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Evaluation of innovative stationary phase ligand chemistries and analytical conditions for the analysis of basic drugs by supercritical fluid chromatography

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ABSTRACT

Similar to reversed phase liquid chromatography, basic compounds can be highly challenging to analyze by supercritical fluid chromatography (SFC), as they tend to exhibit poor peak shape, especially those with high pK_a values. In this study, three new stationary phase ligand chemistries available in sub –2 μm particle sizes, namely 2-picolylamine (2-PIC), 1-aminoanthracene (1-AA) and diethylamine (DEA), were tested in SFC conditions for the analysis of basic drugs. Due to the basic properties of these ligands, it is expected that the repulsive forces may improve peak shape of basic substances, similarly to the widely used 2-ethypyridine (2-EP) phase. However, among the 38 tested basic drugs, less of 10% displayed Gaussian peaks (asymmetry between 0.8 and 1.4) using pure CO_2/methanol on these phases. The addition of 10 mM ammonium formate as mobile phase additive, drastically improved peak shapes and increased this proportion to 67% on 2-PIC. Introducing the additive in the injection solvent rather than in the organic modifier, gave acceptable results for 2-PIC only, with 31% of Gaussian peaks with an average asymmetry of 1.89 for the 38 selected basic drugs.

These columns were also compared to hybrid silica (BEH), DIOL and 2-EP stationary phases, commonly employed in SFC. These phases commonly exhibit alternative retention and selectivity. In the end, the two most interesting ligands used as complementary columns were 2-PIC and BEH, as they provided suitable peak shapes for the basic drugs and almost orthogonal selectivities.

1. Introduction

Over the last few years, a number of technical improvements were brought to supercritical fluid chromatography (SFC) instrumentation [1]. This makes SFC approach on par with the most recent stationary phase technologies, such as columns packed with fully porous sub −2 μm particles which generate kinetic performance similar to those observed in UHPLC [2–5]. SFC is also valued among chromatographers for its low solvent consumption [6] and favorable applicability to preparative chromatography [7,8].

Modern SFC still has to cope with the challenge of basic compounds analysis, similarly to liquid chromatography (LC) some years ago [9,10]. Many molecules with basic pK_a values above 7 tend to exhibit poor peak shape when analyzed in CO_2/methanol mobile phase (i.e. tailing, broadening, shouldering, splitting peaks) [11], which can strongly compromise resolution and sensitivity. This behavior is related to the possible interactions of positively charged basic drugs with acidic silanols present at the surface of the stationary phase. This is particularly critical in the pharmaceutical industry, where a significant proportion of drugs present basic functionalities with high pK_a range (>8) as free bases.

Several solutions have been reported to tackle this issue, such as the use of specific columns designed to analyze basic compounds [12], but none of them has really emerged as a tremendous improvement in the manner of modern end-capped or polar embedded C18 column in RPLC [13]. Very few applications have been published for the analysis of basic drugs by SFC without mobile phase additives, and their use is warmly recommended to get symmetrical peaks [14–17]. These additives are believed to act in different ways, by increasing analytes' mobile phase solubility and by ion pairing mechanisms with the stationary phase surface as well as with the analytes [18]. However, the drawbacks of these additives are numerous: baseline drift with UV detection at low wavelength, adsorption at the surface of the stationary phase leading to undesired changes in selectivity, difficulty of evaporation on preparative scale, and reduction of sensitivity with mass

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Table 1
Average values for co-solvent percentage at elution, asymmetry and peak width at half height for the six SFC stationary phase chemistries with each of the three tested analytical conditions.

	Retention Average % of co-solvent at elution			Peak shape Average asymmetry			Peak Width Average peak width at half heights (s)		
	No AmF	AmF in inj. solvent	AmF in mob. phase	No AmF	AmF in inj. solvent	AmF in mob. phase	No AmF	AmF in inj. solvent	AmF in mob. phase
Acquity UPC^2 Torus 2-PIC	15.1	14.8	14.6	2.96	1.89	1.34	2.40	1.64	1.38
Acquity UPC^2 Torus DEA	11.1	11.1	11.7	2.95	2.32	1.41	2.69	1.99	1.66
Acquity UPC^2 Torus 1-AA	24.4	24.2	20.7	3.23	2.99	1.66	3.85	3.53	1.71
Acquity UPC^2 Torus Diol	20.8	20.6	16.2	5.44	4.25	1.47	4.46	3.82	1.48
Acquity UPC2 BEH 2-EP	16.7	16.5	16.3	5.24	4.39	1.83	4.07	3.37	1.53
Acquity UPC2 BEH	25	24.9	23.4	4.99	5.48	1.35	5.96	4.72	1.78

spectrometry [19,20]. Hence, the search for a ligand capable to perform "no additive SFC" is still in progress.

In this study, three new commercially available stationary phases were evaluated in "additive-free" analytical conditions and their overall performance in terms of retention and peak shape were compared. Additional experiments were also carried out in presence of ammonium formate (AmF) in the mobile phase. A set of 38 basic pharmaceutical drugs was purposely selected to cover a large range of chemical properties (log *P* and pK_a) with the majority of them having high pK_a values (above 8), as they represent the most problematic compounds to be analyzed. The possibility to place the additive in the injection solvent instead of in the organic modifier was also evaluated. Finally, the same experiments were performed with widely used SFC stationary phases, namely bare hybrid silica, DIOL and 2-ethylpyridine. The six columns were ranked according to their capability of giving Gaussian peaks and achievable selectivity.

2. Material and methods

2.1. Chemicals and reagents

Methanol (MeOH) HPLC grade was purchased from Fisher Scientific (Loughborough, UK). Isopropanol (IpOH) of ULC/MS grade was provided by Biosolve (Chemie Brunschwig, Basel, Switzerland) and heptane (extra dry 99%+) was purchased from Acros Organics (Geel, Belgium). Pressurized liquid CO_2, 3.0 grade, (99.9%) was purchased from PanGas (Dagmerstellen, Switzerland). Ammonium formate (AmF), ammonium acetate (AmAc), ammonium hydroxide (NH_4OH) and formic acid (FA) were purchased from Sigma-Fluka (Buchs, Switzerland).

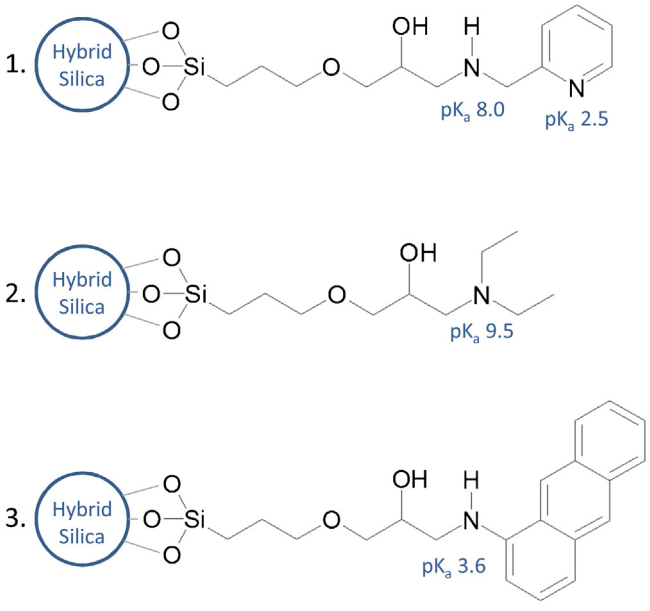

Fig. 1. Representation of the three new stationary phases available for UHPSFC and their respective calculated basic pK_a: 2-Picolylamine (1), diethylamine (2) and 1-Aminoanthracene (3).
Source: www.chemicalize.org

2.2. Instrumentation and columns

All experiments were performed on a Waters Acquity UPC^2 system (Waters, Milford, MA, USA) equipped with a binary solvent delivery pump, an autosampler that included a 10 μL loop for partial loop injection, a column oven, a UV detector fitted with an 8 μL flow-cell and a two-step (active + passive) backpressure regulator (BPR). The active component allows fine backpressure adjustments, while the passive component maintains pressure higher than 104 bar. Different wavelengths were monitored: 210, 220, 254 and 280 nm to systematically work at the best absorbance for each compound. The extra-column volume of the system was measured at 59 μL, while the gradient delay volume was 440 μL. Data acquisition, data handling and instrument control were performed by Empower v. 4.1 software (Waters).

The four Acquity UPC^2 Torus columns employed in this study were a generous gift from Waters: Acquity UPC^2 Torus 2-Picolylamine (2-PIC), Acquity UPC^2 Torus Diethylamine (DEA), Acquity UPC^2 Torus 1-Aminoanthracene (1-AA) and Acquity UPC^2 Torus DIOL. The two other columns, namely Acquity UPC^2 BEH 2-ethylpyridine (2-EP) and Acquity UPC^2 BEH, were purchased from Waters. All these UHPSFC columns have dimensions of 100 × 3.0 mm and particle size of 1.7 μm.

2.3. Analytical procedure

A set of thirty-eight basic compounds was chosen to evaluate the performance of the new stationary phases. This set included purposely different pharmaceutical families such as benzodiazepines, narcotics, stimulants, antidepressants, anesthetics, beta-blockers, antipsychotics and antihistaminics, and covered a broad spectrum of chemical properties (pK_a and log *P*, as shown in Table S-1 and Fig. S-1). Midazolam (1), bromazepam (2), diazepam (3), alprazolam (4), triazolam (5), nortriptyline hydrochloride (6), oxycodone hydrochloride (7), morphine hydrochloride (8), codeine hydrochloride (9), ephedrine hydrochloride (10), cocaine hydrochloride (11) and methadone hydrochloride (12) were purchased from Lipomed (Arlesheim, Switzerland), whereas noscapine hydrochloride monohydrate (13), papaverine hydrochloride (14), pethidine hydrochloride (15), thebaine hydrochloride (16), fentanyl citrate (17), dextromethorphan hydrochloride (18), naloxone hydrochloride dihydrate (19), buprenorphine hydrochloride (20), methylphenidate hydrochloride (21), imipramine hydrochloride (22), amitriptyline hydrochloride (23), procaine hydrochloride