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(54) Title: HIGH EFFICIENCY, ULTRA-STABLE, BONDED HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC) MATRIX ON SUPERFICIALLY POROUS PARTICLES (SPPS)
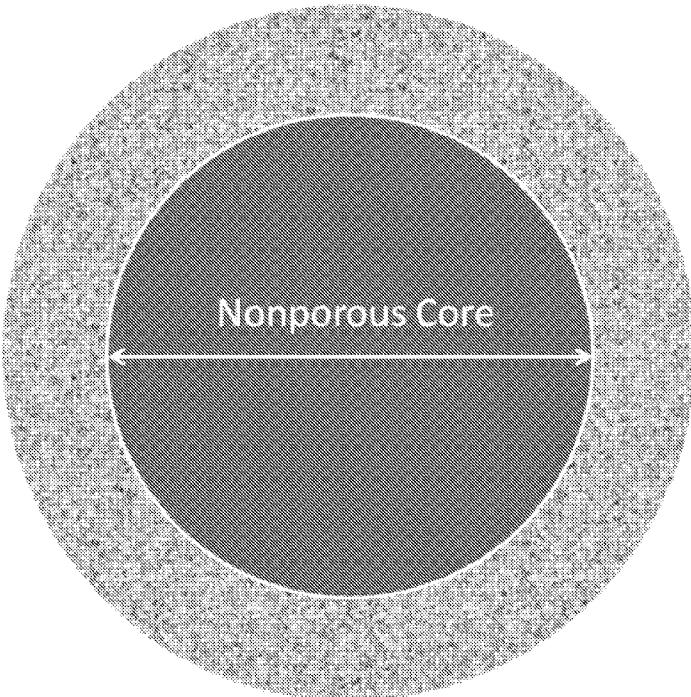
(57) Abstract: The present invention relates to superficially porous particles (SPPs), also called core-shell, porous shell or fused core particles, which are state-of-the-art support materials used in the production of HPLC columns. Hydrolytically stable, highly selective superficially porous particle (SPP) hydrophilic interaction liquid chromatographic (HILIC) stationary phases having higher efficiencies and shorter retention times than analogous stationary phases on fully porous particles (FPP) is provided.

FIG. 1

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HIGH EFFICIENCY, ULTRA-STABLE, BONDED HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC) MATRIX ON SUPERFICIALLY POROUS PARTICLES (SPPs)

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority from US Provisional Application No. 62/025,990 filed July 17, 2014, the contents of which is incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention relates to superficially porous particles (SPPs), also called core-shell, porous shell or fused core particles, which are state-of-the-art support materials used in the production of HPLC columns.

Historically, the concept of shell particles was firstly proposed by Horvath *et al.* during the 1960s and they were developed as ion exchange materials for the analysis of large biological molecules. See, e.g., J.J. Destefano, T.J. Langlois and J.J. Kirkland, J. Chromatogr Sci. **46** (2008) 254, X.L. Wang, W.E. Barber and W.J. Long, J. Chromatogr. A. **1228** (2012) 72, F. Gritti and G. Guiochon, LCGC North America. **7** (2012) 586 and C.G. Horvath, B.A. Preiss and S.R. Lipsky, Anal Chem. **39** (1967) 1422, the contents of each of which are incorporated herein by reference.

SPP technology was advanced by Kirkland, who prepared 50 μm particles in the 1970s and 5 μm particles in the 1990s. See e.g., J.J. Kirkland, Anal Chem. **41** (1969) 218, J.J. Kirkland, Anal Chem. **43** (1971) A36 and J.J. Kirkland, Anal Chem. **64** (1992) 1239, the contents of each of which are incorporated by reference.

Concurrent improvements in the manufacturing of high-quality fully porous particles (FPP) inhibited the manufacture, application, and use of SPPs. See e.g., B. Chauve, D.

Guillarme, P. Cleon and J.L. Veuthey, J Sep Sci. **33** (2010) 752, the contents of which is

incorporated by reference. FPPs with diameters of 3 μm (1990s) and sub-2 μm (2004) came in

vogue along with liquid chromatographs that could operate at higher pressures (i.e., 1000 bar).

However, recent improvements to SPP technology have moved them to the forefront of HPLC

5   packing materials. For example, columns are now available with SPP sizes of 1.7, 2.6 or 2.7 μm

and porous shell thicknesses of 0.23, 0.35 and 0.5 μm, respectively. See, e.g., S. Fekete, E. Olah

and J. Fekete, J. Chromatogr. A. **1228** (2012) 57, the contents of which is incorporated herein by

reference.

          This generation of SPP markedly improved chromatographic performance, due its

10   morphology, which consists of a solid inner core surrounded by a porous layer. See, e.g., Figure

1. SPPs are able to decrease all contributions to band broadening including those from

longitudinal diffusion, eddy dispersion and stationary phase mass transfer contributions. The

presence of the solid core leads a to shorter path for analytes to travel and decreases band

broadening caused by poor mass transfer leading to the ability to carry analysis out under high

15   flow rates without a significant loss in efficiency. Further, SPPs are generally produced with very

narrow particle size distributions and decrease band broadening due to eddy diffusion. Columns

packed with superficially porous particles have been used for high throughput separations by

improving efficiency while keeping methods robust. See, e.g., A. Periat, I. Kohler, J.L. Veuthey

and D. Guillarme, LCGC Europe. **5** (2013) 17, the contents of which is incorporated herein by

20   reference.

          In recent years, the number of publications involving HPLC columns based on SPPs has

increased. See, e.g. J. Ruta, D. Zurlino, C. Grivel, S. Heinisch, J.L. Veuthey and D. Guillarme,

Journal of Chromatography A. **1228** (2012) 221, J.J. Kirkland, S.A. Schuster, W.L. Johnson and

B.E. Boyes, Journal of Pharmaceutical Analysis. **3** (2013) 303, D.V. McCalley, J. Chromatogr. A. **1193** (2008) 85, E. Olah, S. Fekete, J. Fekete and K. Ganzler, J. Chromatogr. A. **1217** (2010) 3642, F. Gritti, A.D. Pereira, P. Sandra and G. Guiochon, J. Chromatogr. A. **1217** (2010) 683 and F. Gritti, I. Leonardis, D. Shock, P. Stevenson, A. Shalliker and G. Guiochon, Journal of

5   Chromatography A. **1217** (2010) 1589, the contents of each of which are incorporated herein by reference.

Many to relatively few SPP HILIC columns can be purchased from different companies, but the vast majority of the marketed HILIC packing material is simply unmodified silica. See, e.g. Fekete, E. Olah and J. Fekete, J. Chromatogr. A. **1228** (2012) 57. Silica gel does not always

10   offer acceptable HILIC separations. See e.g., H.X. Qiu, L. Loukotkova, P. Sun, E. Tesarova, Z. Bosakova and D.W. Armstrong, J. Chromatogr. A. **1218** (2011) 270 and H. Qiu, et al., J. Chromatogr. A. **1218** (2011) 8075-8082, the contents of each of which are incorporated herein by reference.

Furthermore it is not stable when utilized with the water containing mobile phases

15   necessary for HILIC separations. This instability manifests itself by the slow dissolution of the underlying silica and the ever changing retention of the separated analytes (see Table 1 in Experimental). This is completely unacceptable for a commercial HILIC stationary phase. Further this trend is even more critical for SPPs. Thus, it is both timely and important to produce and evaluate newer, more promising, HILIC separating agents on SPP supports (see, e.g. Figures 1-3).

20   As is shown, this is accomplished by using ultra stable, covalently bonded HILIC phases that both protect the surface from dissolution and have broader HILIC selectivities and higher efficiencies than bare supports like silica or other unprotected or less protected supports.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: schematic showing of a superficially porous particle (SPP), a.k.a. core-shell particle (nonporous core surrounded by porous shell).

5   Figure 2: transmission electron micrograph of a superficially porous particle. The ratio of the non-porous core diameter to the total particle diameter is defined as ρ.

Figure 3: scanning electron micrographs of examples of 3 different diameter superficially porous particles.

Figure 4: examples of cyclofructans and cyclofructan derivatives.

10   Figure 5: examples of cyclodextrins and cyclodextrin derivatives.

Figure 6: examples of macrocyclic glycopeptides.

Figure 7: examples of ionic HILIC selectors.

Figure 8: examples of a zwitterionic HILIC selector.

Figure 9: example of the use of non-conventional silanes to form hydrolytically stable, high

15   efficiency, bonded zwitterionic SPP HILIC phases.

Figure 10: example of the use of multipoint attachment to form hydrolytically stable, high efficiency, bonded ionic, derivatized cyclofructan, SPP HILIC phases.

Figure 11: retention time drift of bare SPP silica.

Figure 12: retention time drift when using non-conventional silanes to form hydrolytically stable,

20   high efficiency, bonded zwitterionic SPP HILIC phases.

Figure 13: retention time drift when using multipoint attachment to form hydrolytically stable, high efficiency, bonded ionic, derivatized cyclofructan, SPP HILIC phases.

Figure 14: separation of the polar analytes 5-phenylvaleric acid and ferulic acid in HILIC.

DETAILED DESCRIPTION OF THE INVENTION

In the present application, HILIC stationary phases based on native and derivatized cyclofructans, cyclodextrins, macrocyclic antibiotics and zwitterions were evaluated for selectivity and stability when bonded to SPPs as the support material (see Fig. 1-3). The columns were also

tested in the normal phase (NP) mode, to evaluate the influence of aqueous and non-aqueous containing mobile phases in performance. Results in terms of stability, efficiency, analysis time and resolution were evaluated, demonstrating clear advantages of the new HILIC columns based on SPPs.

5   Further, such HILIC bonded SPPs can be produced on SPPs that range in size from about 0.5 to about 20 micron in total particle diameter, preferably from about 1.3 to about 10 micron, more preferably from about 1.7 to about 5.0 micron. SPP-CSPs according to the invention can be produced on SPPs having total particle diameter of, for example, about 0.5 micron, about 0.6 micron, about 0.7 micron, about 0.8 micron, about 0.9 micron, about 1.0 micron, about 1.1 micron,

10   about 1.2 micron, about 1.3 micron, about 1.4 micron, and so on. Scanning electron micrographs of some particles, e.g. about 1.7, about 2.7 and about 4.0 micron, are shown in Figure 3.

Native cyclofructan-6 (CF6) has been reported to be a powerful selector in separation of polar compounds in the HILIC mode. The column based on CF6 chemically bonded to FPP is commercially available (FRULIC-N) and it has demonstrated advantages over popular

15   commercial columns in separating several compounds such as nucleic acid bases, nucleosides, nucleotides, xanthines, β-blockers, carbohydrates, etc.

Likewise, cyclodextrins and derivatized cyclodextrins (CDs) are shown to make exceptional HILIC phases. Two other types of bonded HILIC selectors included are the zwitterionic types (i.e., those containing both cationic and anionic moieties) and the macrocyclic

20   antibiotic types like vancomycin. See, e.g. R. J. Soukup-Hein, et al. Chromatographia **66** (2007) 461 and US 4,539,399, the contents of each of which are incorporated herein by reference. Table 1 list the classes of HILIC selectors which are presented in this application. Representative/example structures for each class of HILIC selector are shown in Figure 4-8.

Table 1 – list of the classes and types of HILIC selectors. Figures 4-8 provide structures.

Bonded brush-type HILIC Selector Class	Examples of HILIC Selectors
Carbohydrates, oligosaccharides, cyclic oligosaccharides, and their derivatives	Cyclodextrins
	Derivatized cyclodextrin
	Cyclofructans
	Derivatized cyclofructans
Peptides, glycopeptides, macrocyclic glycopeptides, and their derivatives	Teicoplanin
	Vancomycin
	Teicoplanin aglycone
	Ristocetin A
	Dalbavancin
	Boromycin
Ionic	Sulfonated cyclofructans and cyclodextrins
Zwiterionic	3-P,P-diphenylphosphoniumpropylsulfonate

The present invention provides for the preparation of the first hydrolytically stable, covalently bonded, highly selective SPP HILIC stationary phases. Further these SPP HILIC stationary phases have higher efficiencies and shorter retention times than the analogous stationary phases on fully porous particles (FFPs)

EXAMPLES

Anhydrous N,N-dimethylformamide (DMF), anhydrous toluene, anhydrous pyridine, 3-(triethoxysilyl)propylisocyanate, ammonium acetate (NH_4OAc), trifluoracetic acid (TFA) and all analytes tested in this work (5-phenylvaleric acid, ferulic acid, pyridoxine, L-ascorbic acid, uracil, adenosine, cytosine, thymidine 3':5'cyclic monophosphate (cTMP), adenosine 2':3'cyclic

monophosphate (cAMP), guanosine 2':3'cyclic monophosphate (cGMP), and cytidine 2':3'cyclic

monophosphate (cCMP) were purchased from Sigma–Aldrich (Milwaukee, WI). The 1,2-

bis(trichlorosilyl)ethane was obtained from Aldrich. The 1,3-bis(chlorodimethylsilyl))propane and

bis (3-trimethoxysilylpropyl)amine were obtained from Gelest (Morrisville, PA). The CF6,

5   cyclodextrins and macrocyclic glycopeptides were provided by AZYP, LLC (Arlington, TX).

Acetonitrile (ACN), heptane (Hep), isopropyl alcohol (IPA) and ethanol (EtOH), used for the

chromatographic separations, were obtained from EMD (Gibbstown, NJ). Water was purified by

a Milli-Q Water Purification System (Millipore, Billerica, MA).

All the chromatographic separations were conducted on an Agilent HPLC series 1260

10   system (Agilent Technologies, Santa Clara, CA), equipped with a quaternary pump, an

autosampler and a multiwavelength UV–Vis detector. For data acquisition and analysis, the

Chemstation software version Rev. B.03.02 [341] was used. The injection volume was 0.5 μL for

all analyses. The temperature was maintained at 30 °C. The mobile phases (MP) used in the HILIC

mode were composed of 75-95% of ACN and 5-25% of 25 mM NH_4OAc, except for the cyclic

15   nucleotides, for which the MP was composed of ACN/100 mM NH_4OAc (70/30, v/v). For the

separations carried out in the normal phase, Hep/IPA and Hep/EtOH in different ratios were used

and 0.1% of TFA was added to the EtOH phase for the analysis of ferulic acid.

Native CF6 was chemically bonded to silica gel according to the disclosure of H.X. Qiu,

20   L. Loukotkova, P. Sun, E. Tesarova, Z. Bosakova and D.W. Armstrong, J. Chromatogr. A. **1218**

(2011) 270. The cyclodextrin based CSPs were synthesized according to D.W. Armstrong as

disclosed in US 4,539,399. The macrocyclic antibiotic CSP were synthesized according to D.W.

Armstrong as disclosed in US 5,626,757, the content of which is incorporated herein by

reference. The zwitterionic stationary phases were synthesized according to the disclosure of

Qiu, et al., Journal of Chromatography A **1218** (2011) 8075-8082.

The following examples are syntheses for the preparation of a variety of stable, bonded,

5   superficially porous particle (SPP) based HILIC stationary phases.

<u>Example 1 - Preparation of cyclofructan based stable, bonded, SPP HILIC stationary phases</u>

Cyclofructans are cyclic oligosaccharides that possess 18-24 hydroxyl groups. These

hydroxyl groups can be used as reactive functionalities to covalently bond the cyclofructan (or

cyclofructan derivative) to SPP silica. Cyclofructans can be used as HILIC selectors in their native

10   form or in a derivatized format. Derivatization of the cyclofructan molecules can take place before

or after they are immobilized on the SPP silica. The derivatizing groups are typically, but not

limited to: linear alkane C_1-C_{30}, branched alkane C_1-C_{30}, unsaturated alkane C_1-C_{30}, cyclic alkane

C_1-C_{30}, linear and/or cyclic alkane containing heteroatoms (e.g. N, S, O) C_1-C_{30}) and are bonded

to the cyclofructan by a number of chemical linkages (e.g. ether, carbamate, thioether,

15   thiocarbamate, ester, triazole, and urea).

Native and derivatized cyclofructans are linked to SPP silica in the same way. First, in a

250 mL round-bottom flask cyclofructan (3 mmol) was dissolved in anhydrous DMF (60 mL)

under and argon blanket. Then, 3-triethoxysilylpropyl isocyanate (12 mmol) and anhydrous

pyridine (5 mL) were added and the reaction vessel was heated to 90 °C for 5 hours. Meanwhile,

20   the SPP silica (4 grams) was first dried in an oven (120 °C) for 4 hours and later azeotropically

distilled (toluene, 125 mL) using a Dean-stark trap and a 250 mL, 2-neck round-bottom flask. Once

both reaction vessels were allowed to cool to room temperature, the cyclofructan/DMF solution

was added to the SPP silica-toluene slurry, and the resulting suspension was refluxed for 16 hours.

After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). This method gives a carbamate linked cyclofructan HILIC phase. The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube. Alternatively, a second binding chemistry which also forms a carbamate linker can be employed.

5   Here, SPP silica (3 grams) was dried at 120 °C for 4 hours. Next, toluene was added and residual water was removed using a Dean-stark trap to azeotropically distill the toluene-SPP silica slurry. The suspension was allowed to cool (<40 °C) and 1 mL of (3-aminopropyl)triethoxysilane was added to the silica (3.3 grams)-toluene (125 mL) slurry and the reaction mixture was refluxed for 4 hours. After which, the suspension was filtered, washed (toluene, dichloromethane, isopropanol,

10   methanol, water, acetone), and dried to yield the amino-functionalized SPP silica. Then, 1,6-diisocyanatohexane (2 mL) was added to a dry amino-silica toluene slurry (under argon), which was cooled with an ice bath. After all the diisocyanate was added, the reaction mixture was heated to 70 °C for 5 hours. After this time, the suspension was filtered, washed (toluene) and finally re-suspended in toluene (anhydrous, 125 mL) and TEA (10 mL). Finally, cyclofructan (1 mmol) was

15   dissolved in anhydrous DMF (25 mL) and the solution was added to the SPP silica suspension and the resulting slurry was refluxed for 16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). This method gives a carbamate and urea linked cyclofructan HILIC phase. The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

20   A third bonding strategy will give an example of how cyclofructan and its derivatives can be immobilized with an ether linkage. First, in a 500 mL round-bottom flask cyclofructan (10 mmol) was dissolved in 300 mL of anhydrous DMF under and argon blanket. Then, 1 gram of NaH was added to the solution and the resulting suspension was stirred in an inert environment at

room temperature for 30 minutes. Next, any unreacted NaH was filtered off and the filtrate was transferred to a clean, dry, 500 mL round-bottom flask to which 8 mL of 3-glycidoxypropyl trimethoxysilane was added. This solution was heated to 90 °C for 5 hours and then allowed to cool to room temperature. Next, the solution was transferred to a 500 mL 2-neck round bottom flask containing dry SPP silica (21 grams) and the resulting suspension was heated to 110 °C for 16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

Example 2 - Preparation of cyclodextrin based stable, bonded, SPP HILIC stationary phases

Cyclodextrins are cyclic oligosaccharides that possess 18-24 hydroxyl groups. These hydroxyl groups can be used as reactive functionalities to covalently bond the cyclodextrin (or cyclodextrin derivative) to SPP silica. Cyclodextrins can be used as HILIC selectors in their native form or in a derivatized format. Derivatization of the cyclodextrin molecules can take place before or after they are immobilized on the SPP silica. As was noted earlier, the derivatizing groups are typically, but not limited to: linear alkane C_1-C_{30}, branched alkane C_1-C_{30}, unsaturated alkane C_1-C_{30}, cyclic alkane C_1-C_{30}, linear and/or cyclic alkane containing heteroatoms (e.g. N, S, O) C_1-C_{30}) and are bonded to the cyclodextrin by a number of chemical linkages (e.g. ether, carbamate, thioether, thiocarbamate, ester, triazole, and urea) as noted earlier.

Native and derivatized cyclodextrins are linked to SPP silica in the same way. First, in a 250 mL round-bottom flask cyclodextrin (3 mmol) was dissolved in anhydrous DMF (60 mL) under and argon blanket. Then, 3-triethoxysilylpropyl isocyanate (12 mmol) and anhydrous pyridine (5 mL) were added and the reaction vessel was heated to 90 °C for 5 hours. Meanwhile,

the SPP silica (4 grams) was first dried in an oven (120 °C) for 4 hours and later azeotropically distilled (toluene, 125 mL) using a Dean-stark trap and a 250 mL, 2-neck round-bottom flask. Once both reaction vessels were allowed to cool to room temperature, the cyclodextrin/DMF solution was added to the SPP silica-toluene slurry, and the resulting suspension was refluxed for 16 hours.

5   After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). This method gives a carbamate linked cyclodextrin HILIC phase. The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube. Alternatively, a second binding chemistry which also forms a carbamate linker can be employed. Here, SPP silica (3 grams) was dried at 120 °C for 4 hours. Next, toluene was added and residual

10   water was removed using a Dean-stark trap to azeotropically distill the toluene-SPP silica slurry. The suspension was allowed to cool (<40 °C) and 1 mL of (3-aminopropyl)triethoxysilane was added to the silica (3.3 grams)-toluene (125 mL) slurry and the reaction mixture was refluxed for 4 hours. After which, the suspension was filtered, washed (toluene, dichloromethane, isopropanol, methanol, water, acetone), and dried to yield the amino-functionalized SPP silica. Then, 1,6-

15   diisocyanatohexane (2 mL) was added to a dry amino-silica toluene slurry (under argon), which was cooled with an ice bath. After all the diisocyanate was added, the reaction mixture was heated to 70 °C for 5 hours. After this time, the suspension was filtered, washed (toluene) and finally re-suspended in toluene (anhydrous, 125 mL) and TEA (10 mL). Finally, cyclodextrin (1 mmol) was dissolved in anhydrous DMF (25 mL) and the solution was added to the SPP silica suspension and

20   the resulting slurry was refluxed for 16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). This method gives a carbamate and urea linked cyclodextrin HILIC phase. The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

A third bonding strategy will give an example of how cyclodextrin and its derivatives can be immobilized with an ether linkage. First, in a 500 mL round-bottom flask cyclodextrin (10 mmol) was dissolved in 300 mL of anhydrous DMF under and argon blanket. Then, 1 gram of NaH was added to the solution and the resulting suspension was stirred in an inert environment at

5   room temperature for 30 minutes. Next, any unreacted NaH was filtered off and the filtrate was transferred to a clean, dry, 500 mL round-bottom flask to which 8 mL of 3-glycidoxypropyl trimethoxysilane was added. This solution was heated to 90 °C for 5 hours and then allowed to cool to room temperature. Next, the solution was transferred to a 500 mL 2-neck round bottom flask containing dry SPP silica (21 grams) and the resulting suspension was heated to 110 °C for

10   16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

Example 3 - Preparation of glycopeptide based stable, bonded, SPP HILIC stationary phases

15   Macrocyclic glycopeptides (e.g. teicoplanin, boromycin, ristocetin A, dalbavancin, and vancomycin) possess amine and hydroxyl functionalities which can be used as reactive groups to covalently bond the glycopeptide (or glycopeptide analog; e.g. teicoplanin aglycone) to SPP silica. There are a number of bonding chemistries that can be used to chemically immobilize macrocyclic glycopeptides on SPP silica (e.g. ether, carbamate, thioether, thiocarbamate, ester, triazole, and

20   urea). The following lists example bonding strategies, using teicoplanin as the model HILIC selector.

First, in a 250 mL round-bottom flask teicoplanin (3 mmol) was dissolved in anhydrous DMF (60 mL) under and argon blanket and TEA (3 mL) was added. Then, 3-triethoxysilylpropyl

isocyanate (12 mmol) was added and the reaction vessel was heated to 90 °C for 5 hours. Meanwhile, the SPP silica (4 grams) was first dried in an oven (120 °C) for 4 hours and later azeotropically distilled (toluene, 125 mL) using a Dean-stark trap and a 250 mL, 2-neck round-bottom flask. Once both reaction vessels were allowed to cool to room temperature, the

5   teicoplanin/DMF solution was added to the SPP silica-toluene slurry, and the resulting suspension was refluxed for 16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). This method gives a carbamate linked teicoplanin HILIC phase. The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

10   Alternatively, a second binding chemistry which also forms a carbamate linker can be employed. Here, SPP silica (3 g) was dried at 120 °C for 4 hours. Next, toluene was added and residual water was removed using a Dean-stark trap to azeotropically distill the toluene-SPP silica slurry. The suspension was allowed to cool (<40 °C) and 1 mL of (3-aminopropyl)triethoxysilane was added to the silica (3.3 grams)-toluene (125 mL) slurry and the reaction mixture was refluxed

15   for 4 hours. After which, the suspension was filtered, washed (toluene, dichloromethane, isopropanol, methanol, water, acetone), and dried to yield the amino-functionalized SPP silica. Then, 1,6-diisocyanatohexane (2 mL) was added to a dry amino-silica toluene slurry (under argon), which was cooled with an ice bath. After all the diisocyanate was added, the reaction mixture was heated to 70 °C for 5 hours. After this time, the suspension was filtered, washed (toluene) and

20   finally re-suspended in toluene (anhydrous, 125 mL) and TEA (10 mL) was added. Finally, teicoplanin (1 mmol) was dissolved in anhydrous DMF (25 mL) and the solution was added to the SPP silica suspension and the resulting slurry was refluxed for 16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water,

acetone). This method gives a carbamate and urea linked teicoplanin HILIC phase. The resulting

SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

A third bonding strategy will give an example of how teicoplanin and its derivatives can

be immobilized with an ether linkage. First, in a 500 mL round-bottom flask teicoplanin (10 mmol)

5   was dissolved in 300 mL of anhydrous DMF under and argon blanket. Then, 1 gram of NaH was

added to the solution and the resulting suspension was stirred in an inert environment at room

temperature for 30 minutes. Next, any unreacted NaH was filtered off and the filtrate was

transferred to a clean, dry, 500 mL round-bottom flask to which 8 mL of 3-glycidoxypropyl

trimethoxysilane was added. This solution was heated to 90 °C for 5 hours and then allowed to

10   cool to room temperature. Next, the solution was transferred to a 500 mL 2-neck round bottom

flask containing dry SPP silica (21 grams) and the resulting suspension was heated to 110 °C for

16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane,

isopropanol, methanol, water, acetone). The resulting SPP HILIC phase was dried and

subsequently slurry packed into a stainless steel tube.

15

Example 4 - Preparation of ionic type stable, bonded, SPP HILIC stationary phases

Charged or ionic type stable, bonded, HILIC stationary phases can be made via a number

of synthetic and binding techniques. Herein, sample derivatizing and binding strategies are given

to produce an anionic cyclofructan based HILIC stationary phase that has been bonded to SPP

20   silica through the use of multipoint attachment. The first model HILIC selector described is

benzoic acid functionalized cyclofructan 6. This example represents a technique that can be used

to produce and number of ionic SPP HILIC phases.

Benzoic acid derivatized cyclofructan 6 was produced and later bonded to SPP silica by any of the following binding methods. First, cyclofructan 6 (4.2 grams) as dissolved in 200 mL of anhydrous DMF at 75 °C and NaH (2.12 gram) was added. The suspension was stirred at 75 °C

5   for 45 min. Then, methyl 4-(bromomethyl)benzoate (20 grams) was dissolved in anhydrous DMF (50 mL) and the solution was carefully added to the cyclofructan–NaH suspension and the reaction was heated to 75 °C for 20 hours, After which, the reaction was cooled and any solids were filtered. Solvent was removed from the filtrate to yield a yellowish crude material. The crude esterified product was then hydrolyzed to the carboxylic form using water, methanol, and sodium hydroxide. The derivatized cyclofructan was then linked to SPP silica. First, in a 250 mL round-bottom flask

10   cyclofructan (3 mmol) was dissolved in anhydrous DMF (60 mL) under and argon blanket. Then, 3-triethoxysilylpropyl isocyanate (12 mmol) and anhydrous pyridine (5 mL) were added and the reaction vessel was heated to 90 °C for 5 hours. Meanwhile, the SPP silica (4 grams) was first dried in an oven (120 °C) for 4 hours and later azeotropically distilled (toluene, 125 mL) using a Dean-stark trap and a 250 mL, 2-neck round-bottom flask. Once both reaction vessels were

15   allowed to cool to room temperature, the cyclofructan/DMF solution was added to the SPP silica-toluene slurry, and the resulting suspension was refluxed for 16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). This method gives a carbamate linked cyclofructan HILIC phase. The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

20   Alternatively, a second binding chemistry which also forms a carbamate linker can be employed. Here, SPP silica (3 grams) was dried at 120 °C for 4 hours. Next, toluene was added and residual water was removed using a Dean-stark trap to azeotropically distill the toluene-SPP silica slurry. The suspension was allowed to cool (<40 °C) and 1 mL of (3-

aminopropyl)triethoxysilane was added to the silica (3.3 grams)-toluene (125 mL) slurry and the reaction mixture was refluxed for 4 hours. After which, the suspension was filtered, washed (toluene, dichloromethane, isopropanol, methanol, water, acetone), and dried to yield the amino-functionalized SPP silica. Then, 1,6-diisocyanatohexane (2 mL) was added to a dry amino-silica

5   toluene slurry (under argon), which was cooled with an ice bath. After all the diisocyanate was added, the reaction mixture was heated to 70 °C for 5 hours. After this time, the suspension was filtered, washed (toluene) and finally re-suspended in toluene (anhydrous, 125 mL) and TEA (10 mL). Finally, cyclofructan (1 mmol) was dissolved in anhydrous DMF (25 mL) and the solution was added to the SPP silica suspension and the resulting slurry was refluxed for 16 hours. After

10   that time, the reaction was filtered and washed (toluene, dichloromethane, isopropanol, methanol, water, acetone). This method gives a carbamate and urea linked cyclofructan HILIC phase. The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

A third bonding strategy will give an example of how cyclofructan and its derivatives can be immobilized with an ether linkage. First, in a 500 mL round-bottom flask cyclofructan (10

15   mmol) was dissolved in 300 mL of anhydrous DMF under and argon blanket. Then, 1 gram of NaH was added to the solution and the resulting suspension was stirred in an inert environment at room temperature for 30 minutes. Next, any unreacted NaH was filtered off and the filtrate was transferred to a clean, dry, 500 mL round-bottom flask to which 8 mL of 3-glycidoxypropyl trimethoxysilane was added. This solution was heated to 90 °C for 5 hours and then allowed to

20   cool to room temperature. Next, the solution was transferred to a 500 mL 2-neck round bottom flask containing dry SPP silica (21 grams) and the resulting suspension was heated to 110 °C for 16 hours. After that time, the reaction was filtered and washed (toluene, dichloromethane,

isopropanol, methanol, water, acetone). The resulting SPP HILIC phase was dried and subsequently slurry packed into a stainless steel tube.

Example 5 - Preparation of zwitterionic type stable, bonded, SPP HILIC stationary phases

5   Zwitterionic HILIC phases are hydrolytically unstable unless certain measures are taken to protect the HILIC ligand from leaching. There are several approaches to do this (as described earlier). In this example, a phosphoniumsulfonate zwitterion (3-P,P-diphenylphosphoniumpropylsulfonate) is chemically bonded to SPP silica in a stable format through the use of a bipodal silane. Specifically, in a 500 mL round-bottom flask, 25.8 mL of 0.5

10   M potassium diphenylphosphide was transferred under air-free conditions. Next, 120 mL of anhydrous THF was added while stirring the phosphide under argon. The phosphide was converted into the zwitterion by slowly adding 2.35 grams of propanesultone dissolved in 10 mL of anhydrous THF. The sultone was added drop by drop in 3.3 minutes. The resulting white suspension was kept in a freezer (to further precipitate the zwitterion). The white suspension was

15   filtered under argon on glass frit funnel and washed with diethyl ether.

          The resulting zwitterion (5.3 grams) was transferred to a 500 mL flask and dissolved in 60 mL of anhydrous DMF. When the solid dissolved completely, 2.3 g of 3-bromopropionic acid (in 10 mL DMF) was added and the mixture heated overnight at 100 °C. After the reaction, the KBr was filtered and the resulting viscous liquid was rotary evaporated at 80 °C. The brown liquid was

20   washed with hot THF, ACN, and heptane. After overnight drying under vacuum, white crystals of 3-P,P-diphenylphosphinopropylsulfonate were obtained. In the next step, 5.24 grams of crude 3-P,P-diphenylphosphinopropylsulfonate was reacted with 1.55 grams of oxalyl chloride in anhydrous dichloromethane for 4 hours on crushed ice. The solvent was removed at room

temperature in vacuo. The acylated product was immediately mixed with 4.1 grams of bis(3-trimethoxysilylpropyl)amine in anhydrous DMF. Hunig's base was added to absorb HCl. The resulting modified silane was refluxed with 7 grams of SPP silica in toluene, which was previously dried by azeotropic distillation with a Dean-Stark trap. The resulting SPP HILIC phase was dried

5   and subsequently slurry packed into a stainless steel tube.

Two schematics showing typical attachment strategies for stabilizing HILIC phases are shown in Figures 9 & 10. These combined with using HILIC selectors of sufficient steric bulk to protect the silica surface are essential for providing enhanced stability HILIC stationary phases.

Stability properties from the presented stabilization techniques are listed in Table 2.

10   **Table 2** – Stability properties of bonded SPP-HILIC materials tested with benzyltrimethylammonium chloride as a silanol marker for leaching of the ligand

Type	Initial retention time (min)	Final retention time (min)	Percent change – retention time drift
Commercial Silica	14.668	15.552	6.0
Commercial Zwitterion	7.263	7.491	3.0
New, bonded, stabilized, zwitterionic SPP material	2.090	2.092	0.1
New, bonded, stabilized, oligosaccharide SPP material	6.139	6.136	0.0

Figures 11-13 provide additional representative data indicating the success of our "stability

15   strategy". Figure 11 shows the retention time drift of bare SPP silica. Figure 12 shows an example of the use of non-conventional silanes to form hydrolytically stable, high efficiency, bonded

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zwitterionic SPP HILIC phases. Figure 13 shows an example of the use of multipoint attachment to form hydrolytically stable, high efficiency, bonded ionic, derivatized cyclofructan, SPP HILIC phases.

Table 3 lists some particle properties and surface coverage data for native CF6 bonded to FPPs and SPPs to serve as a model example for such stable bonded HILIC selectors. Note the much lower surface area for the SPP compared to the FPP. Yet, an equivalent relative coverage (i.e. μmol/m^2) of HILIC selector is obtained on the SPPs.

Table 3 - Example of particle properties and elemental analysis for stable HILIC phases produced on FPPs and SPPs. [1]Values calculated starting with the % C measured by elemental analysis.

	Particle diameter (μm)	Porosity %	Pore size (Å)	Surface Area (m²/g)	CF6 content (μmol/m²)[1]	CF6 content (mass %)[1]
FPP 5 μm	4.3	100%	93	465	0.72	32.2
FPP 3 μm	3.0	100%	100	300	0.91	27.9
SPP 2.7 μm	2.7	75%	120	120	0.86	12.8

According to the invention, SPPs have pore size ranging from about 100 angstroms to about 300 angstroms, preferably from about 100 angstroms to about 150 angstroms, more preferably from about 110 angstrom to about 130 angstrom. SPP according to the invention includes a pore size of about 120 angstrom. Also, according to the invention, SPPs have surface area ranging from about 100 m^2/g to about 500 m^2/g, preferably from about 100 m^2/g to about 400 m^2/g, or from about 100 m^2/g to about 300 m^2/g, or from about 100 m^2/g to about 200 m^2/g, more preferably from about 110 m^2/g to about 150 m^2/g. SPP according to the invention has a surface area of about 120 m^2/g

The following paragraphs with their respective figures describe examples of the superiority of SPP-HILIC based stable, bonded materials in producing separations of greater efficiency (higher theoretical plate number (N) and shorter retention (analysis times).

Figure 14 shows the separation of the polar analytes 5-phenylvaleric acid and ferulic acid in HILIC using a stabilized, bonded, HILIC selector based on cyclofructan 6. The example shows a comparison of separations obtained when the stabilized phase is bound to FPPs and SPPs. The values on the top of the peaks correspond to the efficiency in terms of number of plates (N) on column. The mobile phase was composed of ACN/25 mM NH$_4$OAc (85/15); flow rate = 750 μL/min; T = 30 °C. Note the equivalent resolution observed in all three chromatograms, while the separation using the SPP-HILIC phase was complete in a much shorter time. Such an advantage is a result of the increased efficiency afforded by the SPPs, without a concomitant loss of selectivity.

Figure 15 shows the separation of polar nucleobases (uracil and cytosine) and nucleosides (adenosine) in HILIC using a stabilized, bonded, HILIC selector based on cyclofructan 6. The example shows a comparison of separations obtained when the stabilized phase is bound to FPPs and SPPs. The values on the top of the peaks correspond to the efficiency in terms of number of plates (N) on column. The mobile phase was composed of ACN/25 mM NH$_4$OAc (75/25); flow rate = 750 μL/min; T = 30 °C. Note the equivalent resolution observed in all three chromatograms, while the separation using the SPP-HILIC phase was complete in a much shorter time. Such an advantage is a result of the increased efficiency afforded by the SPPs, without a concomitant loss of selectivity.

Figure 16 shows the separation of polar cyclic nucleotides in HILIC using a stabilized, bonded, HILIC selector based on cyclofructan 6. The example shows a comparison of separations

obtained when the stabilized phase is bound to FPPs and SPPs. The values on the top of the peaks correspond to the efficiency in terms of number of plates (N) on column. The mobile phase was composed of ACN/25 mM NH$_4$OAc (70/30); flow rate = 750 µL/min; T = 30 °C. Note the equivalent/improved resolution observed when using the SPP-HILIC phase. Also, the separation

5   using the SPP-HILIC phase was complete in a much shorter time. Such an advantage is a result of the increased efficiency afforded by the SPPs, without a concomitant loss of selectivity.

Figure 17 shows the separation of the polar analytes pyridoxine and ascorbic acid in HILIC using a stabilized, bonded, HILIC selector based on cyclofructan 6. The example shows a comparison of separations obtained when the stabilized phase is bound to FPPs and SPPs. The

10   values on the top of the peaks correspond to the efficiency in terms of number of plates (N) on column. The mobile phase was composed of ACN/25 mM NH$_4$OAc (75/25); flow rate = 750 µL/min; T = 30 °C. Note the equivalent resolution observed in all three chromatograms, while the separation using the SPP-HILIC phase was complete in a much shorter time. Such an advantage is a result of the increased efficiency afforded by the SPPs, without a concomitant loss of

15   selectivity.

Figure 18 shows separation of polar nucleobases (uracil and cytosine) and nucleosides (adenosine) in HILIC using a stabilized, bonded, HILIC selector based on cyclodextrin. The example shows a comparison of separations obtained when the stabilized phase is bound to FPPs and SPPs. The values next to each peak correspond to the efficiency in terms of number of plates

20   (N) on column. The mobile phase was composed of ACN/25 mM NH$_4$OAc (90/10); flow rate = 750 µL/min; T = 25 °C. Note the equivalent resolution observed in all three chromatograms, while the separation using the SPP-HILIC phase was complete in a much shorter time. Such an advantage

is a result of the increased efficiency afforded by the SPPs, without a concomitant loss of selectivity.

Figure 19 shows separation of polar xanthines (xanthine and hypoxanthine) in HILIC using a stabilized, bonded, HILIC selector based on cyclodextrin. The example shows a comparison of separations obtained when the stabilized phase is bound to FPPs and SPPs. The values next to each peak correspond to the efficiency in terms of number of plates (N) on column. The mobile phase was composed of ACN/25 mM NH4OAc (90/10); flow rate = 750 μL/min; T = 25 °C. Note the equivalent resolution observed in all three chromatograms, while the separation using the SPP-HILIC phase was complete in a much shorter time. Such an advantage is a result of the increased efficiency afforded by the SPPs, without a concomitant loss of selectivity.

Figure 20 shows separation of polar nucleosides and nucleobases in HILIC using a stabilized, bonded, ionic HILIC selector based on benzoic acid functionalized cyclofructan. The example shows the extremely high efficiency obtained when the stabilized phase is bound to SPPs. The mobile phase was composed of CAN/10nM NH4OAc (85/15); flow rate = 1.5 mL/min; T = 25°C. Also note that the reduced plate height of 1.7 is the best ever found for a HILIC separation.

Figure 21 shows separation of polar peptides (enkephalins) with single amino acid polymorphisms in HILIC using a stabilized, bonded, macrocyclic glycopeptide zwitterionic HILIC selector based on teicoplanin. The example shows the extremely high efficiency obtained when the stabilized phase is bound to SPPs. The mobile phase was composed of ACN/water (80/20); flow rate = 1.0 mL/min; T = 25°C.

Another way to demonstrate the advantages of stable HILIC SPP columns is via kinetic plots (van Deemter and related plots). Figures 22 and 23 show van Deemter plots for an ultra stable HILIC SPP stationary phase (based on cyclofructan 6) compared to 2 analogous fully porous

particle stationary phases in both the HILIC mode and the classic normal phase mode respectively. Note that the SPP stationary phase produces the lowest HETP (or H) curve at all flow rates and has the smallest slope at higher flow rates. Consequently these SPP phases produce the greatest number of theoretical plates (N) per minute (Figure 24). Thus analyses can be completed in less time on these phases.

More particularly, Figure 22 shows the dependence of efficiency (in terms of HETP) on the flow rate of the mobile phase for ferulic acid in normal phase mode using a stabilized, bonded, selector based on cyclofructan 6. The example shows a comparison of FPPs and SPPs. As can be seen, the minimum HETP was at higher flow rates for the SPP material. At all tested flow rates, the efficiency of the SPP column was the greatest among the tested columns and the SPP column showed a notable improvement in the mass transfer band broadening effect at high flow rates.

Figure 23 shows the dependence of efficiency (in terms of HETP) on the flow rate of the mobile phase for ferulic acid in HILIC using a stabilized, bonded, HILIC selector based on cyclofructan 6. The example shows a comparison of FPPs and SPPs. As can be seen, the minimum HETP was at higher flow rates for the SPP material. At all tested flow rates, the efficiency of the SPP column was the greatest among the tested columns and the SPP column showed an improvement in the mass transfer band broadening effect at high flow rates.

Figure 24 shows the dependence of plate numbers per minute on the flow rate of the mobile phase for ferulic acid in HILIC using a stabilized, bonded, HILIC selector based on cyclofructan 6. The example shows a comparison of FPPs and SPPs. It should be noted that, the SPP column leads to a decrease in the total analysis time. This example clearly shows this, by displaying the number of plates per unit time versus the mobile phase flow rate for each compound. As can be

seen, the number of plates afforded per time spent on the analysis is much higher for the SPP columns than the FPPs columns.

Finally, Figure 25 shows selectivity values of neighboring peaks for all the analytes separated in Figures 14-17 in HILIC using a stabilized, bonded, HILIC selector based on cyclofructan 6. The example shows a comparison of FPPs and SPPs and the selectivity values were calculated based on the following pairs of separated analytes: 1) uracil-adenosine; 2) adenosine-cytosine; 3) 5-phenylvaleric acid-ferulic acid; 4) pyridoxine ascorbic acid; 5) cTMP-cAMP; 6) cAMP-cGMP; 7) cGMP-cCMP. It can be seen, selectivity values are essentially the same between the FPP and SPP columns. Clearly, selectivity does not follow the absolute mass % loading of CF6 in the column (Table 2). This invention shows, for the first time, that for stabilized, bonded, HILIC phases on SPPs, having an equivalent *relative* coverage (μmol/m^2) will yield equivalent selectivities.

The following are some embodiments according to the present invention.

Embodiment 1. Covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases comprising superficially porous particle (SPP) linked to a HILIC selector.

Embodiment 2. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein SPP has a particle diameter from about 0.5 microns to about 20 microns.

Embodiment 3. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein SPP has a particle diameter from about 1.3 microns to about 10 microns.

Embodiment 4. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein SPP has a particle diameter from about 1.7 microns to about 5.0 microns.

Embodiment 5. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein SPP has a particle diameter selected from the group consisting of about 1.7, about 2.7 and about 4.0 microns.

Embodiment 6. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein SPP has a particle diameter of about 1.7 microns.

Embodiment 7. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein SPP has a particle diameter of about 2.7 microns.

Embodiment 8. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein the SPP has a particle diameter of about 4.0 microns.

Embodiment 9. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein the SPP has a pore size from about 100 angstroms to about 300 angstroms.

Embodiment 10. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein the SPP has a pore size from about 100 angstroms to about 150 angstroms.

Embodiment 11. The covalently bonded ultra-stable hydrophilic interaction

chromatography (HILIC) phases of embodiment 1, wherein the SPP has a pore size from about

110 angstroms to about 130 angstroms.

Embodiment 12. The covalently bonded ultra-stable hydrophilic interaction

chromatography (HILIC) phases of embodiment 1, wherein the SPP has a pore size of about 120

angstroms.

Embodiment 13. The covalently bonded ultra-stable hydrophilic interaction

chromatography (HILIC) phases of embodiment 1, wherein the SPP has a surface area from

about 150 m^2/g to about 500 m^2/g.

Embodiment 14. The covalently bonded ultra-stable hydrophilic interaction

chromatography (HILIC) phases of embodiment 1, wherein the SPP has a surface area of about

120 m^2/g.

Embodiment 15. The covalently bonded ultra-stable hydrophilic interaction

chromatography (HILIC) phases of embodiment 1, wherein the HILIC selector is selected from

the group consisting carbohydrates and derivatives, oligosaccharides and derivatives, cyclic and

derivatives oligosaccharides, peptides and derivatives, glycopeptides and derivatives,

macrocyclic glycopeptides and derivatives, charged or ionic type and zwitterionic type.

Embodiment 16. The covalently bonded ultra-stable hydrophilic interaction

chromatography (HILIC) phases of embodiment 15, wherein the HILIC selector is selected from

the group consisting cyclodextrins, derivatized cyclodextrin, cyclofructans, derivatized

cyclofructans, teicoplanin, vancomycin, teicoplanin aglycone, ristocetin A, dalbavancin,

boromycin, sulfonated cyclofrutans, sulfonated cyclodextrins, and 3-P,P-

diphenylphosphoniumpropylsulfonate.

Embodiment 17. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein the HILIC selector is selected from the group consisiting of native or derivatized cyclofrutans, native or derivatized cyclodextrins,

Embodiment 18. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 17, wherein derivatizing groups of the derivatized cyclofrutans and the derivatized cyclodextrins are selected from the group consisting of linear alkane C_1-C_{30}, branched alkane C_1-C_{30}, unsaturated alkane C_1-C_{30}, cyclic alkane C_1-C_{30}, linear and/or cyclic alkane containing heteroatoms (e.g. N, S, O) C_1-C_{30})

Embodiment 19. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein the superficially porous particle (SPP) is linked to a HILIC selector via ether, carbamate, thioether, thiocarbamate, ester, triazole, or urea linkages.

Embodiment 20. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of embodiment 1, wherein the retention time drift is less than 3.0 % change.

Embodiment 21. The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the retention time drift is less than 1.0 % change.

Embodiment 22. A superficially porous particle (SPP) comprising high efficiency, ultra-stable hydrophilic interaction chromatography (HILIC) matrix covalently bonded thereto.

Embodiment 23. The superficially porous particle (SPP) of embodiment 22, wherein the matrix comprises HILIC selector.

Embodiment 24. Hydrolytically stable, highly selective superficially porous particle (SPP) hydrophilic interaction liquid chromatography (HILIC) stationary phases having higher efficiencies and shorter retention times than analogous stationary phases on fully porous particles (FPP).

Embodiment 25. The hydrolytically stable highly selective SPP HILIC phases of embodiment 3, wherein the HILIC packing material is not unmodified silica thereby protecting the SPP surface from dissolution and having broader HILIC selectivity and higher efficiencies than bare supports and fully porous supports comprising silica.

Embodiment 26. A method for separation of polar molecules comprising contacting a molecule with covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases comprising superficially porous particle (SPP) linked to a HILIC selector.

Embodiment 27. A method of making ultra-stable hydrophilic interaction chromatography (HILIC) phases comprising superficially porous particle (SPP) linked to a HILIC selector comprising selecting a HILIC selector selected from the group consisting of cyclodextrins, derivatized cyclodextrin, cyclofructans, derivatized cyclofructans, teicoplanin, vancomycin, teicoplanin aglycone, ristocetin A, dalbavancin, boromycin, sulfonated cyclofrutans, sulfonated cyclodextrins, and 3-P,P-diphenylphosphoniumpropylsulfonate; and covalently bonding the chiral selector to a superficially porous particle, thereby obtaining ultra stable HILIC SPP stationary phase.

Although the preferred embodiments of the present invention have been described herein, the descriptions are merely illustrative. Further modification of the invention herein disclosed will occur to those skilled in the respective arts and all such modifications are deemed to be within the scope of the invention as defined by the attached claims.

WHAT IS CLAIMED:

1.   Covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases comprising superficially porous particle (SPP) linked to a HILIC selector.

2.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein SPP has a particle diameter from about 0.5 microns to about 20 microns.

3.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein SPP has a particle diameter from about 1.3 microns to about 10 microns.

4.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein SPP has a particle diameter from about 1.7 microns to about 5.0 microns.

5.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein SPP has a particle diameter selected from the group consisting of about 1.7, about 2.7 and about 4.0 microns.

6.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein SPP has a particle diameter of about 1.7 microns.

7.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein SPP has a particle diameter of about 2.7 microns.

8.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the SPP has a particle diameter of about 4.0 microns.

9.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the SPP has a pore size from about 100 angstroms to about 300 angstroms.

10.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the SPP has a pore size from about 100 angstroms to about 150 angstroms.

11.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the SPP has a pore size from about 110 angstroms to about 130 angstroms.

12.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the SPP has a pore size of about 120 angstroms.

13.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the SPP has a surface area from about 150 m^2/g to about 500 m^2/g.

14.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the SPP has a surface area of about 120 m^2/g.

15.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the HILIC selector is selected from the group consisting carbohydrates and derivatives, oligosaccharides and derivatives, cyclic and derivatives oligosaccharides, peptides and derivatives, glycopeptides and derivatives, macrocyclic glycopeptides and derivatives, charged or ionic type and zwitterionic type.

16.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 15, wherein the HILIC selector is selected from the group consisting cyclodextrins, derivatized cyclodextrin, cyclofructans, derivatized cyclofructans, teicoplanin, vancomycin, teicoplanin aglycone, ristocetin A, dalbavancin, boromycin, sulfonated cyclofrutans, sulfonated cyclodextrins, and 3-P,P-diphenylphosphoniumpropylsulfonate.

17.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the HILIC selector is selected from the group consisiting of native or derivatized cyclofrutans, native or derivatized cyclodextrins,

18.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 17, wherein derivatizing groups of the derivatized cyclofrutans and the derivatized cyclodextrins are selected from the group consisting of linear alkane C_1-C_{30}, branched alkane C_1-C_{30}, unsaturated alkane C_1-C_{30}, cyclic alkane C_1-C_{30}, linear and/or cyclic alkane containing heteroatoms (e.g. N, S, O) C_1-C_{30})

19.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the superficially porous particle (SPP) is linked to a HILIC selector via ether, carbamate, thioether, thiocarbamate, ester, triazole, or urea linkages.

20.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the retention time drift is less than 3.0 % change.

21.   The covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC) phases of claim 1, wherein the retention time drift is less than 1.0 % change.

22.   A superficially porous particle (SPP) comprising high efficiency, ultra-stable hydrophilic interaction chromatography (HILIC) matrix covalently bonded thereto.

23.   The superficially porous particle (SPP) of claim 22, wherein the matrix comprises HILIC selector.

5   24.   Hydrolytically stable, highly selective superficially porous particle (SPP) hydrophilic interaction liquid chromatography (HILIC) stationary phases having higher efficiencies and shorter retention times than analogous stationary phases on fully porous particles (FPP).

25.   The hydrolytically stable highly selective SPP HILIC phases of claim 3, wherein the

10   HILIC packing material is not unmodified silica thereby protecting the SPP surface from dissolution and having broader HILIC selectivity and higher efficiencies than bare supports and fully porous supports comprising silica.

26.   A method for separation of polar molecules comprising contacting a molecule with covalently bonded ultra-stable hydrophilic interaction chromatography (HILIC)

15   phases comprising superficially porous particle (SPP) linked to a HILIC selector.

27.   A method of making ultra-stable hydrophilic interaction chromatography (HILIC) phases comprising superficially porous particle (SPP) linked to a HILIC selector comprising selecting a HILIC selector selected from the group consisting of cyclodextrins, derivatized cyclodextrin, cyclofructans, derivatized cyclofructans,

20   teicoplanin, vancomycin, teicoplanin aglycone, ristocetin A, dalbavancin, boromycin, sulfonated cyclofrutans, sulfonated cyclodextrins, and 3-P,P-diphenylphosphoniumpropylsulfonate; and covalently bonding the chiral selector to a

superficially porous particle, thereby obtaining ultra stable HILIC SPP stationary

phase.

Nonporous Core

FIG. 1

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Porous layer

Non-porous core

FIG. 2

Diameter = 1.7 µm

Diameter = 2.7 µm

Diameter = 4.0 µm

FIG. 3

FIG. 4

L = linker type (examples)	R = derivative group (examples)
Ether	Hydrogen
Carbamate	Linear alkane C_1-C_{30}
Thioether	Branched alkane C_1-C_{30}
Thiocarbamate	Unsaturated alkane C_1-C_{30}
Ester	Cyclic alkane C_1-C_{30}
Triazole	Cyclic or linear alkane with heteroatoms (e.g. N, S, O) C_1-C_{30}

FIG. 4 (cont'd)

$$n = 1\ (\alpha\text{-CD}),\ 2\ (\beta\text{-CD}),\ 3\ (\gamma\text{-CD})$$

FIG. 5

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L = linker type (examples)	R = derivative group (examples)
Ether	Hydrogen
Carbamate	Linear alkane C_1-C_{30}
Thioether	Branched alkane C_1-C_{30}
Thiocarbamate	Unsaturated alkane C_1-C_{30}
Ester	Cyclic alkane C_1-C_{30}
Triazole	Cyclic or linear alkane with heteroatoms (e.g. N, S, O) C_1-C_{30}

FIG. 5 (cont'd)
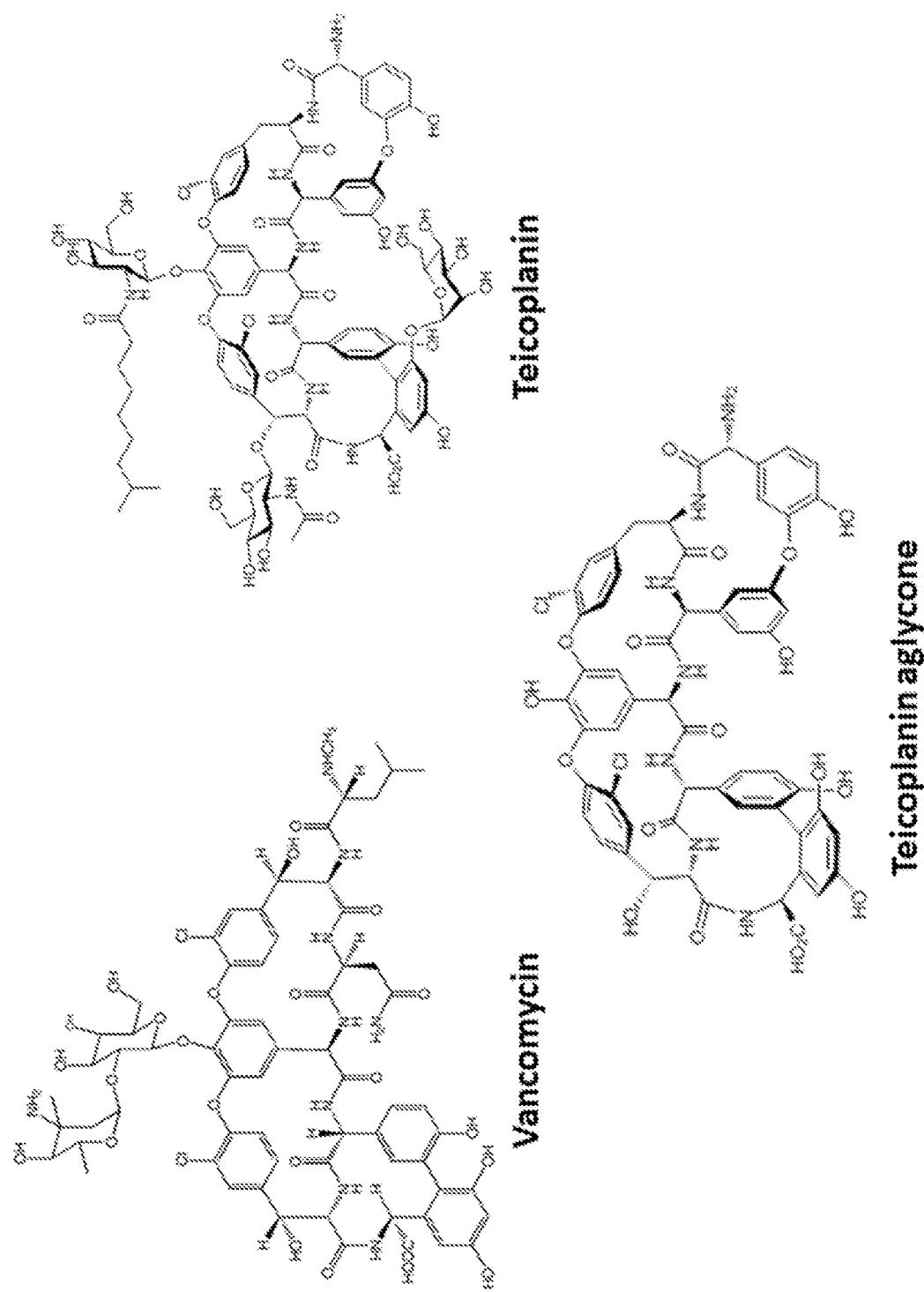
Teicoplanin

Teicoplanin aglycone

Vancomycin

FIG. 6
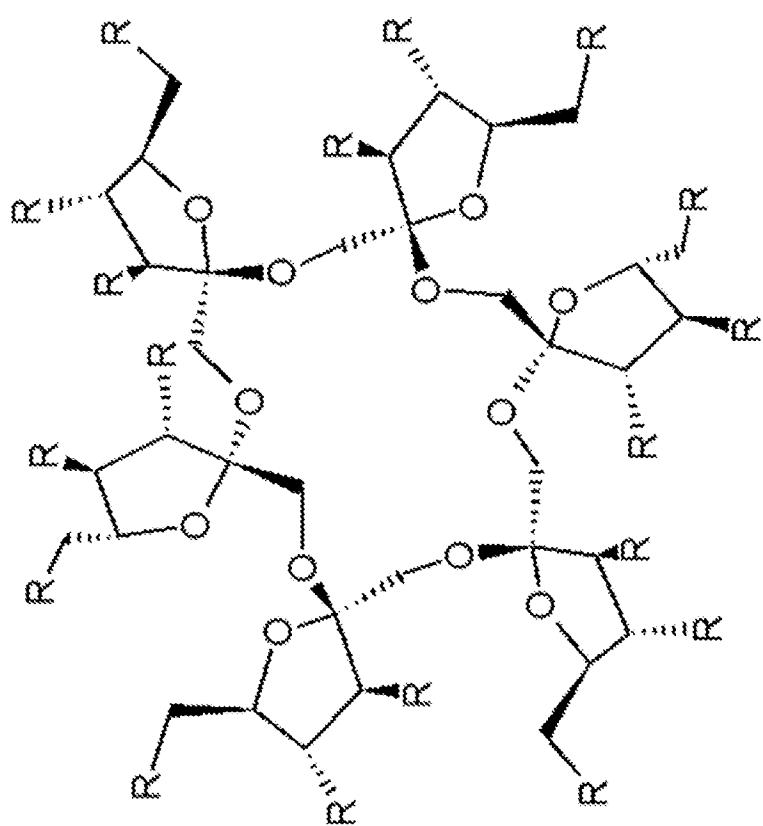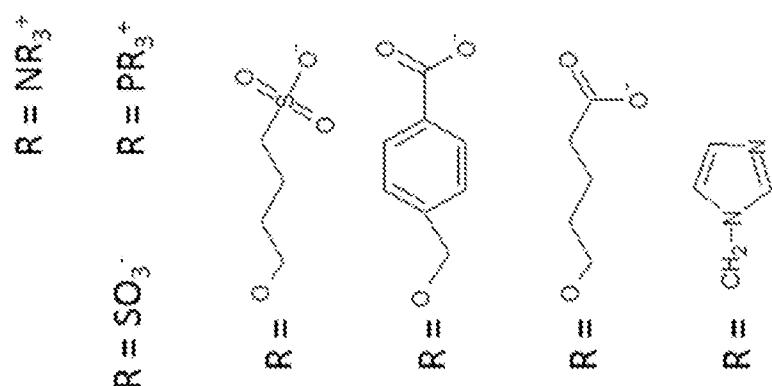
FIG. 7
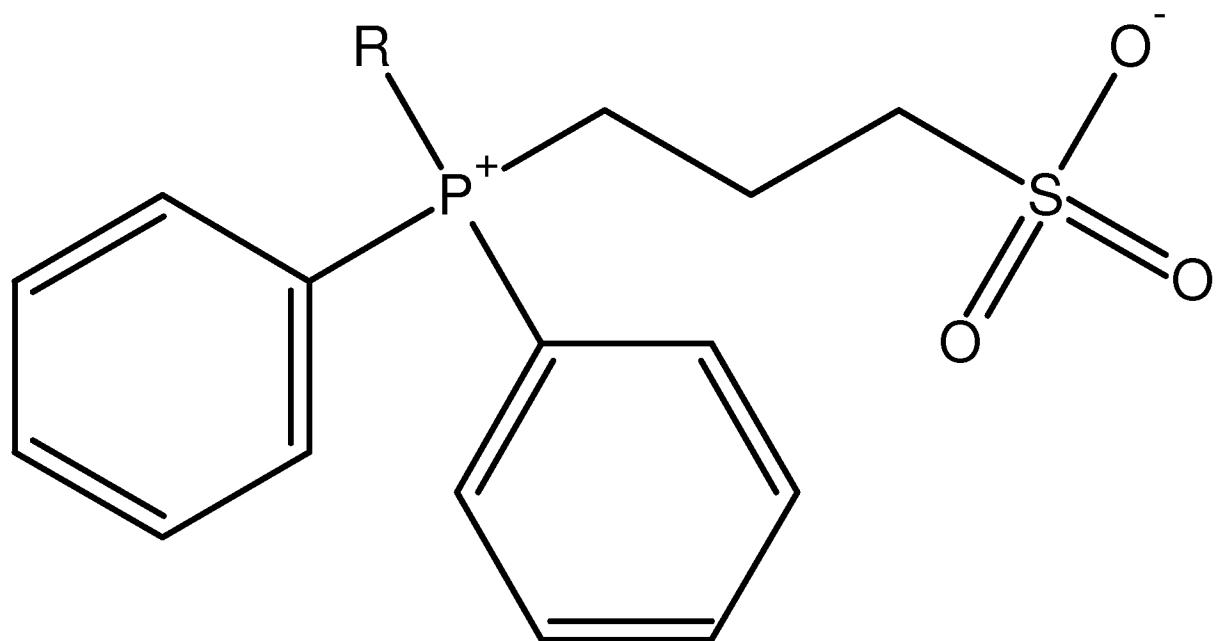
FIG. 8
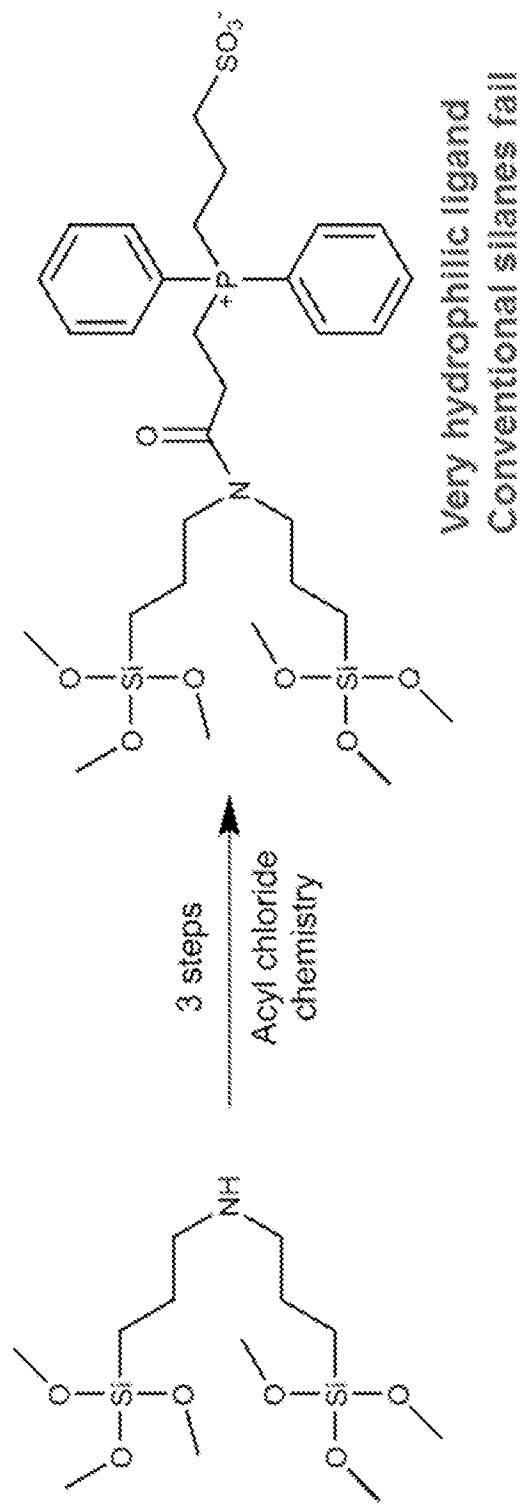
FIG. 9

Modified Cyclofructans for HILIC
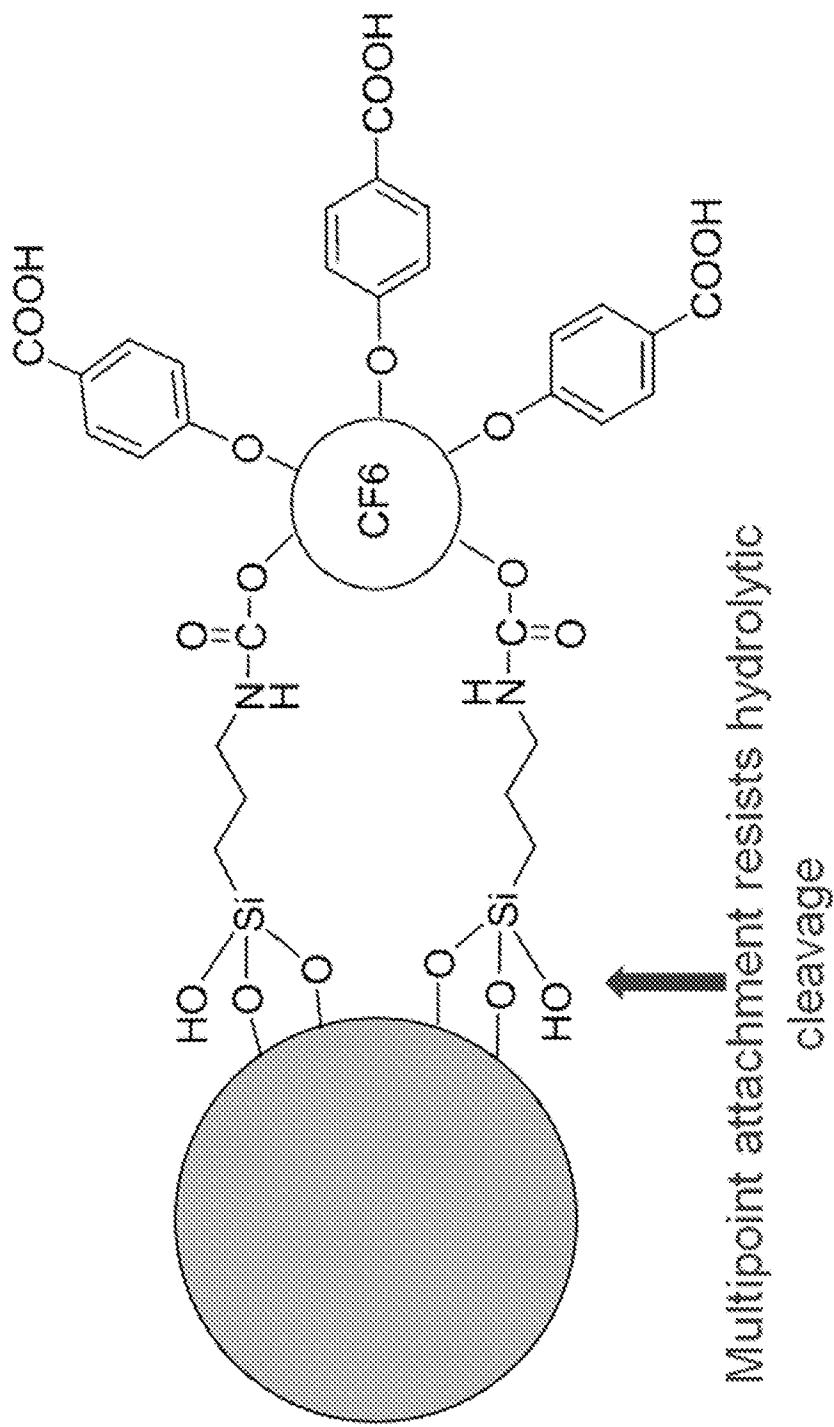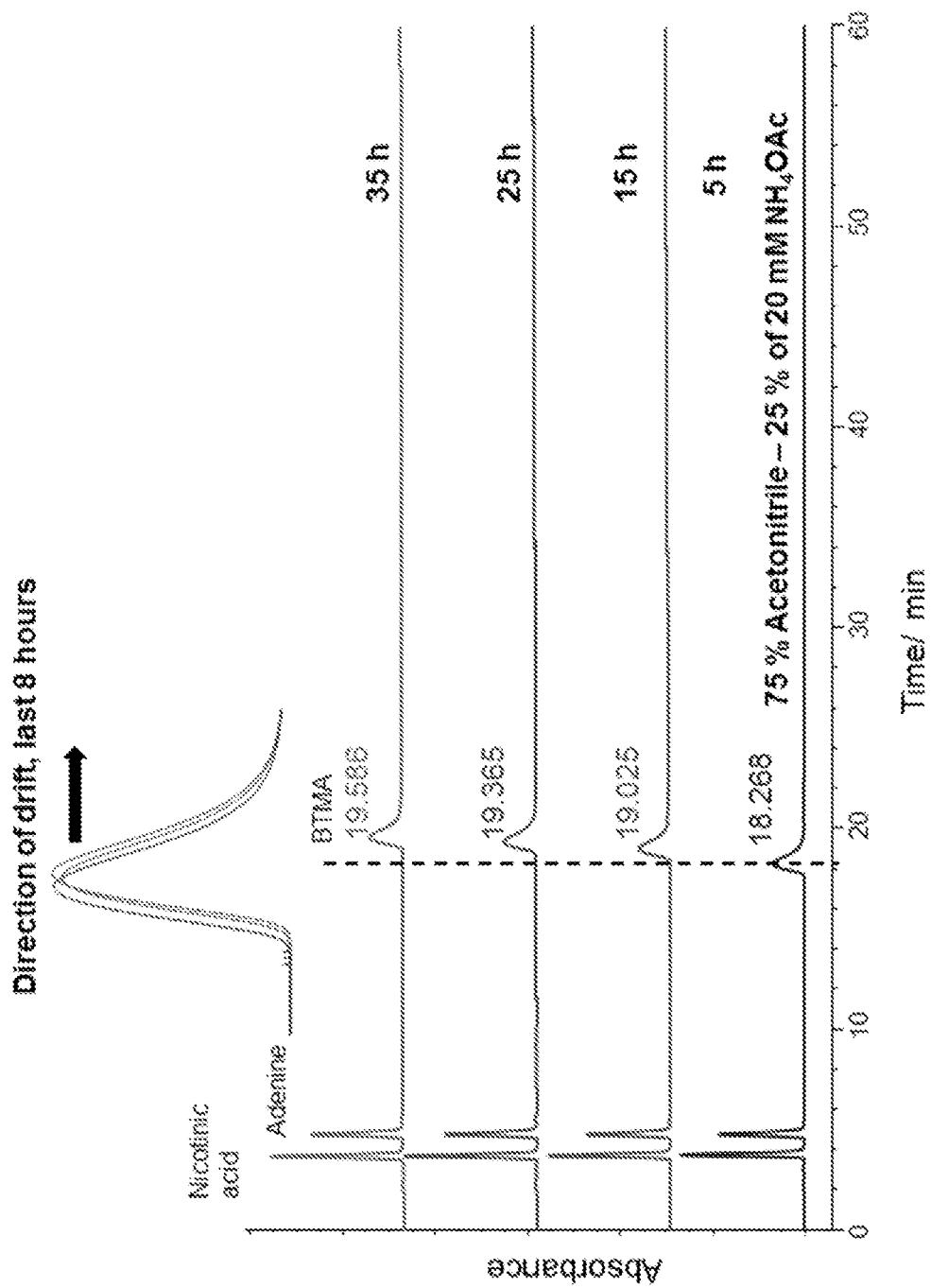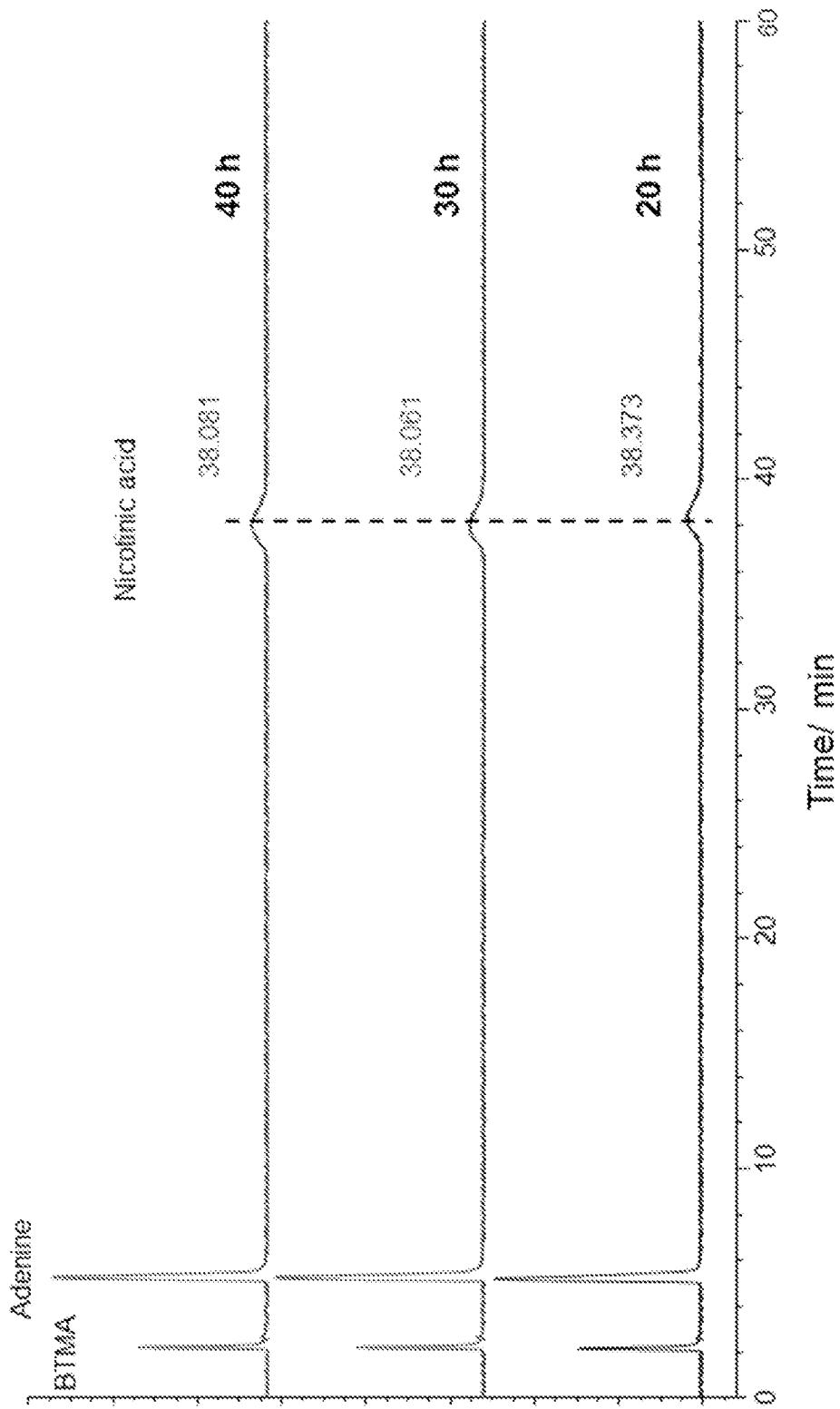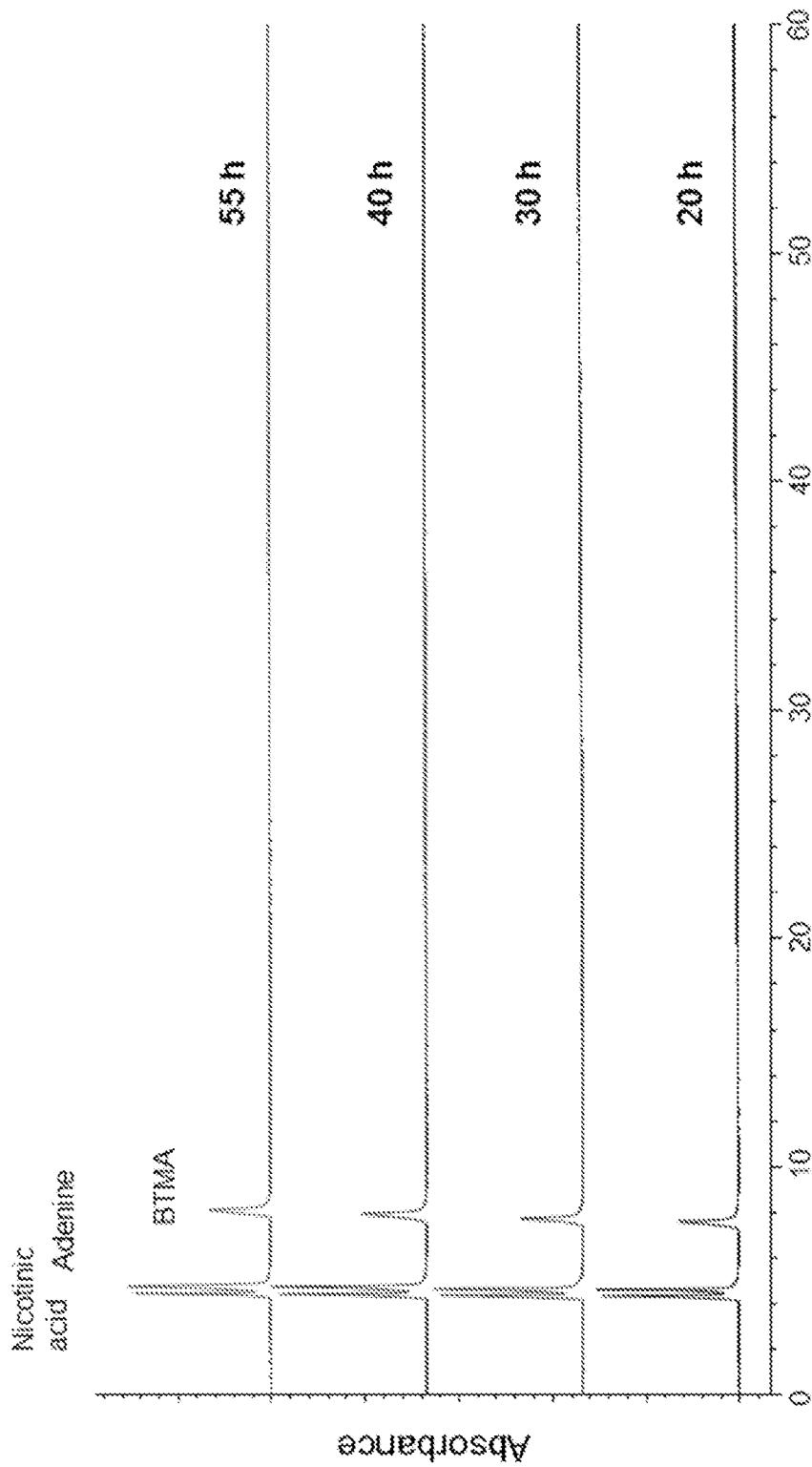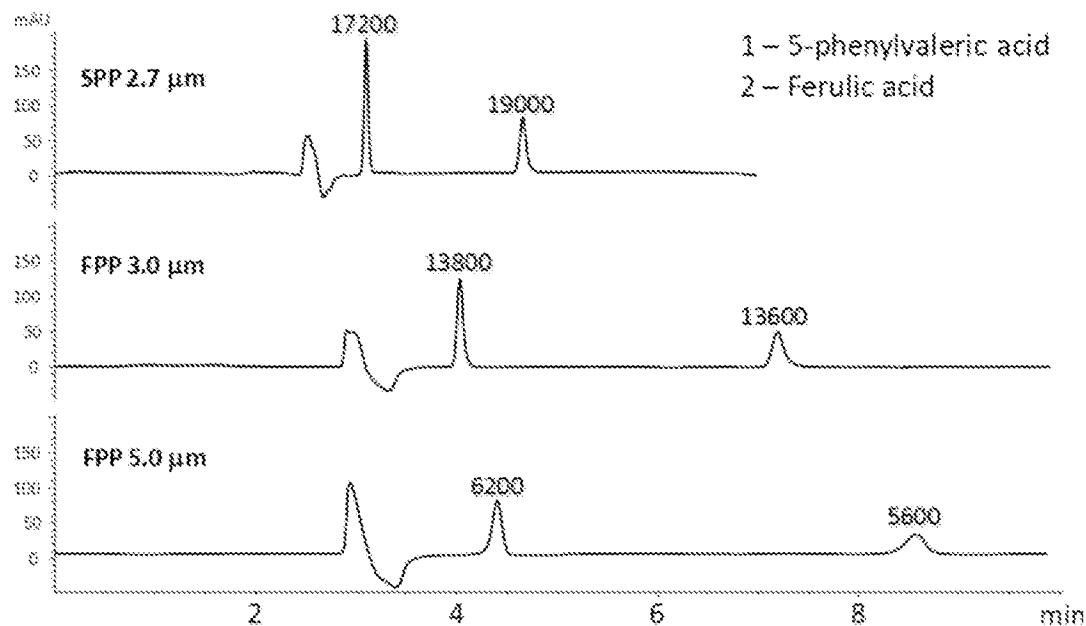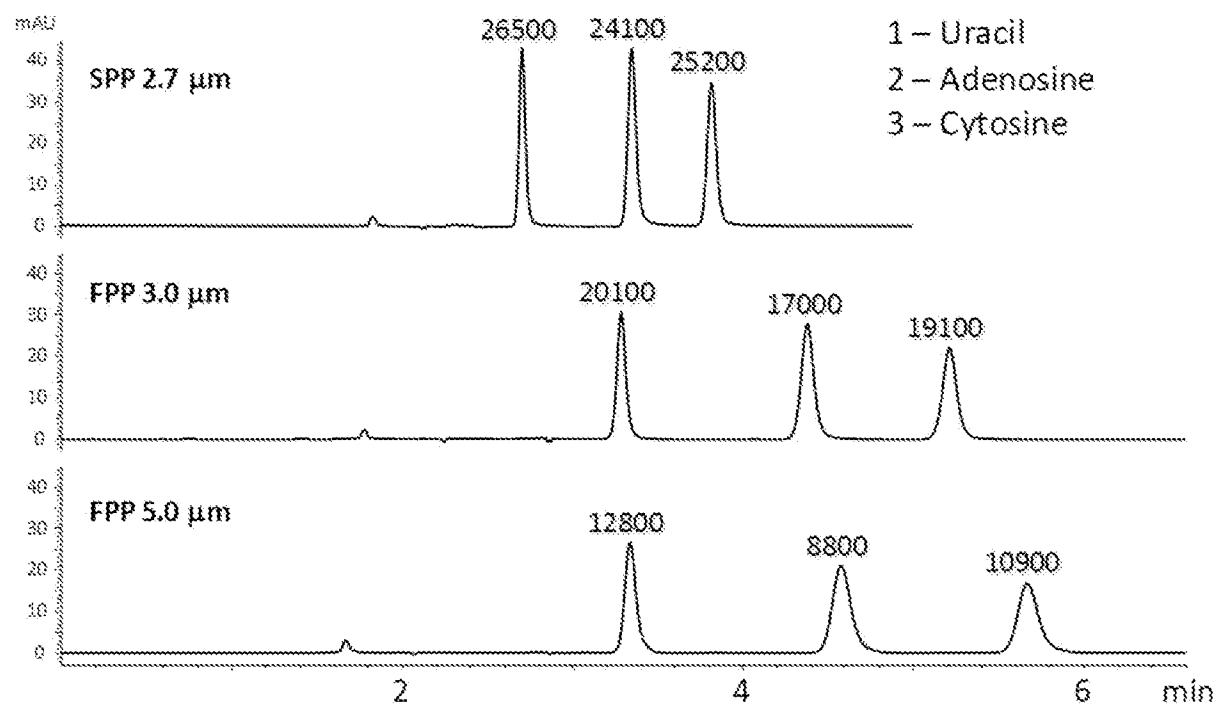
Multipoint attachment resists hydrolytic cleavage

FIG. 10

FIG. 11

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FIG. 12

FIG. 13

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FIG. 14

FIG. 15

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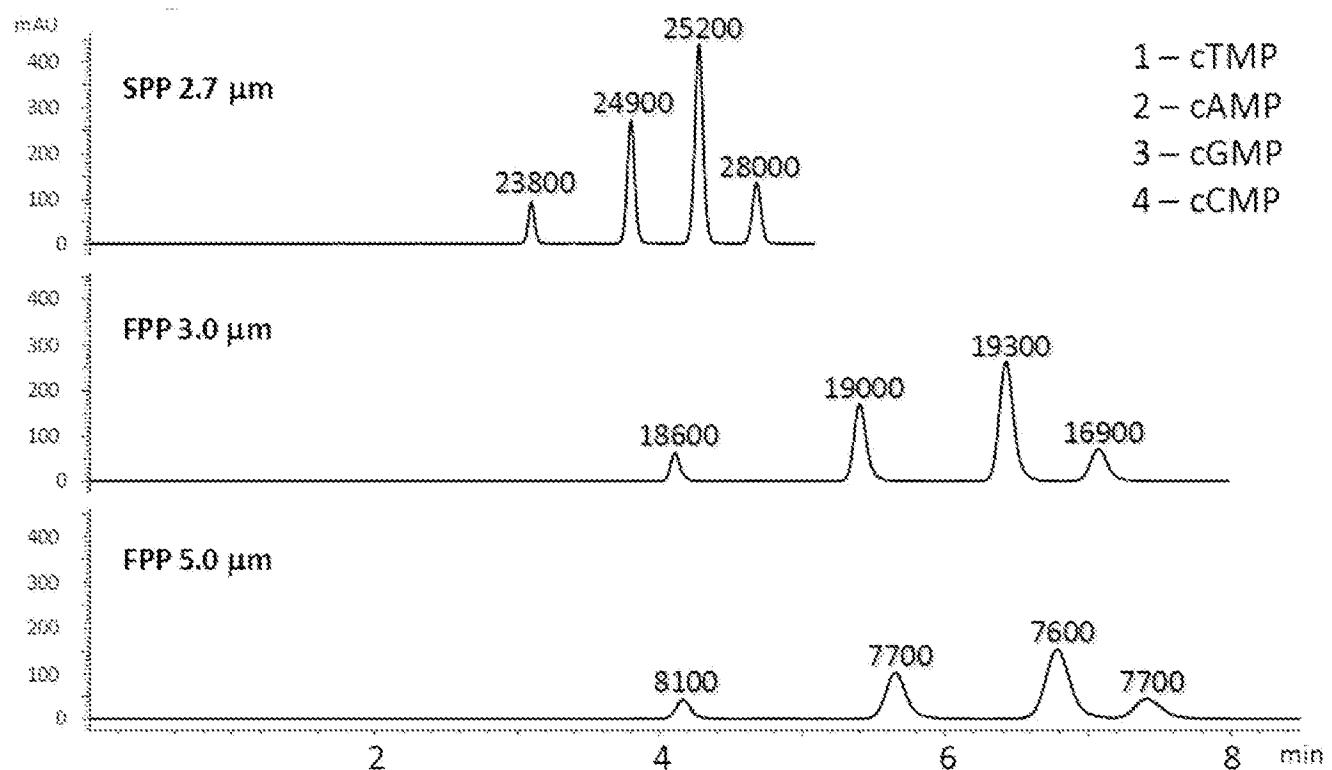
FIG. 16
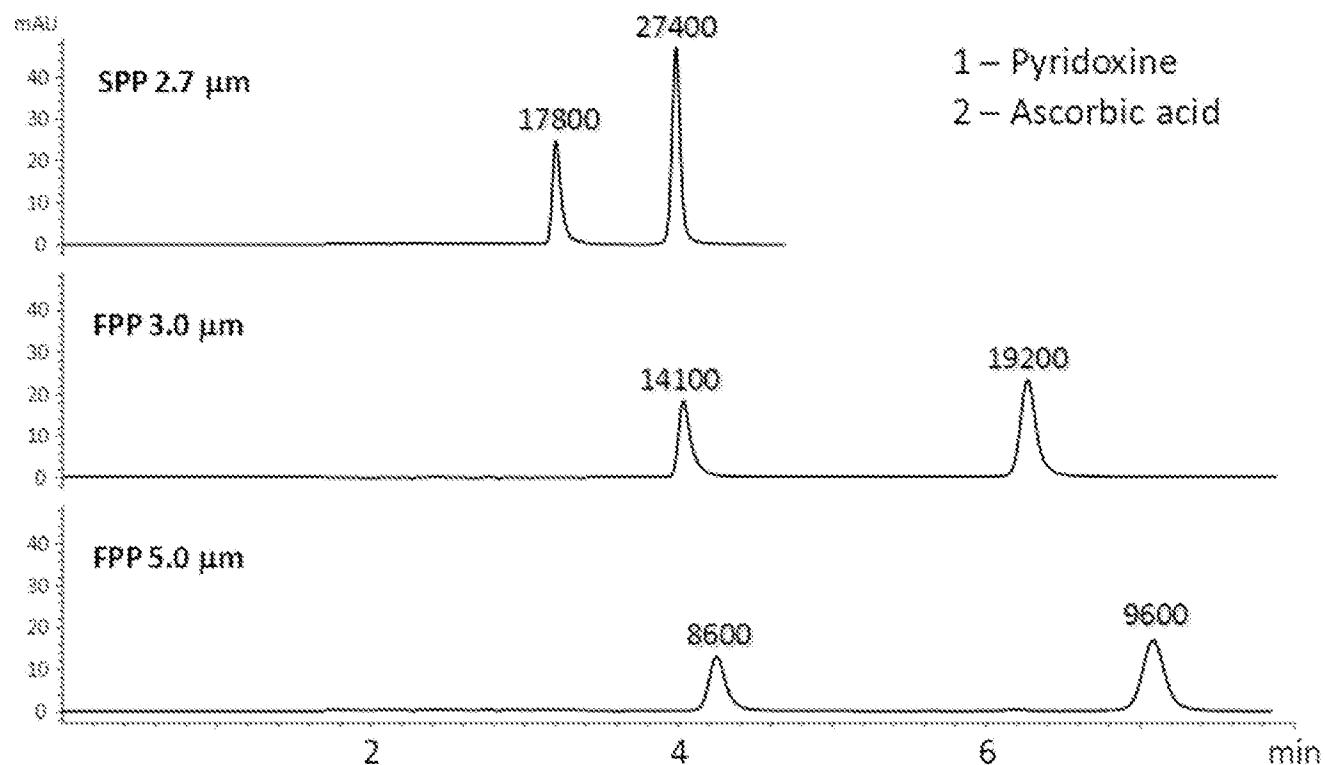
FIG. 17
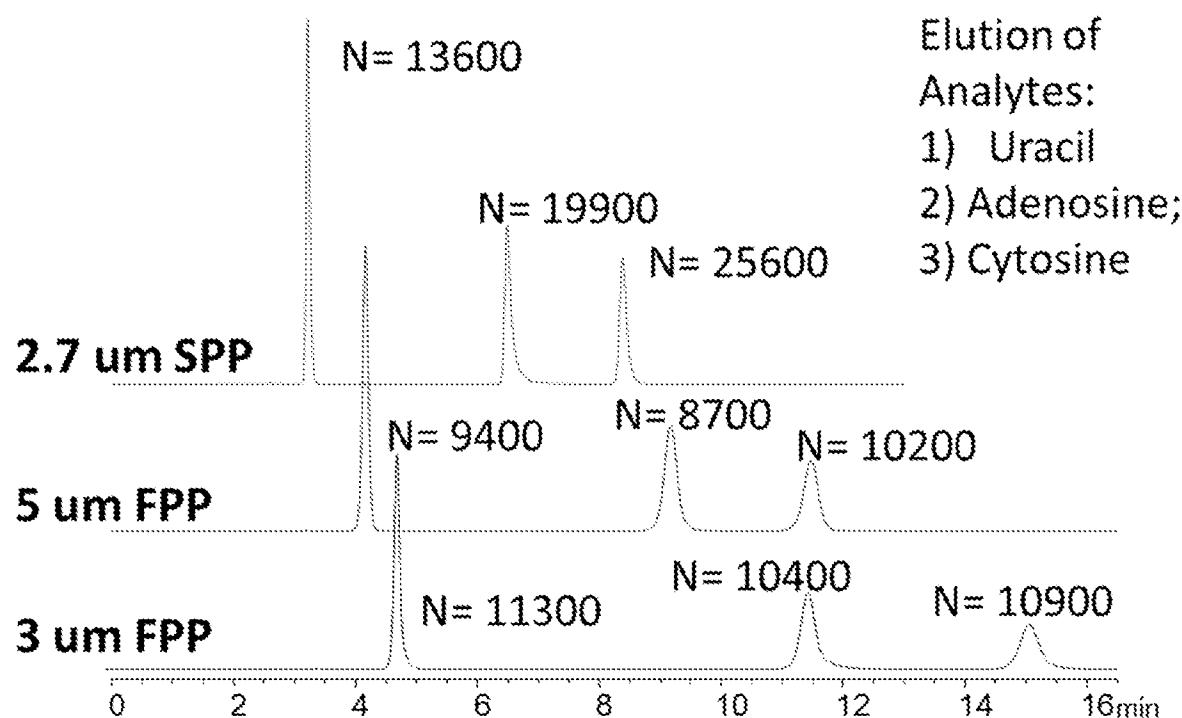
FIG. 18
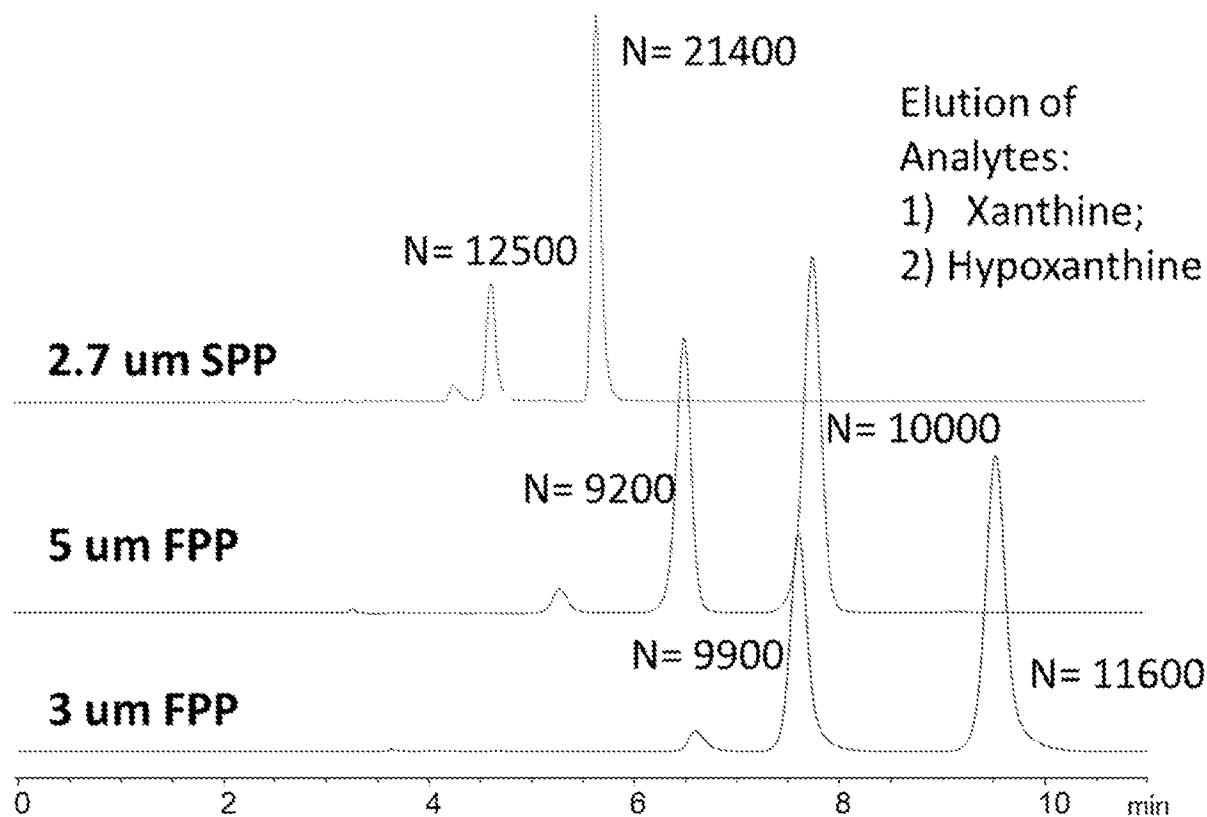
FIG. 19
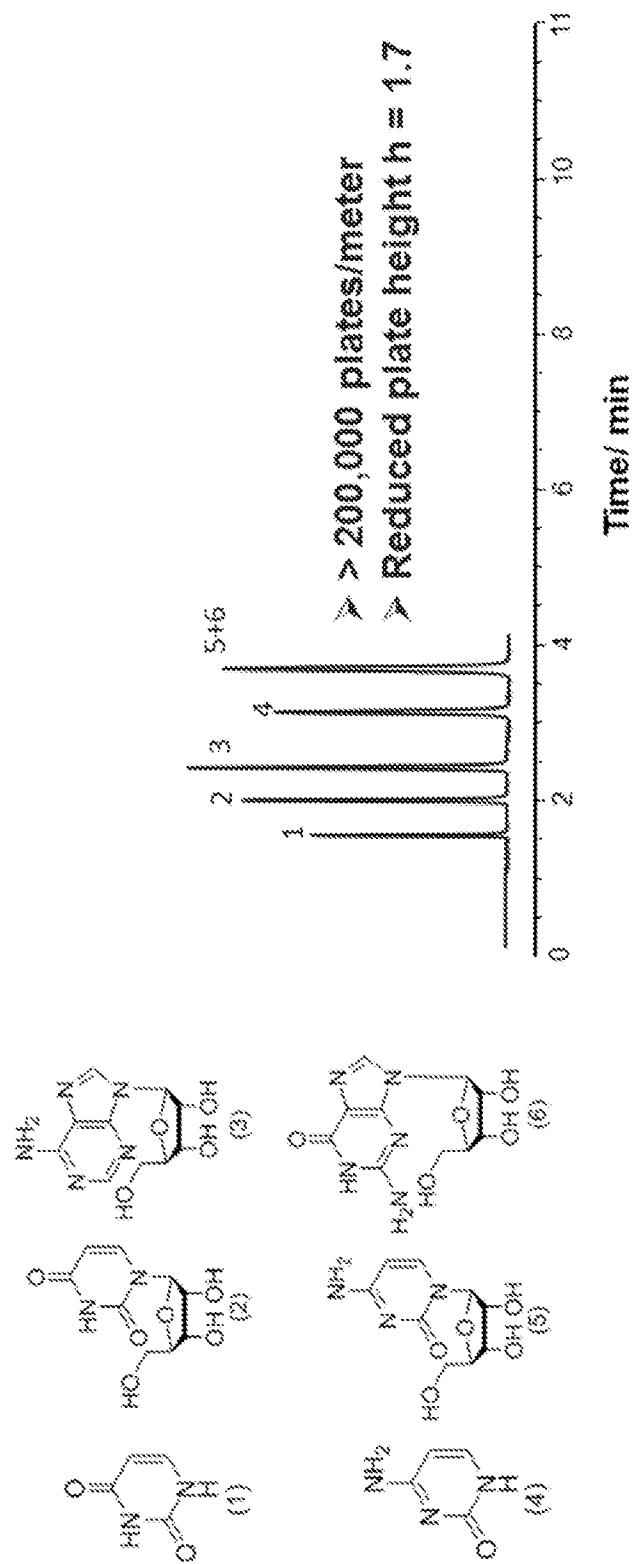
FIG. 20

Single Amino acid polymorphism - Enkephalins
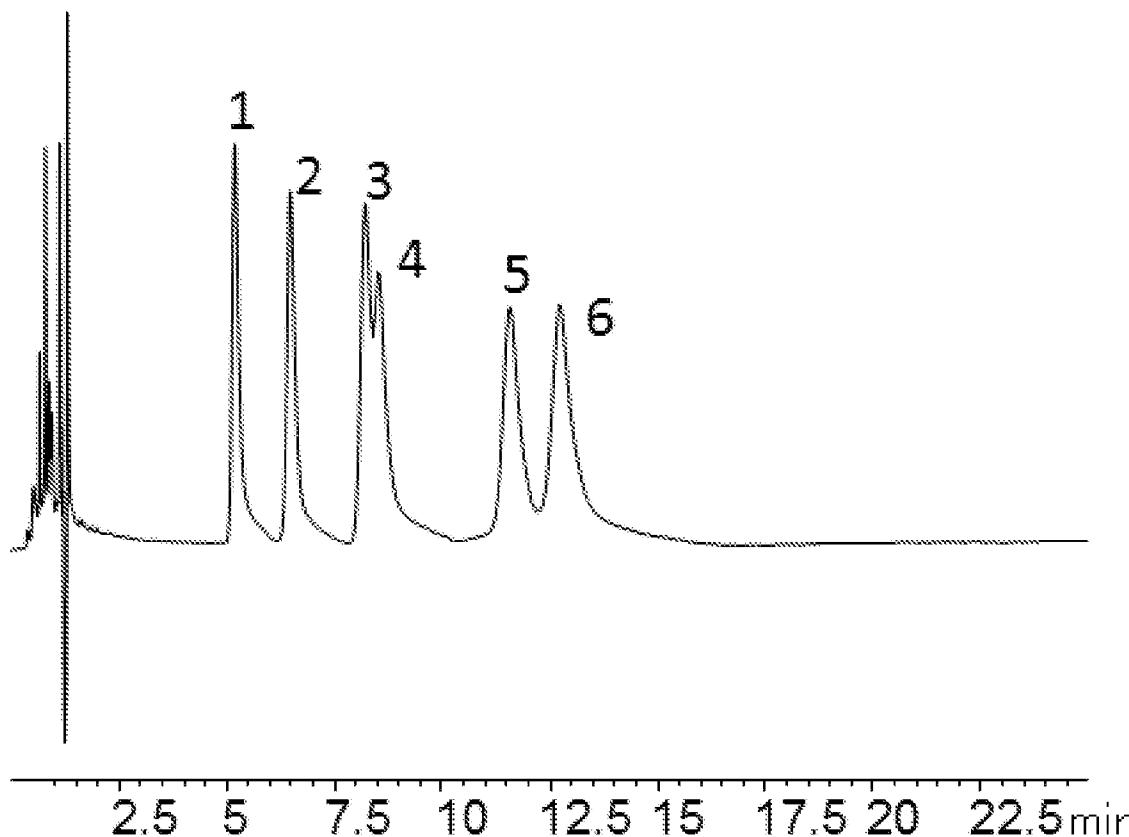
1- [D-Ala2,D-Leu5]-Enkephalin
Tyr-D-Ala-Gly-Phe-D-Leu

2- Met-Enkephalin [D-Ala2]
Tyr-D-Ala-Gly-Phe-Met

3- Met-Enkephalin
Tyr-Gly-Gly-Phe-Met

4- [D-Ala2,Leu5]-Enkephalin
Tyr-D-Ala-Gly-Phe-Leu

5- Leu-Enkephalin
Tyr-Gly-Gly-Phe-leu

6- [D-Ala2,Leu5]-Enkephalin
Tyr-Ala-Gly-Phe-Leu

FIG. 21
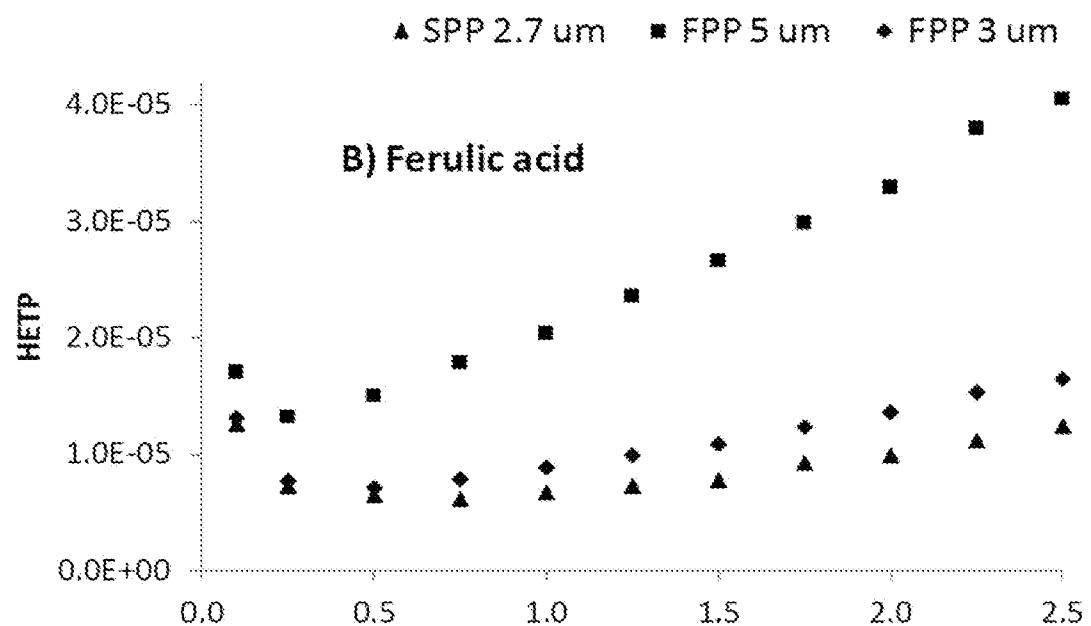
FIG. 22
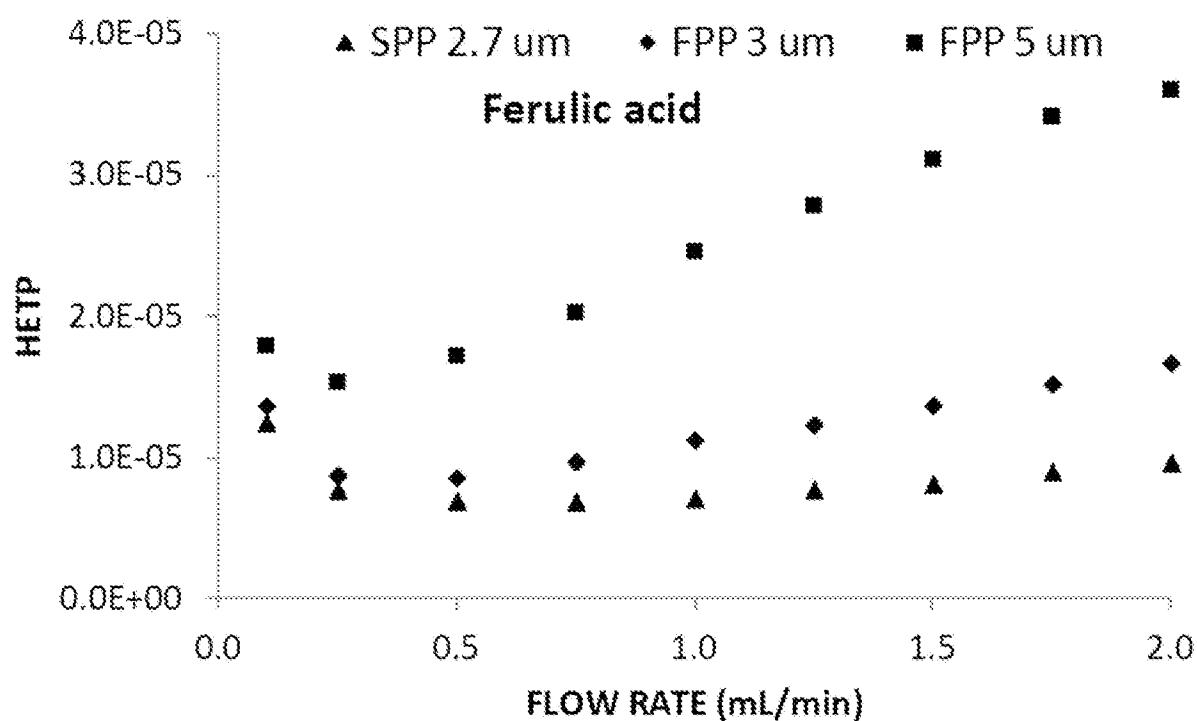
FIG. 23
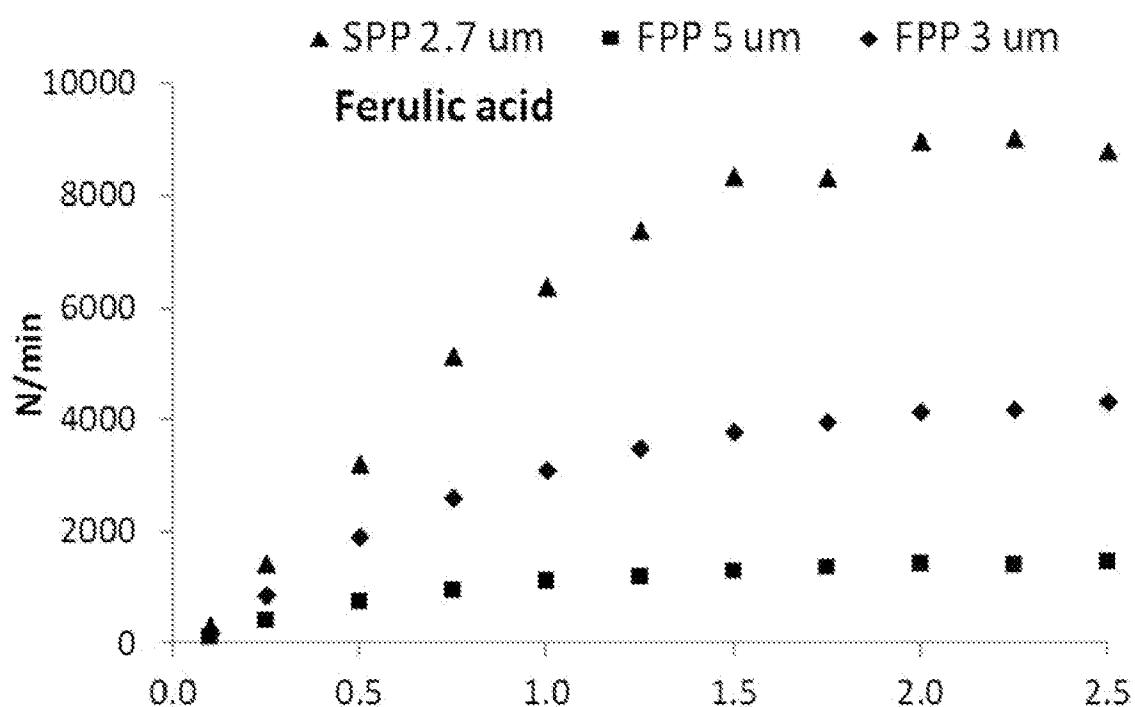
FIG. 24
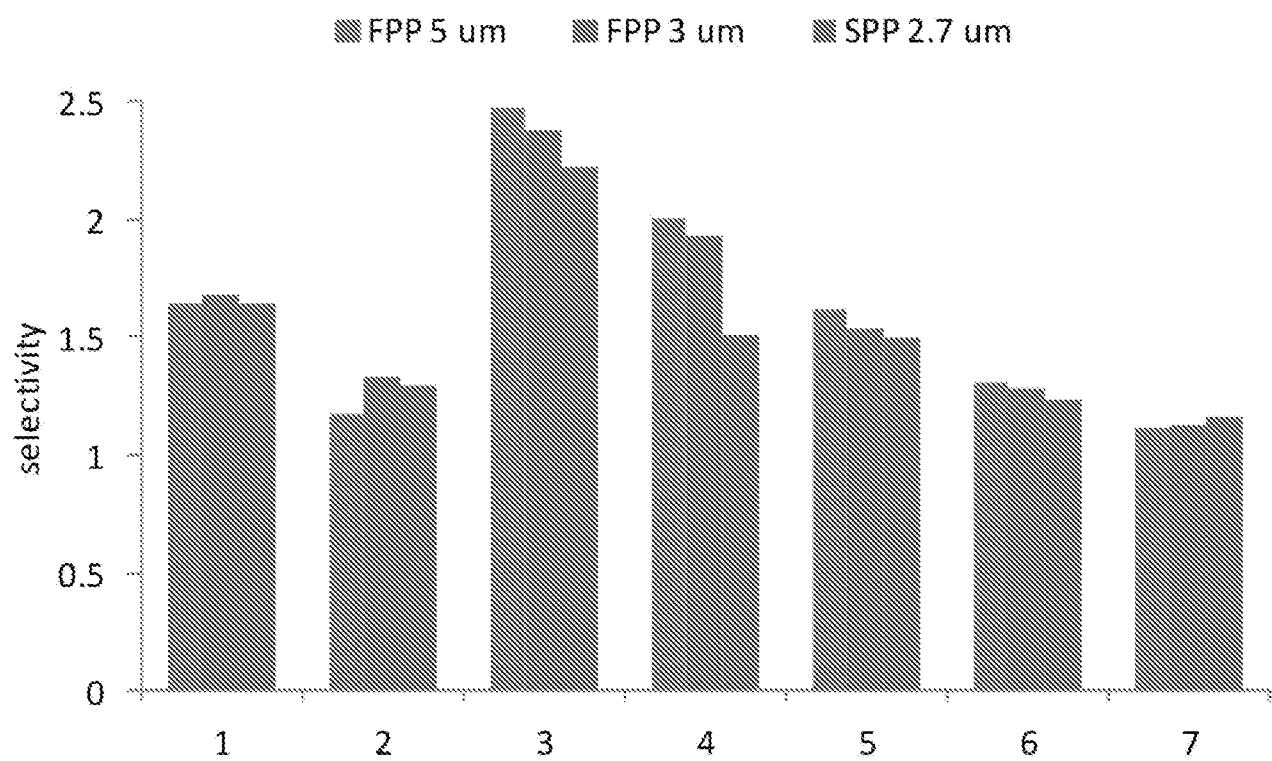
FIG. 25

	International application No.
	PCT/US2015/041028

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - B01J 20/288 (2015.01)
CPC - B01J 20/288 (2015.09)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - B01D 15/00, 15/08; B01J 20/00, 20/281, 20/282, 20/283, 20/286, 20/288 (2015.01)
CPC - B01D 15/00, 15/08; B01J 20/00, 20/281, 20/282, 20/283, 20/286, 20/288 (2015.09)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 210/198.1, 298.2, 600, 656; 502/400, 401; IPC(8) - B01D 15/00, 15/08; B01J 20/00, 20/281, 20/282, 20/283, 20/286, 20/288;
CPC - B01D 15/00, 15/08; B01J 20/00, 20/281, 20/282, 20/283, 20/286, 20/288 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit, Google Patents, Google
Search terms used: chromatography superficially porous particles diameter covalent HILIC hydrophilic interaction retention time efficiency selectivity drift

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0021136 A1 (QIU et al) 23 January 2014 (23.01.2014) entire document	1-23, 26, 27
Y	US 2013/0112605 A1 (WATERS TECHNOLOGIES CORPORATION) 09 May 2013 (09.05.2013) entire document	1-23, 26, 27
Y	WO 2013/173501 A2 (WATERS TECHNOLOGIES CORPORATION) 21 November 2013 (21.11.2013) entire document	20, 21
Y	DESTEFANO et al. Performance characteristics of new superficially porous particles. Journal of Chromatography A. Vol. 1258. 05 October 2012. [retrieved on 15 September 2015]. Retrieved from the Internet. <URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3606024/>. pp. 76-83	24, 25
Y	IBRAHIM et al. Hybrid carbon nanoparticles modified core-shell silica: A high efficiency carbon-based phase for hydrophilic interaction liquid chromatography. Analytica Chimica Acta. Vol. 820. 11 April 2014. [retrieved on 15 September 2015]. Retrieved from the Internet. <URL: http://www.sciencedirect.com/science/article/pii/S0003267014002475>. entire document	24, 25
Y	US 2013/0324700 A1 (DAILIAN INSTITUTE OF CHEMICAL PHYSICS et al) 05 December 2013 (05.12.2013) entire document	25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2015	2 0 OCT 2015

Name and mailing address of the ISA/	Authorized officer
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Facsimile No. 571-273-8300	PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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