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Development of an achiral supercritical fluid chromatography method with ultraviolet absorbance and mass spectrometric detection for impurity profiling of drug candidates. Part I: Optimization of mobile phase composition

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ABSTRACT

Supercritical fluid chromatography (SFC) is a very useful tool in the purpose of impurity profiling of drug candidates, as an adequate selection of stationary phases can provide orthogonal separations so as to maximize the chances to see all impurities. The purpose of the present work is to develop a method for chemical purity assessment. The first part, presented here, focuses on mobile phase selection to ensure adequate elution and detection of drug-like molecules, while the second part focuses on stationary phase selection for optimal separation and orthogonality.

The use of additives in the carbon dioxide – solvent mobile phase in SFC is now commonplace, and enables in particular to increase the number of eluted compounds and to improve peak shapes. The objective of this first part was to test different additives (acids, bases, salts and water) for their chromatographic performance assessed in gradient elution with a diode-array detector, but also for the mass responses obtained with a single-quadrupole mass detector, equipped with an electrospray ionization source (Waters ACQUITY QDa).

In this project, we used a selection of one hundred and sixty compounds issued from Servier Research Laboratories to screen a set of columns and additives in SFC with a Waters ACQUITY UPC^2 system. The selected columns were all high-performance columns (1.7–1.8 μm with totally porous particles or 2.6–2.7 μm with superficially porous particles) with a variety of stationary phase chemistries.

Initially, eight additives dissolved in the methanol co-solvent were tested on a UPC^2 ACQUITY UPC^2 HSS C18 SB column. A Derringer desirability function was used to classify the additives according to selected criteria: elution capability, peak shapes, UV baseline drift, and UV and mass responses (signal-to-noise ratios). Following these tests, the two best additives (ammonium acetate and ammonium hydroxide) were tested on a larger number of columns (10) where the two additives appeared to provide very comparable overall scores. However, ammonium acetate was selected for slightly better chromatographic quality.

In a second step, we investigated the effects of ammonium acetate concentration (between 0 and 25 mM in the methanol co-solvent) on retention and peak efficiency. Two types of silica supports were tested by working with ACQUITY UPC^2 HSS C18 SB and BEH columns. 20 mM ammonium acetate in methanol with 2% water was finally selected as the best co-solvent composition.

1. Introduction

Impurity profiling of organic products that are synthesized as possible drug candidates is a significant concern. For this purpose, it is necessary to have complementary high-performance analytical methods to ensure that all impurities are identified. A general

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screening method for impurity profiling of drug candidates should naturally allow the elution of a maximum of species with good peak shapes. In addition, while detection is most often carried out with a UV detector, mass spectrometric (MS) detection is desirable to confirm peak identity and support peak purity. Some single-quadrupole mass spectrometers with a small footprint are now available at a rather low price. Some of these instruments allow for "push-button" operating mode with most parameters having been optimized to ensure reasonably good response to a large array of analytes. Such apparatus are bound to expand the number of routine methods with MS detection, especially as limited expertise is necessary to operate them.

SFC (usually expanded as Supercritical Fluid Chromatography, although the fluid employed is now rarely in the supercritical state) makes use of liquid mobile phases comprising a significant portion of pressurized carbon dioxide mixed to another solvent (most often an alcohol as methanol) [1]. The high-throughput capability and economic benefits of the method, but also the "green" aspect of a non-toxic solvent together render SFC very attractive for a wide range of applications, whenever a replacement or complement to HPLC is desired. Thus the recent introduction of improved analytical SFC systems that take full advantage of all these features is currently causing a revival of the technique.

It was shown in numerous occasions that SFC is an adequate tool for small molecules of pharmaceutical interest: active pharmaceutical ingredients, impurities or degradation products [2–6]. Additionally, it was proven already some 10 years ago that SFC-MS could efficiently compete with LC–MS for the purpose of screening libraries of pharmaceutical compounds [7].

Because active pharmaceutical ingredients are most often basic molecules, and the carbon dioxide – methanol mixture is acidic, adequate elution of such analytes is preferably achieved with an adjusted mobile phase composition comprising a small percentage (typically 0.1–1% in the co-solvent) of a polar additive [8]. The additive may be a base (like isopropylamine [9] or ammonium hydroxide [10,11]), an acid (formic acid [10], ethanesulfonic acid [12] or citric acid), a combination of an acid and a base [13,14], or a salt (most often ammonium formate or ammonium acetate [15,16]). Water is also increasingly cited as an additive to improve elution of polar analytes [17]. While the effect of additive nature and concentration in SFC was often discussed as regards chromatographic features (retention or peak shapes) [15,18] and is considered to cause most significant changes to SFC chromatograms than usually observed in RPLC [6], the impact on MS detection was rarely addressed [10,16,19].

The present study aims at developing a rapid screening method for impurity profiling of drug candidates with SFC-ESI-MS. The first part presented in this paper will focus on the selection of a versatile mobile phase composition to ensure elution of the largest proportion of drug-like compounds with good peak shape and the best possible ESI-MS response. Several additives introduced in the CO_2-methanol mobile phase were thus tested with a wide range of stationary phases to assess their capabilities for successful chromatography and MS detection. Because the method aims at direct applicability in a pharmaceutical company, a large selection (160) of drug candidates (further presented in Section 3.1) were evaluated.

The second part, presented in a subsequent paper, will focus on stationary phase selection to achieve orthogonal methods.

2. Material and method

2.1. Chemicals, solvents and reagents

160 drug candidates were obtained from Servier Research Laboratories (Suresnes, France) whose structures are confidential, but they are further described in Section 3.1. For the additives: ammonium acetate, ammonium formate, diethylamine, diethanolamine and isopropylamine were obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France); ammonium hydroxide solution was provided by Fisher Scientific (Illkirch, France); ultra-pure water was provided by an Elga UHQ system from Veolia (Wissous, France) and trifluoroacetic acid was obtained from VWR (Fontenay-sous-Bois, France). Solvents used were HPLC-grade methanol (MeOH) and ethanol provided by VWR (Fontenay-sous-Bois, France). Formic acid was obtained from VWR (Fontenay-sous-Bois, France). Carbon dioxide of industrial grade 99.5% was provided by Messer (Puteaux, France).

2.2. Stationary phases

For this study, eleven commercialized columns were compared. The known features of the stationary phase chemistries and dimensions are gathered in Table 1. The columns selected were all high efficiency phases (1.7 or 1.8 μm fully porous and 2.6 or 2.7 μm superficially porous particles) with a variety of stationary phase chemistries. The columns were kindly provided by Waters (Guyancourt, France), Phenomenex (Le Pecq, France), Thermo Fisher Scientific (Villebon, France) and Macherey-Nagel (Hoerdt, France).

2.3. Instrumentation

The supercritical fluid chromatography system was a Waters Corporation (Millford, MA, USA) ACQUITY Ultra Performance Convergence Chromatography™ (UPC²®). It was equipped with a binary solvent delivery pump compatible with mobile phase flow rates up to 4 mL/min and pressures up to 414 bar, an autosampler that included partial loop volume injection system, a back pressure regulator, 4-position column oven compatible with 150 mm length columns and two detectors: a photodiode-array (PDA) detector and an ACQUITY QDa® single-quadrupole mass detector with electrospray ionization source. An isocratic solvent manager was used as a make-up pump and was positioned before the mass detector. The main flow stream was then splitted by the on-board flow-splitter assembly. With this system, most of the column flow goes to the back-pressure regulator and only an unknown portion goes to the MS. MassLynx® software (V4.1) was used for system control and data acquisition. Empower® 3 was used for integration of peaks for column efficiency measurements. Waters Data Converter (V2.1) was used to convert data from MassLynx to Empower.

2.4. Chromatographic conditions

The screening of the different additives with the selection of stationary phases was performed in a gradient elution program in the following conditions:

(1) For columns with 100 × 3.0 mm dimensions (1.7–1.8 μm fully porous particles), the mobile phase composition was CO_2 with 5–50% MeOH (+additive) in 10 min, flow rate was fixed at 1 mL/min, temperature was set at 25 °C and the outlet pressure was maintained at 150 bar. Inlet pressure at the beginning and end of the gradient program varied from 215 to 330 bar respectively.
(2) For columns with 150 × 4.6 mm dimensions (2.6 μm superficially porous particles), the mobile phase composition was CO_2 with 5–50% MeOH (+additive) in 15 min, flow rate was fixed at 2.35 mL/min, temperature was set at 25 °C and the outlet pressure was maintained at 150 bar. Inlet pressure at the beginning