Journal of Chromatography A, 1408 (2015) 227–235

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Development of an achiral supercritical fluid chromatography method with ultraviolet absorbance and mass spectrometric detection for impurity profiling of drug candidates. Part II. Selection of an orthogonal set of stationary phases

Elise Lemasson[a], Sophie Bertin[b], Philippe Hennig[b], Hélène Boiteux[c], Eric Lesellier[a], Caroline West[a,*]

[a] Université d'Orléans et CNRS, Institut de Chimie Organique et Analytique (ICOA), UMR 7311, Pôle de Chimie, rue de Chartres, B.P. 6759, 45067 Orléans, Cedex 2, France
[b] Institut de Recherches Servier, 11 rue des Moulineaux, 92150 Suresnes, France
[c] Waters Corporation, 5 rue Jacques Monod, 78280 Guyancourt, France

ARTICLE INFO

Article history:
Received 8 April 2015
Received in revised form 3 July 2015
Accepted 8 July 2015
Available online 14 July 2015

Keywords:
Derringer desirability functions
Ranking
Mass detection
Pharmaceutical ingredients
Screening process
Supercritical fluid chromatography

ABSTRACT

Impurity profiling of organic products that are synthesized as possible drug candidates requires complementary analytical methods to ensure that all impurities are identified. Supercritical fluid chromatography (SFC) is a very useful tool to achieve this objective, as an adequate selection of stationary phases can provide orthogonal separations so as to maximize the chances to see all impurities.

In this series of papers, we have developed a method for achiral SFC-MS profiling of drug candidates, based on a selection of 160 analytes issued from Servier Research Laboratories.

In the first part of this study, focusing on mobile phase selection, a gradient elution with carbon dioxide and methanol comprising 2% water and 20 mM ammonium acetate proved to be the best in terms of chromatographic performance, while also providing good MS response [1].

The objective of this second part was the selection of an orthogonal set of ultra-high performance stationary phases, that was carried out in two steps. Firstly, a reduced set of analytes (20) was used to screen 23 columns. The columns selected were all 1.7–2.5 μm fully porous or 2.6–2.7 μm superficially porous particles, with a variety of stationary phase chemistries. Derringer desirability functions were used to rank the columns according to retention window, column efficiency evaluated with peak width of selected analytes, and the proportion of analytes successfully eluted with good peak shapes. The columns providing the worst performances were thus eliminated and a shorter selection of columns (11) was obtained. Secondly, based on 160 tested analytes, the 11 columns were ranked again. The retention data obtained on these columns were then compared to define a reduced set of the best columns providing the greatest orthogonality, to maximize the chances to see all impurities within a limited number of runs. Two high-performance columns were thus selected: ACQUITY UPC^2 HSS C18 SB and Nucleoshell HILIC.

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. SFC (usually expanded as Supercritical Fluid Chromatography, although the fluid employed is now rarely in the supercritical state) is one such method. SFC makes use of liquid mobile phases comprising a significant portion of carbon dioxide mixed to a co-solvent [2]. CO_2 has major advantages over more conventional chromatographic solvents, as it has a low viscosity allowing for high diffusivities of the analytes (hence high efficiencies) and limited pressure drop over packed columns. As a result, high flow rates can be used without strongly affecting efficiency, and columns packed with sub-2 μm particles can be employed with relatively low-pressure pumping systems (400 bar) [3]. Consequently, the recent progresses in stationary phase technology (small particles [4,5], but also superficially porous particles [6]) has also benefited to SFC.

* Corresponding author. Tel.: +33 238 49 47 78; fax: +33 238 41 72 81.
E-mail addresses: caroline.west@univ-orleans.fr, west.caroline@yahoo.fr (C. West).

http://dx.doi.org/10.1016/j.chroma.2015.07.035
0021-9673/

Table 1
23 columns used in this study.

Column Name	Manufacturer	Support	Bonded ligand	Dimensions (mm)	Particle size (μm)
ACQUITY UPC^2 HSS C18 SB	Waters	Fully porous silica	Octadecyl, non endcapped	100 × 3.0	1.8
ACQUITY UPC^2 BEH	Waters	Fully porous hybrid silica	–	100 × 3.0	1.7
ACQUITY UPLC BEH Shield RP18	Waters	Fully porous hybrid silica	Alkyl with embedded carbamate group	100 × 3.0	1.7
ACQUITY UPC^2 BEH 2-EP	Waters	Fully porous hybrid silica	2-ethylpyridine	100 × 3.0	1.7
ACQUITY UPC^2 CSH Fluorophenyl	Waters	Fully porous hybrid silica	Pentafluorophenyl	100 × 3.0	1.7
ACQUITY UPC^2 Torus 1-AA	Waters	Fully porous hybrid silica	1-Amino-anthracene	100 × 3.0	1.7
ACQUITY UPC^{2}Torus 2-PIC	Waters	Fully porous hybrid silica	2-Picolyl-amine	100 × 3.0	1.7
ACQUITY UPC^2 Torus DEA	Waters	Fully porous hybrid silica	Diethylamine	100 × 3.0	1.7
ACQUITY UPC^2 Torus DIOL	Waters	Fully porous hybrid silica	Propanediol	100 × 3.0	1.7
Synergi Polar RP	Phenomenex	Fully porous silica	Phenyl-oxypropyl	100 × 3.0	2.5
Kinetex HILIC	Phenomenex	Superficially porous silica	–	150 × 4.6	2.6
Kinetex PFP	Phenomenex	Superficially porous silica	Pentafluorophenyl	150 × 4.6	2.6
Kinetex Biphenyl	Phenomenex	Superficially porous silica	Biphenyl	150 × 4.6	2.6
Kinetex XB C18	Phenomenex	Superficially porous silica	Octadecyl, endcapped	150 × 4.6	2.6
Accucore HILIC	Thermo	Superficially porous silica	–	150 × 4.6	2.6
Accucore Phenyl-X	Thermo	Superficially porous silica	Phenyl-alkyl	150 × 4.6	2.6
Accucore Phenyl-hexyl	Thermo	Superficially porous silica	Phenyl-hexyl	150 × 4.6	2.6
Accucore C18	Thermo	Superficially porous silica	Octadecyl	150 × 4.6	2.6
Accucore PFP	Thermo	Superficially porous silica	Pentafluorophenyl	150 × 4.6	2.6
Ascentis Express OH5	Supelco	Superficially porous silica	Penta-hydroxyl	150 × 4.6	2.7
Ascentis Express F5	Supelco	Superficially porous silica	Pentafluorophenyl	150 × 4.6	2.7
Nucleoshell HILIC	Macherey-Nagel	Superficially porous silica	Sulfobetaine	150 × 3.0	2.7
Nucleoshell PFP	Macherey-Nagel	Superficially porous silica	Pentafluorophenyl	150 × 3.0	2.7

An interesting feature of SFC is that, in addition to possibly providing an orthogonal method to a reversed-phase HPLC one [5–10], it can also be orthogonal to itself, when stationary phases are adequately selected [11]. Indeed, all columns that are marketed for HPLC, whether for reversed-phase (RP), normal-phase (NP), hydrophilic interaction (HILIC) or ion-exchange modes, can also be used with mobile phases comprising carbon dioxide [12–16]. Chemical diversity of the available stationary phases is currently significantly improving, with rising interest of the column manufacturers and research groups to produce original phases dedicated to SFC use [17–19]. Moreover, while different operating modes in HPLC require different mobile phase composition (for instance, hydro-organic in RP, alkane-alcohol in NP), the same CO_2-co-solvent mobile phase may be used with all of them. As a result, two columns with different surface chemistry can be employed with the same operating conditions and provide orthogonal selectivity [4,20].

The present work aims at developing a rapid screening method for impurity profiling of drug candidates with SFC-ESI-MS. The first part presented in the previous paper focused 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 UV and ESI-MS responses. 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 provided by Servier Research Laboratories was evaluated. We finally settled our choice on a gradient elution of methanol comprising 2% water and 20 mM ammonium acetate [1].

The second part, described in the present paper, will focus on stationary phase selection to achieve orthogonal methods.

2. Material and methods

2.1. Chemicals, solvents and reagents

160 drug candidates were obtained from Servier Research Laboratories (Suresnes, France) whose structures are confidential. More details about the compounds selected can be found in the first part of this study. Ammonium acetate was obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France) and ultra-pure water was provided by an Elga UHQ system from Veolia (Wissous, 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, 23 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, 1.8 or 2.5 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, Phenomenex, Thermo, Supelco and Macherey-Nagel.

2.3. Instrumentation

The supercritical fluid chromatography system was a Waters Corporation (Millford, MA, USA) ACQUITY Ultra Performance Convergence Chromatography™ (UPC$^{2®}$). 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 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 peak width measurements. Waters Data