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Investigation of the effect of mobile phase composition on selectivity using a solvent-triangle based approach in achiral SFC

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ABSTRACT

Defining a method development methodology for achiral drug impurity profiling in SFC requires a number of steps. Initially, diverse stationary phases are characterized and a small number of orthogonal or dissimilar phases are selected for further method development. In this paper, we focus on a next step which is the investigation of the modifier composition on chromatographic selectivity. A solvent-triangle based approach is used in which blends of organic solvents, mainly ethanol (EtOH), propanol (PrOH), acetonitrile (ACN) and tetrahydrofuran (THF) mixed with methanol (MeOH) are tested as modifiers on six dissimilar stationary phases. The tested modifier blends were composed to have equal eluotropic strengths as calculated on bare silica. The modifier leads to minor changes in terms of elution order, retention and mixture resolution. However, varying only the modifier composition on a given stationary phase does not lead to the creation of dissimilar systems. Therefore the modifier composition is an optimization parameter, with the stationary phase being the factor determining most the selectivity of a given mixture in achiral SFC.

1. Introduction

The characterization of impurities in a new drug substance is important to protect the patient against their adverse events. Impurities can arise during synthesis, purification or storage, and can include starting materials, by-products, catalysts, reagents and/or intermediates from synthesis, degradation products and/or ligands [1] . Currently, high performance liquid chromatography (HPLC), more specifically reversed-phase liquid chromatography (RPLC), is the benchmark technique for drug impurity profiling [2].

To maximize the possibility that no impurity remains unnoticed under the peak of the active substance or under that of another impurity, several chromatographic systems i.e. combinations of a stationary and a mobile phase, are simultaneously screened [3–5]. These systems should be as orthogonal or dissimilar as possible. Pinkston et al. [6] compared SFC and HPLC for the screening of a library of pharmaceutical compounds. 3.7% of the compounds observed by SFC/MS were not detected by LC/MS, while 8.1% of compounds were observed by LC/MS but not by SFC/MS, showing the orthogonal selectivities of the two techniques. Another study [7] noted a 1–2% improvement in success rate when SFC is used together with HPLC for the purification of pharmaceutical library compounds. Wang et al. [8] developed an SFC method capable of trace level impurity analysis for mometasone furoate and compared it to the complementary HPLC method. The two chromatographic techniques provide significant selectivity differences which is reflected in the R^2 value of the retention factors obtained from the two methods ($R^2 = 0.2$).

In HPLC, selectivity can be varied by maintaining a given polarity or elution strength, but using solvents having different properties [9,10]. Snyder's solvent-selectivity triangle [10] includes solvents with different properties. Solvent selectivity is controlled by the proton donor, proton acceptor and dipole interactions of the different solvents. Keeping the solvent strength constant while changing the mobile phase components (using solvents from different groups) leads to alteration in the solvent selectivity, which in turn can lead to the separation of co-eluting components. Thus using organic solvents with different properties may lead to selectivity differences. The mobile phase in SFC consists of CO_2, generally together with a co-solvent and a small percentage of additive. Most frequently, methanol is used as co-solvent; its presence is necessary to increase the polarity of the mobile phase since CO_2 is

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non-polar. Properties of organic solvents, such as density, polarity, degree of adsorption onto the stationary phase surface and hydrogen-bond donating or accepting abilities, are responsible for the solutes' retention and elution. This study investigates whether the modifier composition in achiral SFC is responsible for rather global selectivity differences or more for local changes, within the context of drug impurity profiling.

The effect of mobile phase optimization in SFC by variation of the modifier composition was only marginally investigated by other research groups. Brunelli et al. [11] determined the optimal MeOH/ACN modifier ratio by plotting the adjusted retention times of diverse pharmaceuticals on a 2-ethylpyridine column as a function of the MeOH/ACN ratio. The optimal composition, as determined from a selectivity-tuning plot, i.e. the one in which the mixture components showed the least co-elutions and maximal resolutions, was then verified experimentally. However in that study, only two modifier compositions were investigated, and only on one stationary phase. West et al. [12] compared retention and selectivity changes observed when changing the nature of the modifier (methanol (MeOH), ethanol (EtOH), propanol (PrOH) and acetonitrile (ACN)), on seven different stationary phase chemistries. The different modifiers change the elution strength of the mobile phase and adsorb to the stationary phase surface to different degrees, which in turn lead to retention and separation alterations. But straightforward comparisons based on the data from this study are difficult since not all four mobile phase compositions were tested on the seven stationary phases. In addition, the solutes used do not possess strong basic or acidic functional groups, which are typically present in pharmaceutical compounds.

This study is part of a project evaluating SFC as an alternative technique to RPLC in drug impurity profiling. Differences in selectivity in SFC originate mainly from the stationary phase chemistry [13]. Therefore in impurity profiling the mixture to separate is screened on several dissimilar phases [4,5] in a first step, followed by further method optimization in a second step. In the context of defining proper optimization steps for our SFC methodology, the aim of this study was to investigate the effect of different modifier blends composed of organic solvents with different properties. A Snyder solvent triangle-based approach was applied on a set of dissimilar stationary phases. The modifier mixtures have the same theoretical eluotropic strength on silica, ensuring a fair comparison between the different systems. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) are used to understand the effect of a change in mobile phase composition on retention and selectivity. Test mixtures were finally used to demonstrate how this approach can be used to optimize separation in SFC.

2. Materials and methods

2.1. Instrumentation

SFC analysis was performed on an Acquity Ultra Performance Convergence Chromatography (UPC^2) from Waters® The system was equipped with a binary solvent delivery pump, an autosampler with a fixed loop of 10 μL, a convergence manager, an external Acquity column oven without active pre-heating, a PDA detector and a back-pressure regulator. For all analyses, partial loop injections were done and the sample compartment was thermostated at 10 °C.

2.2. Materials

CO_2 quality 4.5 (purity ≥99.995%) was from Messer (Sint-Pieters-Leeuw, Belgium). MeOH, EtOH, PrOH and ACN, type HPLC gradient grade, were purchased from VWR Chemicals (Fontenay-sous-Bois, France). HPLC-grade inhibitor-free THF was purchased from Sigma-Aldrich (Steinheim, Germany). Ammonium formate (purity ≥99.0%), used as mobile-phase additive, was also from Sigma-Aldrich. Five phenothiazine derivatives were used to demonstrate column dissimilarity. These were phenothiazine, promethazine, acetopromazine and chlorprothixene, all from Sigma-Aldrich, and chlorpromazine from Fluka (Neu-Ulm, Switzerland). A mixture with the phenothiazines at 0.2 mg/mL was prepared in MeOH.

2.3. Compound set

To evaluate the orthogonality of systems, diverse test substances, being mostly pharmaceuticals, given the eventual application of the systems in drug impurity profiling, were injected. A total of 64 compounds, covering broad ranges of pKa (0.00–13.5) and logP (−1.65 to 10.23) values, obtained through SciFinder (American Chemical Society, Columbus, OH), were used. They vary also in structure (functional groups, ring structures), molecular weight and originate from different pharmacological and chemical classes. It was also taken into account that approximately 80% of the pharmaceuticals are basic. The compounds, the used concentrations, their nature (A-acidic, B-basic, N-neutral) and manufacturers are found in Table 1. Solutions were prepared in methanol.

2.4. Stationary phases

Given that the aim is to develop a method for drug impurity profiling, an orthogonal set of stationary phases was used in this study. Screening on these phases maximizes the possibility that no impurity remains unnoticed. The six stationary phases implemented in this study were Luna CN, Luna NH2 and Luna Silica from Phenomenex, and Inertsil Phenyl from GL Sciences, all with dimensions 100 mm × 4.6 mm i.d., 3 μm particles; Viridis Silica 2-ethylpyridine from Waters having dimensions 100 mm × 4.6 mm i.d., 5 μm particles and FluoroSep-RP Phenyl from ES Industries (West Berlin, NJ, USA) with dimensions 150 mm × 4.6 mm i.d., 5 μm particles.

2.5. Chromatographic conditions

Gradient elution was applied because of the diversity of the test compounds. The mobile phase was composed of CO_2 mixed with modifier, which contained 10 mM ammonium formate. The organic modifier was made up as specified in section 2.5 and added gradually from 5% to 40% over 10 min. The mobile phase composition was held at 40% for 5 min and then decreased to 5% over 0.5 min. Two minutes of reconditioning with 5% organic modifier was done, resulting in a run-time of 17.5 min. Analyses were performed at constant flow rate of 3.0 mL/min. The temperature was kept constant at 25 °C and the back-pressure at 150 bar. Detection was performed at 220 and 254 nm. All compounds were injected individually on all columns with an injection volume of 5 μL.

The void time (t_o) was marked as the first baseline disturbance after injection of the solvent. Retention factors (k) were calculated as $(t_r - t_o)/t_o$ with t_r the retention time of the compound.

2.6. Organic modifier composition

The reference organic modifier was pure methanol (100%) which was added to carbon dioxide. Initially, the aim was to use isoeluotropic fractions of the other modifiers (EtOH, PrOH, ACN and THF). However, ACN and THF show poor elution strengths when used as modifiers. To overcome this problem, binary mixtures of organic solvents were used, taking into account their solvent strengths on bare silica [14]. The binary mixtures always included methanol and one other solvent under study, namely the proton acceptor