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Short communication

Comparison of chromatographic techniques for diastereomer separation of a diverse set of drug-like compounds

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ABSTRACT

Preparative supercritical fluid chromatography (SFC) has proven value for isocratic bulk separation of both diastereomers and enantiomers. With the recent availability of SFC equipment suitable for rapid gradient separation [Ebinger et al. JALA (2011) 16 (3) 241], we have become interested in comparing the effectiveness of traditional reverse phase high performance liquid chromatography (HPLC) with SFC using non-chiral columns for the separation of diastereomeric mixtures. The success rates for separation of a diverse set of 258 synthetic diastereomer pairs were used to determine the relative utility of the aforementioned two techniques. Our results suggest that gradient non-chiral SFC was more successful than the traditional non-chiral HPLC technique for diastereomer separations of the diverse sample set of 258 drug-like compounds.

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1. Introduction

Numerous studies have been reported demonstrating that typical drug-like compounds can be chromatographed and detected by gradient supercritical fluid chromatography (SFC) as well as by high performance liquid chromatography (HPLC) [1–4]. Previous work by our research group compared the value of achiral SFC and HPLC techniques by analyzing the success rate for separation of target drugs from associated impurities in over 1000, mostly achiral, crude reaction mixtures [4]. In light of the importance of diastereomer separation in the pharmaceutical industry, a new study was designed as a continuation of the previous evaluation, with focus on the separation of mixtures of diastereomers. The primary goal of the current study was to determine the relative utility of two techniques, non-chiral SFC and non-chiral HPLC, as measured by success rate rates for separation of 258 synthetic drug-like diastereomeric mixtures using two stationary phases per technique.

2. Experimental

A collection of 258 synthetic research samples containing diastereomeric pairs from ongoing drug discovery synthesis at Bristol-Myers Squibb Company was selected for evaluation. Compounds used in the present study were purified using common techniques (i.e. normal phase flash chromatography, reverse phase HPLC, ion exchange chromatography, etc.). Diastereomers were typically not separated, either intentionally or due to separation difficulty. The presence of diastereomers in each sample was indicated by chiral chromatography or NMR.

SFC-MS analysis was performed on a Waters Thar SFC Resolution analytical system controlled by Waters MassLynx 4.1 software (Waters Corporation, Milford, MA, USA).Analytical HPLC–MS analysis was performed on a Waters MassLynx 4.0 driven analytical LC–MS system equipped with a Waters ZQ mass spectrometer CTC-Leap HTS-PAL autosampler, an Agilent 1100 quaternary pump and, and an Agilent 1100 photodiode array (PDA) detector.

Stationary phases for HPLC separation were unused prior the experiment. In light of literature data [5–7], suggesting that new columns used in SFC elution should be pre-conditioned with additive, all SFC columns were equilibrated with 100 column volumes of 10 mM ammonium acetate in methanol. The necessity of excessive column volume of mobile phase for pre-equilibration was suggested by the results of injection repeatability tests (unpublished data of our laboratory).

All columns had the dimensions of 4.6 mm × 150 mm and were packed with 5-μm particles unless stated otherwise in Table 1. Columns were purchased from the following manufacturers:

Princ. EP (PrincetonSFC 2-Ethylpyridine), Princ. HA-Dipyr (PrincetonSFC HA-Dipyridyl) from Princeton (Princeton Chromatography, Cranbury, NJ, USA), XBridge C18 (XBridge™ C18),

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Table 1
Stationary phases used in the study.

Technique	Column	Nature of the stationary phase	End capped	Gradient range (% organic)	Gradient time (min)	Flow rate (ml/min)	Peak capacity
Achiral HPLC	XBridge C18	Trifunctional C18	Yes	20–95	11	1.2	137
Achiral HPLC	Syn. Polar RP	Ether embedded phenyl	Yes polar	20–95	11	1.2	137
Achiral SFC	Zorbax Rx Sil	Silica gel	n/a	5–60	6	5	150
Achiral SFC	YMC PVA Sil	Polyvinyl alcohol coated silica gel	n/a	5–60	6	5	143
Achiral SFC	Xbridge HILIC	Silica gel ethylene bridged hybrid	n/a	5–60	6	5	158
Achiral SFC	Luna HILIC	Cross-linked diol bonded silica gel	No	5–60	6	5	120
Achiral SFC	Zorbax SB-CN	Cyanopropyl-bonded silica gel	No	5–60	6	5	120
Achiral SFC	Zorbax NH2	Aminopropyl-bonded silica gel	No	5–60	6	5	143
Achiral SFC	Princ. EP	2-Ethylpyridine bonded-silica gel	No	5–60	6	5	143
Achiral SFC	Princ. HA-Dipyr	HA-dipyridyl bonded silica gel	No	5–60	6	5	114
Achiral SFC	Syn. Polar RP	Ether embedded phenyl	Yes polar	5–60	6	5	141
Achiral SFC	Cosmosil PYE	2-Pyrenyl-ethyl	Yes	5–60	6	5	113

Xbridge HILIC (XBridge™ HILIC) from Waters (Waters Corporation, Milford, MA, USA), Syn. Polar RP (Synergi 4 um Polar RP), Luna HILIC (Luna 5 μm HILIC) from Phenomenex (Phenomenex Torrance CA, USA), Zorbax Rx Sil, (Zorbax Rx-SIL), Zorbax SB-CN (Zorbax SB-CN), Zorbax NH2 (Zorbax NH2) from Agilent (Agilent Technologies, Wilmington, DE, USA), Cosmosil PYE, (Cosmosil 5PYE) from Nacalai (Nacalai USA Inc., San Diego, CA, USA), YMC PVA Sil (YMC-Pack PVA-SIL-NP) from YMC America (Allentown, PA, USA).

For all SFC/MS separations, the mobile phase A was carbon dioxide and mobile phase B was methanol with 10 mM ammonium acetate additive. Temperature was 35 °C with outlet pressure at 100 bar. For all achiral HPLC/MS separations, the mobile phase A was water with 10 mM ammonium acetate, mobile phase B was methanol with 10 mM ammonium acetate. Acetonitrile as a possible choice of mobile phase for HPLC separation was briefly evaluated. The separation success rate of 33 diasteromeric mixtures using both methanol and acetonitrile organic modifiers was compared. The result of this comparison, along with literature data [8], did not favor either of the two organic modifiers, therefore, to keep the mobile phases as consistent as possible between the two techniques, the methanol was selected. Due to the significant differences in column volumes of mobile phase traditionally used for elution by the two techniques, gradient times and flow rates for HPLC and SFC were chosen in an attempt to approximately match peak capacities [9], while still being representative of typical high throughput analysis methods. Peak capacity (PC) was measured using a standard test mix, across two columns used in HPLC and ten columns used in SFC with methods shown in Table 1. Test mixture of Sulconazole, Caffeine, Bendroflumethiazide, Propranolol and Perphenazine were used for PC calculation in SFC, and test compounds of Vanillin, Prednisolone, Bendroflumethiazide, Flavone and Phenylurea was used for PC calculation in HPLC. The same column geometry, particle size, and methods resulting in similar peak capacity, along with the same choice of buffer and organic solvent ensured the pronounced influence of column selectivity on resolution. All data files were analyzed using Waters MassLynx OpenLynx software, and the retention times were obtained from UV trace at wavelength 220 nm. Baseline resolution (i.e. Rs > 1.5) of each band-pair is preferred for easy scaling for purification, therefore Rs > 1.5 was used to define a successful separation and enable comparison of the success rate of the separation on each stationary phase [10].

3. Results and discussions

3.1. Column selection process

To develop orthogonal separation two columns with different selectivity [11–14] were chosen for achiral HPLC separation. Considering the frequency of use in our laboratory a traditional C18 stationary phase, Xbridge C18 and a phenyl column with embedded polar group (Polar RP) were selected. Our previous paper [4] suggested the need for further research to identify a suite of columns most appropriate for SFC separation. Therefore, before narrowing the field to two columns, a collection of ten SFC columns was chosen for an initial performance scan. The ten columns, as shown in Table 1, were selected considering orthogonality [15–17] and using previous results of injection repeatability tests conducted in our laboratory.

The performance of these ten columns for isomer separation was evaluated by the separation success rate of a small diverse set of 33 diastereomeric mixtures. Resolution of 1.5 or greater, providing sufficient separation for transfer to preparative scale, was defined as the measure of successful separation [4].

The separation success rate of the 33 compounds is summarized in Fig. 1. Effective separations (% of compounds separated by Rs > 1.5) were the most pronounced on non-end-capped amine bonded (67%), on bare silicas (Rx Sil: 73%, XBridge HILIC: 76%), and on Cosmosil PYE (91%) columns. Bare rigid silica columns offered better results for isomer separation than the polar-bonded stationary phases due to the fixed position of the strong silanol adsorption sites of the silica surface [18–20]. The main active sites of the polar-bonded stationary phases are the polar groups positioned to the silica surface by a flexible, usually propyl linker. The partially deactivated silanol sites, the less polar than the bare silica-gel nature, and the decreased rigidity of the polar-bonded silica surface, usually reduce the selectivity of polar-bonded columns for isomer separation [19–24]. The increased separation success rate on the amino-propyl (67%) bonded phases vs. the cyano-propyl column (0%) suggests the positive effect of the strong hydrogen-bond acceptor ability [23–26] of the stationary phase on the isomer separation.

Columns with sterically shielded silica surface such as the Princeton HA-dipyridyl, or diol-bonded stationary phases (less

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