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Short communication

Ultrafast separation of fluorinated and desfluorinated pharmaceuticals using highly efficient and selective chiral selectors bonded to superficially porous particles

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ABSTRACT

The separation of fluorinated active pharmaceutical ingredients (APIs) from their desfluoro analogs is a challenging analytical task due to their structural similarity. In this work, fluorine containing APIs and their corresponding desfluorinated impurities were separated on five new 2.7 μm superficially porous particles (SPPs) functionalized with bonded chiral selectors. The unique shape selectivity of bonded macrocyclic glycopeptides and oligosaccharides was utilized to separate seven pairs of fluoro/desfluoro APIs resulting in some unprecedented selectivity values. For example, SPP bonded isopropyl cyclofructan 6 yielded a selectivity of 2.73 for voriconazole and desfluoro voriconazole. Further, the SPP based columns allowed for rapid separations ranging from 9 to 55 s with very high efficiencies ranging from 45,000 to 70,000 plates/m (at high flow rates) in both reversed phase and polar organic modes. Chromatographic separation and detection by HPLC-ESI-MS was demonstrated using ezetimibe and voriconazole and their desfluorinated impurities. Among the tested phases, SPP hydroxypropyl-β-cyclodextrin separated the most fluorinated and desfluorinated analogs with baseline resolution.

1. Introduction

Medicinal chemists frequently alter the chemical structure of natural products to produce new active pharmaceutical ingredients (APIs) with modified biological activity. This strategy often reduces side effects while increasing bioavailability [1]. One of the common strategies to increase the biological activity and bioavailability of an API is to incorporate fluorine atom(s) in the drug's structure which in turn increases the lipophilic character of the API [2,3]. Approximately, one fifth of all Food and Drug Administration (FDA) approved drugs, which are available in the market, contain at least one fluorine atom [3,4]. During the synthesis of fluorinated pharmaceuticals, desfluoro impurities are often generated by hydrodehalogenation from palladium coupling or catalytic hydrogenation reactions [5]. These impurities can contaminate the API with the desfluoro analogue and persist throughout purification steps. Recently, Atici and Karlığa reported that desfluoro ezetimibe, which is one of the impurities generated during the synthesis of the plasma cholesterol lowering drug ezetimibe, was observed consistently in the range of 0.05–0.15% in the final product [6]. Since fluorine and hydrogen have similar sizes and electronic structures, chromatography using ordinary C18 chemistries is often unable to separate these desfluorinated impurities causing them to co-elute with the major product of interest.

To date, only a few publications directly address the separation of fluorinated and desfluorinated mixtures [6–11]. Impurities in the fluorinated drug atorvastatin were separated on a C18 column using reverse phase (RP) gradient elution with THF as the organic modifier [7]. The typical retention time was 30 min for atorvastatin and desflourinated atorvastatin. Alternatively, Turco et al. showed detection and quantification of desfluorinated impurities in casopitant mesylate using ^{19}F NMR and LC–MS [8]. The authors utilized HPLC coupled with ESI-MS and the typical retention time for separation was approximately 4–6 min using RP mode. In 2015, Welch et al. performed a comprehensive study of 132 mobile phase and

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column combinations for the separation of 8 fluorinated APIs and their desfluoro analogues [9]. The authors found that perfluorinated stationary phases produced the best separations in most cases. Sub-2 μm fully porous perfluoro phenyl (PFP) columns were used to achieve separation times in the range of 1.5–4 min. The authors also showed the compatibility of the perfluorinated stationary phase using UHLPC coupled with high resolution electrospray ionization mass spectrometry [10]. Recently, Regalado et al. used 16 fluorinated stationary phases and showed faster separations ranging from 0.4 to 5 min and selectivity values in the range of 1.07–1.75 [11]. For a comprehensive review on fluorinated stationary phases the readers can consult references [12,13].

It was recognized in the early 1980s that macrocyclic oligosaccharides bonded on silica can not only act as chiral phases but they also possesses excellent shape selectivity for diastereomers and structural isomers [14–20]. The authors separated hundreds of achiral compounds from their isomers using hydrolytically stable β-cyclodextrin bonded columns. Using the same column chemistry, nicotine and thirteen structurally related compounds were separated [21]. In 1994, macrocyclic glycopeptides such vancomycin, teicoplanin, and teicoplanin aglycone were introduced as a chiral selectors and it was shown that these chiral selectors have good selectivity for amino acids, β-blockers and heterocyclic compounds [22–24]. In addition these chiral selectors were advantageous in separating diastereomers and large peptides [25,26]. When the same chiral selectors were bonded to 2.7 μm superficially porous particles (SPPs), similar separations could be achieved under 30 s [27–29]. Recently, more than 60 such ultrafast high resolution separations were shown in short 3–5 cm columns [27,30]. Such fast separations are highly advantageous for drug screening where hundreds of pharmacologically active compounds have to be evaluated.

In this work the unique selectivity of vancomycin, teicoplanin, cyclofructan, and hydroxypropyl-β-cyclodextrin bonded on 2.7 μm SPPs was evaluated for the separation of fluorinated and desfluorinated compound mixtures. Given the unusual selectivity of the above mentioned stationary phases, it is logical to test the capability of these columns for ultrafast separation of fluorinated drugs and their desfluorinated impurities. To best of our knowledge this is first example where chiral selectors bonded to SPP supports are employed for the separation of desfluoro impurities from fluorinated APIs.

2. Reagents and materials

Methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), triethylamine (TEA), trifluoroacetic acid (TFA) and ammonium acetate (NH_4OAc) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solvents utilized for separation purpose were HPLC grade or better. Ultrapure water was obtained from Milli-Q water purification system (Millipore, Billerica, MA, USA). Atorvastatin sodium, desfluoro atorvastatin sodium, voriconazole, desfluoro voriconazole, paroxetine, desfluoro paroxetine, ciprofloxacin, desfluoro ciprofloxacin, ofloxacin, desfluro ofloxacin, aprepitant, desfluoro aprepitant (structures shown in Fig. 1), were purchased from Molcan Corporation (Ontario, Canada). The 2.7 μm superficially porous silica particles with an inner core diameter of 1.7 μm and surrounding shell thickness of 0.5 μm were provided by Agilent Technologies (Wilmington, DE). These silica particles have a surface area of 120 m^2/g and pore size of 120 Å. Vancomycin SPP (5 cm and 10 cm × 0.46 cm), teicoplanin SPP (5 cm × 0.46 cm), cyclofructan (CF 6) SPP (15 cm × 0.46 cm), isopropyl bonded cyclofructan (CF6-P) SPP (10 cm × 0.46 cm) and hydroxyl-β-cyclodextrin (RSP) SPP (5 cm × 0.46 cm) chiral columns utilized in these studies were provided by AZYP LLC (Arlington, TX, USA). The commercial Chirobiotic V (10 cm × 0.46 cm) column used for comparative purposes was obtained from Supelco (Sigma–Aldrich).

2.1. Instrumentation

A 1290 UHPLC system from Agilent technologies, Santa Clara, CA was used for all ultrafast separations. The instrument was equipped with an autosampler (G4226A), quaternary pump (G4204A) and a diode array detector (G4212A). For ultrafast separations, it was necessary to achieve low volume dispersion to reduce extra column effects by using 0.075 mm i.d. connection tubing (Agilent Technologies, USA) and an ultralow dispersion kit (Agilent, P/N 5067-5189). This kit includes two stainless steel capillary (0.075 mm × 220 mm and 0.075 mm × 340 mm) and low dispersion needle seat assembly (P/N G4226-87020). To minimize peak broadening from detection electronics, highest possible sampling frequency 160 Hz and shortest response time 0.016 s were used. The instrument was controlled by OpenLAB CDS ChemStation software (Rev. C.01.06 [61], Agilent Technologies 2001–2014) in Microsoft Windows 8.1. Before performing separations all solvents were degassed using sonication under vacuum. Reverse phase chiral HPLC-ESI-MS experiments were performed on Shimadzu LCMS 2020 system (Shimadzu Corporation, Kyoto, Japan) with two LC-20AD pumps, SIL-20AC autosampler, CBM-20A HPLC system controller, SPD-M20A photodiode array detector and LCMS 2020 mass spectrophotometer. The ESI-MS was a single quadrupole mass analyzer. The software to control the mass spectrometer was Shimadzu Labsolution LCMS 5.1.

3. Results and discussion

The drugs selected for these studies are FDA approved APIs and are commercially available (Fig. 1). All drugs are fluorine containing molecules and their corresponding hydrogen containing analog is referred to as their desfluoro impurity. Separation of these desfluoro impurities is very challenging considering the drug and its desfluro analogue have very similar structures. In order to achieve ultrafast separations while maintaining baseline resolution between the drug and the impurity, the combination of ultrahigh efficiency superficially porous supports and the unique selectivity of chiral macrocyclic glycopeptides or oligosaccharides were used to great effect.

3.1. Performance comparison of SPP bonded selectors with commercially available columns

The state of art oligosaccharide and glycopeptide chiral stationary phases (CSPs) utilize 5 μm fully porous silica particles. Fig. 2 provides a comparison of a commercially available 5 μm Chirobiotic V column with the vancomycin SPP column. In Fig. 2A the same mobile phase and flow rates were used whereas in Fig. 2B mobile phase for the Chirobiotic V column was adjusted to achieve the same retention time as the vancomycin SPP column. Better peak shapes were observed with the vancomycin SPP column compared to the Chirobiotic V column in both cases. The USP tailing factor was 1.9 with the vancomycin SPP column compared to 2.4 and 2.1 (at constant mobile phase and constant retention time, respectively) with the Chirobiotic V column. Interestingly, a 4× increase in plate count and a resolution increase from 1.4 to 1.8 was observed for the vancomycin SPP column compared to commercially available column of the same length at constant mobile phase (see Fig. 2A). The increase in resolution on the SPP column is due to the high efficiency of the SPPs rather than selectivity, because the same selectivity was observed for both vancomycin columns under constant mobile phase conditions.

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