressibility of the mobile phase, which makes SFC a “rubber variant of LC” [1]. Everything considered constant in LC is not constant in SFC [2,3], unless if near isopycnic conditions can be achieved [4]. This ultimately results in radial and axial density and temperature gradients in the column that affect the thermodynamics of adsorption and cause a volumetric flow rate gradient through the column [4–6]. The consequences are that it cannot be guaranteed that the set operational conditions reflect the actual conditions of the columns as can be done in LC [7]. These phenomena also result in difficulties predicting scale-up between different column geometries and SFC instruments. The issue is complicated by the fact that most analytical instruments are volume controlled or calibrated while some preparative scale units are mass flow controlled. The latter is a particular problem when performing pilot-scale experiments and optimizations before scaling up to production [8]. Tarafder et al. proposed a strategy for scale-up and method transfer in SFC in which we “ensure that the intrinsic and the extrinsic conditions are the same in both the systems” [9]. This means that the parameters are properly measured and can be accounted for. In the present investigation, external measuring devices are used to provide accurate measurements of mass flows, pressure, and temperature.

Inside the column, the axial density profile is a nonlinear function of pressure and temperature [8]. To calculate the density of the carbon dioxide and methanol fluid inside the column, the pressure at the column inlet and outlet as well as the actual column temperature must be known. Although different manufacturers use

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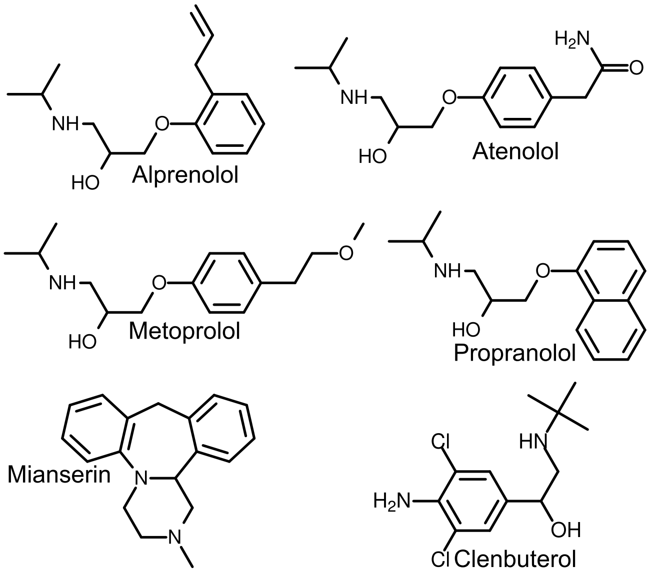

Fig. 1. Molecular structures of the studied basic pharmaceuticals.

different solutions to measure pressure, it is often done only at the pump and the back-pressure regulator. These pressure readings cannot simply be correlated between different systems, because the pressure drop upstream from the back-pressure regulator is divided between the tubing and the column. The pressure drop from the tubing depends on their length and diameter, which cannot be assumed to be identical between different systems. Several reports confirm the existence of temperature gradients along and across SFC columns, with significantly lower temperature at the outlet end of the column than the inlet, caused by the adiabatic decompression of the mobile phase fluid [10]. This underlines the benefits of measuring temperature at least at the column inlet and outlet, and preferably also at several radial positions. Pressure changes will give rise to different retention behaviors for different compounds. Pressure changes also affect the retention, because mobile phase density varies and hence also changes the linear velocity of the mobile phase. The mass flow through the system will, however, remain constant [11].

Free design of the experimental space is not possible in SFC systems, and neither is the structural diversity as suggested by Sköld et al. [12], due to the limited solubility of molecules in the SFC mobile phase [13]. However, many pharmaceutical compounds are still applicable for SFC analysis. Pharmaceuticals from three therapeutic areas are used in this study; four of these are beta blockers, i.e., atenolol, alprenolol, metoprolol, and propranolol, used for treating cardiovascular diseases. Mianserin is prescribed for the treatment of depression and clenbuterol is used as a bronchodilator in the treatment of asthma. The molecular structures of the selected molecules are shown in Fig. 1 and their physicochemical properties are taken from [14] and are presented in Table 1. Most pharmaceuticals are basic so it is very important to study the separation of this particular class of drugs. It is common practice to add diethylamine (DEA) in the eluent to improve the peak symmetries [15] even though other authors has shown it can be done without additive [16]. This was also done in the present investigation (see Section 2, "Experimental"). These charged interactions are pronounced in the SFC due to the low dielectric constant of the eluent.

The poor solubility of the supporting electrolytes makes the system very different from the aqueous mobile phases used in LC. This will have a huge effect on the strength of bases and acids, with the acids shifting to their neutral forms and the bases shifting to their charged forms [17–19]. Some studies find that supercritical phases containing CO_2 and MeOH mixtures may have a slightly acidic pH because they contain small amounts of carbonic acid [20]. However, running an SFC separation in the supercritical mode may cause great variation in the density through the column, which affects both the solubility and the retention factors. These effects can be reduced by running the separation in the subcritical mode, which reduces the variation in compressibility. When the SFC system is run in the subcritical mode, typically by using a larger amount of liquid co-solvent, this will result in a more stable separation system [21]. It has been demonstrated that more polar basic compounds are more sensitive to changes in the co-solvent fraction of the mobile phase [22]. Adding MeOH to the SFC mobile phase affects the retention by shortening the retention time, due to the increased elution strength of the mobile phase. The selectivity in chiral separations can sometimes be controlled by using different alcohols as organic modifiers [23]. However, any change in temperature will cause variation in density, and this is more pronounced with low co-solvent fractions [24]. Unfortunately, most modern commercial SFC instruments are not equipped with mass-flow regulation or monitoring, except for some preparative instruments. Rajendran et al. have illustrated how the pressure drop is distributed through the column [13,25].

When a chemometric approach, such as design of experiments (DoE), is applied to an SFC system, several parameters can be changed simultaneously. Recently, Åsberg et al. [26] used external temperature, pressure and mass-flow sensors to obtain knowledge of these parameters during experiments using neutral model compounds as solutes. They also used a factorial design, which can provide important information about the robustness and productivity of chiral SFC. They concluded that DoE can facilitate improved robustness by giving insight into the experimental space, which will facilitate better method transfer.

There are few systematic investigations on the relative importance of chromatographic operational parameters on the separation basic drugs in SFC; this is remarkable since many of all small molecule drugs today are basic components. None of them are concerned with measuring actual conditions. Grand-Guillaume Perrenoud et al. states that basic compounds often give poor chromatographic performances and can suffer strong peak shape distortions, including tailing, fronting, splitting and shouldering [27]. However, recently Desfontaine et al. investigated the use of novel stationary phases for the achiral analysis of basic drugs in SFC [28]. They for example showed that more than 90% of the studied compounds showed non-symmetrical peak shapes in pure CO_2/methanol mobile phase without additive. In the study by Grand-Guillaume Perrenoud [27] et al. investigated the achiral separation of 92 pharmaceutical basic compounds on five different 2-ethylpyridine columns without mobile phase additive. They noted that the peak shapes of the basic drugs were strongly depen-

Table 1
Properties of the studied drugs, data taken from Ref. [14]. Polar surface area is in vacuum. Vacuum data is an acceptable approximation for qualitative discussions, because dielectric constant of the eluent is close to one.

	Alprenolol	Atenolol	Propranolol	Metoprolol	Clenbuterol	Mianserin
Mw	249.3	266.3	259.3	267.4	277.2	264.4
pKa in water	9.67	9.6	9.42	9.6	9.63	6.92
Polar surface area, Å^2	41.48	84.58	41.49	50.72	58.28	6.48