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A novel positively charged achiral co-monomer for β -cyclodextrin monolithic stationary phase: Improved chiral separation of acidic compounds using capillary electrochromatography coupled to mass spectrometry

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Abstract

The work presented here demonstrates the incorporation of vinylbenzyl trimethylammonium (VBTA) as a novel positively charged achiral co-monomer to a glycidyl methacrylate- β -cyclodextrin (GMA/ β -CD) based monolith, providing anion exchange sites with reversed electroosmotic flow (EOF) for capillary electrochromatography (CEC). The monolithic phases, GMA/ β -CD-VBTA and GMA/ β -CD (without co-monomer) were characterized by scanning electron microscopy, optical microscopy, pressure drop/flow-rate curves and nitrogen adsorption analysis. After optimizing the stationary phase and mobile phase parameters, chiral separations of 41 pairs of structurally diverse anionic chiral analytes were compared individually using the GMA/ β -CD-VBTA and GMA/ β -CD monolithic columns. The GMA/ β -CD-VBTA monolith chiral stationary phase separated significantly more acidic compounds compared to the GMA/ β -CD column. To-date there has been limited work in the development of chiral monolithic column for CEC-mass spectrometry (MS). Because of good electrodriven flow characteristics, which allow the column to maintain a stable current in the absence of outlet vial, GMA/ β -CD-VBTA column was successfully coupled to single quadrupole mass spectrometer for CEC-MS of several chiral test compounds. In addition, the same monolithic CEC column when coupled to a triple quadrupole MS instrument, two orders of magnitude higher sensitivity was observed compared to a single quadrupole MS instrument.

Keywords

Capillary electrochromatography (CEC); GMA- β -CD-VBTA monolithic column; Monolithic characterizations; Acidic compounds screenings; Capillary electrochromatography-coupled to single quadrupole and triple quadrupole mass spectrometers

1. Introduction

The development of new type of monolithic chiral stationary phases (CSPs) for capillary electrochromatography (CEC) is a continuously growing field. Up to date, several chiral selectors have been developed for monolithic CEC [1-17]. However, the design and discovery of new type of monolithic phases is still needed to develop new applications and for development of mass spectrometry (MS) compatible monolithic CEC phases. Cyclodextrins (CDs) have been one of the most popular and successful type of

pseudostationary phase for electrokinetic chromatography (EKC) [7,18] and as CSP in high performance liquid chromatography (HPLC) [19,20]. Because of the presence of multiple chiral centers, CDs are very adaptable to function as a successful monolithic CSP for capillary HPLC [21,22]. While a large number of CDs have been introduced as chiral selectors for HPLC and CE, relatively few CDs are studied in a monolithic format. A monolithic CSP in CEC must fulfill two requirements. One is to provide large number of chiral interaction sites for the analytes to be retained mainly through enantioselective binding, and the other one is to provide fixed charges for electroosmotic (EOF) generation. Those charged groups in the bulk monolithic phase may interact with charged functionalities within the chiral analyte structure. For example, a post modification multistep method is used in which the achiral monolithic column backbone is formed inside the capillary first. Next, the achiral monolithic backbone is modified with the CD selector matrix after the formation of the monolith [9,23,24].

Very recently we have described an alternative and a simple “*one step*” approach for preparing CD based monolithic columns for CEC [25]. In our approach, a glycidyl methacrylate bonded β -cyclodextrin (GMA/ β -CD) was first synthesized as a new synthetic chiral monomer by covalently bonding primary hydroxyl groups of β -CD with the epoxide groups of GMA. By polymerizing GMA- β -CD monomer with ethylene dimethacrylate (EDMA), in the presence of commonly used porogens and a charged monomer, 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) a versatile chiral monolith (GMA/ β CD-AMPS) was formed. One of the advantages of this aforementioned approach in making CD monolith is that the synthesis of the GMA- β -CD outside the column allows for the verification of covalent attachment as well as determination of degrees of substitution of methacrylate groups through NMR spectroscopy. After the verification of the covalent attachment of GMA and degree of substitution, the GMA- β -CD can be copolymerized in a single step with a charged co-monomer in the porogen solution. Under optimum mobile phase conditions, a variety of basic and neutral chiral compounds could be successfully resolved using GMA- β -CD-AMPS monolith phase in separate CEC runs. However, the electrostatic repulsion between the charged co-monomer (AMPS) and negatively charged chiral analytes limited the use of this achiral co-monomer to separate acidic compounds.

To overcome this aforementioned limitation, it is of great importance to use positively charged achiral monomers, which can overcome the problem of electrostatic repulsion, allowing chiral separation in CEC for negatively charged compounds. Therefore, the work presented here looks at the incorporation of a positively charged vinylbenzyl trimethylammonium (VBTA) co-monomer into the monolithic structure along with the GMA- β -CD chiral selector. Comparison of the separations of the chiral compounds with GMA- β -CD versus GMA/ β -CD-VBTA monolithic columns indicated that the charge of the achiral co-monomer significantly influences chiral separation of the negatively charged analytes.

Although novel designs for both fritless [26] and single-frit [27] packed-column have been developed for chiral CEC-MS, progress in applying monolithic chiral columns in CEC-MS has remained slow. The current major challenge in the development of a monolithic chiral CEC column for MS interfacing is the resistance to the flow of liquid, in particular through small pores containing chiral moieties, which results in band broadening. The problem is even more severe when monolith column with smaller internal diameter and cathodic EOF is coupled to MS. In this work, the GMA/ β -CD-VBTA monolithic columns was applied for the hyphenation of CEC to both single quadrupole mass spectrometer (MS) and triple quadrupole MS for several representative acidic compounds. To the best of our knowledge, this is the first work reporting the preparation of GMA/ β -CD-VBTA monolith and its

potential use for the enantioseparation of wide variety of negatively charged chiral analytes in CEC and CEC-MS.

2. Experimental

2.1. Reagents and materials

The HPLC grade solvent acetonitrile (ACN), β -cyclodextrin (β -CD), anhydrous dimethylformamide (DMF), 2,2'-azobis(2-methylpropionitrile) (AIBN), ammonium acetate (NH_4OAc), glacial acetic acid (HOAc), triethylamine (TEA), anhydrous 1-propanol, anhydrous dimethylsulfoxide (DMSO) and 99% acetone were purchased from Sigma (St. Louis, MO, USA). The reagents (vinylbenzyl)-trimethylammonium chloride (VBTA), ethylene glycol dimethacrylate (EDMA), 3-(methacryloyloxy) propyltrimethoxysilane (γ -MAPS), and ammonium formate (NH_4COOH) were obtained from Aldrich (Milwaukee, WI, USA). Glycidyl-methacrylate (GMA), 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), and 1,4-butanediol were purchased from Fluka (Buchs, Switzerland). All reagents were used as received, except EDMA, which was purified by vacuum distillation to remove inhibitors. Water used in the project was purified using a Barnstead Nanopure II Water System (Barnstead International, Dubuque, IA). Reagent grade toluene was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All chiral analytes were purchased as racemic mixtures from Sigma, except for ($\pm$)-lactolactic acid, DL -2-(4-chlorophenyl)glycine, and N -(2,4-dinitrophenyl)- DL -threonine, which were purchased from TCI America (Portland, OR, USA) (Figure S1, SI). Stock solutions of analytes were prepared as 2 mg/mL solutions in ACN and working standard solutions were diluted to appropriate final concentrations in the range of 0.5–1.0 mg/mL with triply deionized water. The fused silica capillaries (50 cm $\times$ 100 μm or 150 μm i.d.) were obtained from Polymicro Technologies, Inc. (Phoenix, AZ).

2.2. Synthesis of glycidylmethacrylate modified β -cyclodextrin (GMA/ β -CD)

The GMA/ β -CD was synthesized according to a procedure previously published [25,28]. Briefly, a 100 mL round bottom flask containing approximately 6 g of β -CD (5.22 mmol) and a clean, dry stir bar, was placed in a vacuum oven and dried for 48 h at 40 °C. After drying, 28 mL of anhydrous DMF was added to the round bottom flask and a moisture trap was attached before sonicating to dissolve the β -CD. Once the β -CD had dissolved, the moisture trap was removed, and 58.1 μL of DBU (1%, w/w β -CD) was added followed by the quick addition of 2.14 mL GMA (15.6 mmol). The moisture trap was reattached and the entire apparatus was placed in a 98 °C oil bath to stir and react for 2.5 h. After 2.5 h, the obtained golden brown solution was vacuum filtered (Whatman 42, 70 mm filters) and any solids were discarded. Next, the filtrate was poured into a 1000 mL beaker containing ~600 mL of toluene to precipitate out the product as a white brown solid. The precipitated solution was stirred gently for 10 min and then allowed to sit for an additional 10 min. After precipitation, the solid product was vacuum filtered and washed with 200 mL of acetone. Finally, the solid was dried in a vacuum oven at 40 °C for 24 h, then stored in a desiccation chamber until used. The GMA/ β -CD material was then characterized by ^1H NMR spectroscopy performed with a 300 MHz Varian Unity spectrometer using deuterium oxide as a solvent (Figure S2, SI).

2.3. Preparation of monolithic columns

Before monolith formation, the fused silica capillary was vinylized by reaction with various % w/w of γ -MAPS in methanol according to procedures outlined previously [25]. Following pretreatment of the capillaries, pre-polymerization solutions were prepared in various ratios consisting of chiral monomers (GMA/ β -CD-VBTA or GMA/ β -CD), EDMA, and AIBN dissolved in the porogen solution of 1,4-butanediol, 1-propanol and DMSO. After the solution was sonicated and purged with nitrogen for 10 min, the 50 cm vinylized

capillary was filled up to a length of 35 cm and capped at both ends with rubber septum. In the case of CEC-MS experiments, a 150 μm i.d. capillary was used to improve the flow through the monolithic bed. The loaded capillary was then placed in a water bath for 24 h at 60 $^{\circ}\text{C}$ for polymerization. The schematic illustration of the polymerized material is shown in Fig. 1. Unless otherwise stated, the following pre-polymerization solution was used for GMA/ β -CD-VBTA column: (% w/w): 14.6% (75 mg) GMA/ β -CD; 0.4% (2 mg) VBTA; 5% (23.8 μL) EDMA, 30% (148 μL) 1,4-Butanediol; 30% (136 μL) DMSO; 20% (125 μL) 1-propanol, 0.5% AIBN. After removing the polymerized monolithic column from the water bath, the septa were carefully removed and a small portion at each end of the capillary was cut to ensure that there were no blockages. To remove any unreacted monomers and porogens, the monolithic column was then flushed with ACN for 12 h using an HPLC pump. For CEC-UV experiments, an on-column detection window was made next to the polymer bed using an in-house coating burner. Before conditioning with mobile phase, the capillary was cut to a final effective length of 25 cm with a total length of 35 cm. In the case of CEC-MS experiments, the capillary (with a 150 μm i.d., an effective length of 25 cm and a total length of 50 cm) with no detection window was used to increase the flow rate and stabilize the current.

2.4. Morphology, porosimetry, and surface area measurements

Scanning electron microscopy (SEM) was carried out to analyze the differences in microscopic morphology between the two different types of monolithic column under study in this work. This was done with a Hitachi X-650 (Hitachi, Japan) SEM apparatus at 7.5 kV and 40 mA filament current. Before imaging, each monolithic column sample was prepared by fracturing and cutting to a length of 2 mm, followed by attachment to an aluminum stub using means a double sided carbon tape. Next each monolithic column sample was sputter-coated with a SPI Sputter (SPI Supplies Division of Structure Probe, West Chester, PA, USA) for 1 min at 30 mA to prevent charging using a gold/palladium alloy.

Using the capillary HPLC flow method the porosity of the two monoliths was determined [29,30]. Briefly, by measuring the void volume of an unretained marker (DMSO) the mobile phase (100% ACN) linear velocity was determined, along with measurements of the volumetric flow rate at 10 $\mu\text{L}/\text{min}$, 14 $\mu\text{L}/\text{min}$, and 18 $\mu\text{L}/\text{min}$. The volumetric flow rate was determined by weighing out the amount of (ACN) that eluted from the column in a given time. The eluted ACN was collected a sealed vial to avoid errors due to the evaporation of the mobile phase. This weight was then converted to volume using density of ACN. Next, based on the dimensions of the empty capillary, the total porosity ε_T was calculated using the following Eq. (1):

$$\varepsilon_T = \frac{V}{\pi r^2 u} \times 100 \quad (1)$$

where ε_T is the total porosity, $V(\text{m}^3/\text{s})$ is the volumetric flow rate of mobile phase, $r(\text{m})$ is the inner radius of the empty column, and $u(\text{m}/\text{s})$ is the linear velocity of mobile phase, which was determined by the unretained compound DMSO. The linear velocity was determined by dividing the effective length of the column by the retention time of the DMSO.

The permeability of a porous medium is a measure of its capacity to transmit a fluid driven by an imposed pressure drop. The specific permeability, K^0 , can be determined by Darcy's law, which is directly proportional to the solvent viscosity and column porosity, and inversely proportional to back pressure (Pa). The column back pressure was measured in the range (1.4–25 MPa) at different flow rates. The K^0 was calculated using the following equation:

$$K^o = \frac{u\eta L \varepsilon_T}{\Delta p} \quad (2)$$

where η (Pa s) is the dynamic viscosity of the eluent, L (m) is the effective column length and Δp is the pressure drop (Pa) [30]. The linearity of the slope of u versus Δp will assure the monolith possess good mechanical stability.

The samples for measurements of BET surface area were prepared in separate glass vials under the same polymerization conditions used for monolithic columns. After polymerization, the hard gel monolith was crushed and soxhlet extraction was performed for each monolith (using methanol for 24 h), dried at 70 °C under vacuum before BET measurements were performed by nitrogen adsorption experiments. Briefly, a multi-point BET method was applied to nitrogen physisorption data obtained on a Micromeritics Tristar 3020 (Micromeritics Instruments, Norcross, GA, USA). All samples were first heated under vacuum at 70 °C for 24 h to remove physisorbed water before being analyzed. The 59 point adsorption/desorption isotherm was then obtained at liquid nitrogen temperatures.

2.5. CEC and CEC-MS instrumentation

The CEC-UV experiments were carried out with an Agilent capillary electrophoresis (CE) system (Palo Alto, CA, USA) interfaced to a diode-array detector. The CEC ESI-MS or CEC-ESI-MS/MS experiments were carried out with a CE instrument interfaced to an Agilent 1100 series single quadrupole mass spectrometer and an Agilent 6410 triple quadrupole MS/MS, respectively. An Agilent 1100 series HPLC pump equipped with a 1:100 splitter was used to deliver the sheath liquid for both types of CE-MS instruments. The interface of the CE to MS and MS/MS was made possible by a G1603A CE MS adapter kit and a G1607 CE-ESI-MS sprayer kit also provided by Agilent Technologies. The Agilent ChemStation and CE-MS add-on software (V 10.02) were used for instrument control and calculation of chiral resolution (R_s), retention time (t_r), and separation efficiency (N) for both CEC-UV and CEC-ESI-MS modes. Resolution was calculated using the equation: $R_s = 2(t_{fb} - t_{ra})/(w_b + w_a)$, where t_{ra} is the retention time of first eluting enantiomer 'a', t_{fb} is the retention time of second eluting enantiomer 'b', w_a is the base width of compound 'a' and w_b is the base width of compound 'b.' Using Agilent MassHunter Optimizer software (V. B. 02.00), the most abundant product ion for multiple reaction monitoring (MRM) experiments of 2,4-dinitrophenylthreonine was determined to be 178 m/z, which was generated from the precursor ion of $[M-H]^- = 284$ m/z.

2.6. CEC and CEC-MS conditions

Stock background electrolyte solutions (BGE) were prepared by dissolution of the appropriate salt (ammonium acetate or ammonium formate) in triply deionized water followed by pH adjustment with acetic or formic acid, respectively. The final mobile phases were then prepared fresh daily by combining volumetric ratios of ACN, water and stock BGE. After the monolithic columns were fabricated, they were connected to an HPLC pump to be conditioned with ACN for 12 h following by conditioning with the mobile phase for 2 h. Next, the monolith columns were placed in the instrument and voltage conditioned. Unless otherwise stated, the GMA/ β -CD and GMA/ β -CD-VBTA columns were run with 75/25 ACN/H₂O, 5 mM NH₄COOH at pH 3.0 mobile phase.

The CEC-UV experiments were all performed at 25 °C with a final voltage of -30 kV, unless otherwise noted. During the runs, 6 bar of pressure was applied to each end of the capillary to suppress bubble formation. Electrokinetic injections were performed at -6 kV for 6 s of the test compounds prepared at 1 mg/mL in 50/50 ACN/H₂O. The CEC-MS

experiments were carried out at 25 °C and –30 kV voltage with 12 bar of pressure applied to the inlet side to reduce bubble formation and improve flow. In addition, with the CEC-MS experiments the 25 cm portion of the capillary containing GMA/β-CD-VBTA monolith was placed in the inlet CE side of the instrument, whereas the open 25 cm portion of the capillary was placed in the MS instrument outlet side. This was done to maintain a stable current, as reversal of this configuration led to current breakdown and poor reproducibility. The following ESI-MS conditions were used. The negatively charged analytes were detected as $[M-H]^-$ deprotonated molecular ions in select ion monitoring (SIM) mode. The spray chamber settings were as follows: drying gas temperature, 150 °C; drying gas flow rate, 3 L/min; nebulizer pressure, 20 psi; capillary voltage, –3500 V; fragmentor voltage 84 V; and gain 3. The MS/MS SIM conditions were the same as those for the SIM MS runs. The MS/MS MRM experiments were conducted using the same spray chamber conditions as the MS SIM runs. For detection in MS/MS, the precursor ion of 284 m/z and product ion of 178 m/z were monitored for 2,4-dinitrophenylthreonine as determined by flow injection analysis using Agilent MassHunter Optimizer Software (V. B.02.00).

3. Results and discussion

First the GMA modified β-CD was synthesized. Next, the monolithic conditions such as percentage of γ-MAPS (column pretreatment reagent) and the concentration of VBTA in the polymerization mixture were optimized for separation of 11 negatively charged chiral compounds. Following monolith column characterization, the mobile phase conditions were optimized for the same eleven chiral compounds. The mobile phase conditions that were explored included buffer (type and concentration), pH, and percent organic modifier. Two different voltages (–15 kV and –30 kV) were studied to see which voltage provide the best compromise between chiral resolution and analysis time. Under optimized conditions, 30 additional negatively charged chiral compounds (Figure S1) were examined using the GMA/β-CD-VBTA column. Additional screening of select compounds was carried out with a GMA/β-CD monolithic column with no co-monomer to see the effects of removing the charged co-monomer from the polymerization mixture on chiral separations. Finally, several model compounds separated with the GMA/β-CD-VBTA column were compared using CEC hyphenated to a single quadrupole mass spectrometer (MS) and triple quadrupole mass spectrometer.

3.1. Synthesis of GMA/β-CD

The ^1H NMR was conducted to confirm the attachment of the GMA to the β-CD and to determine the degree of substitution (DS) (Figure S2, SI). Besides showing the covalent attachment of the GMA to the β-CD, the ^1H NMR spectrum can also be used to calculate the DS of the GMA to β-CD. This is done by taking the ratio of the integral of the H1 methine proton peak of β-CD ring proton to the integral of the H5' methylene proton of GMA. Because the integral ratio represents the number of equivalent protons present in each peak, this allows for the estimation of the actual number of GMA group attached to the β-CD ring. In our case, the DS was determined to be 1.76. This DS was shown to be nearly optimum based on our previous work (data not shown) [25].

3.2. Optimization of monolithic column parameters

The percentage of γ-MAPS, concentration of the VBTA comonomer are stationary phase parameters, which were first optimized [17,25]. After characterizing the GMA/β-CD and GMA/β-CD-VBTA columns, the mobile phase conditions (buffer type, pHs and concentration) and voltage are common parameters that are varied to improve enantioselectivity of acidic compounds.

3.2.1. Optimization of the percentage of γ -MAPS—The γ -MAPS coating on bare-fused silica capillary is used to create anchoring (vinylization) sites within the inner walls of the capillary to allow the monolith to attach to the column. For this reason, it is important to optimize the amount of γ -MAPS by varying the percentage of γ -MAPS in methanol from 10% to 90%. After monolith formation, the capillaries were flushed with ACN for 2 h, then digital microscope images were taken. As detailed in the optical microscopic images at the top of Table 1, in the case of the 10% γ -MAPS column, the monolith bed was nonhomogenous with gaps and could not provide a stable current. Most likely at 10% there were not enough γ -MAPS present on the inside of the capillary to allow a complete monolith formation. Thus, when flushed unanchored pieces of monolith were removed. On the other hand, 70% and 90% γ -MAPS column, showed extensive areas of dryness as represented by the dark areas in the inset digital images. In addition, the 70% γ -MAPS column could only separate one test compound (e.g. 2-(3-chlorophenoxy)propionic acid), whereas the 90% monolithic column could not maintain a stable current for separation of any analytes. Perhaps, higher % γ -MAPS in column causes excessive attachment sites within the capillary causing too tight of a porous network at the outside edges of the monolith restricting the flow of mobile phase in the area where the monolith attaches to the capillary walls. The best monoliths formed were those made with capillaries pretreated with 30% and 50% γ -MAPS in methanol. When compared to 30% γ -MAPS, the 50% γ -MAPS column showed higher resolution and efficiency for all eleven test analytes with shorter retention time and was able to baseline resolve several phenoxypropionic acids. While the current did not completely breakdown for the 30% γ -MAPS column, it was relatively less stable than the 50% γ -MAPS capillary (data not shown). Therefore, 50% γ -MAPS monolith column had the correct number of anchoring sites, allowing for an optimum attachment to the monolithic material.

3.2.2. Optimization of comonomer concentration—As mentioned earlier, we have previously observed that the presence of anionic co-monomer, AMPS, in the β -CD monolith was useful for the separation of positively charged chiral compounds [25]. Hence, for the separation of negatively charged chiral compounds the next obvious parameter to be optimized was the amount of cationic VBTA co-monomer incorporated into the monolithic material. Because the presence of VBTA provided the positively charged site for the reversal of EOF and help in retaining negatively charged chiral analytes, the correct amount of the co-monomer added to the polymerization mixture needed to be determined. As shown in Table 2, the monolith containing 1 mg of VBTA in the polymerization mixture showed the shortest runtime and lowest R_s for all eleven acidic compounds and the worst chiral resolution in almost all cases. As the amount of VBTA was increased in the polymerization mixture to 2 mg, we see a general trend in an increase in retention time for all compounds, which in turn also improves chiral R_s . In fact, at 2 mM VBTA concentration mark, the chiral R_s all compounds are at their best. Further increase in VBTA comonomer showed longer run time but drop in R_s values for 9 out 11 compounds. Hence, the 2 mg VBTA in the polymerization mixture seems appropriate. No further components of the monolith (i.e. % w/w of GMA/ β -CD, EDMA and porogens) were varied due to the optimization of these polymerization components in our previous study [25].

3.2.3. Monolithic columns characterization—Following optimization of the γ -MAPS and VBTA concentrations, preliminary studies were performed using dry-state methods of SEM imaging to elucidate the microscopic morphology of the positively charged GMA/ β -CD-VBTA monolithic column versus GMA/ β -CD column (with no charged co-monomer) (Fig. 2A, B). Looking at the SEM images it appears that GMA/ β -CD monolithic material (Fig. 2B) has much finer structure with more granular well defined clusters of particles. On the other hand, the GMA/ β -CD-VBTA (Fig. 2A) material appears to be globular particles,

which is buried more within the monolithic matrix. The porosity values of GMA/ β -CD-VBTA and GMA/ β -CD-VBTA is close to what we expect at a fixed weight ratio of 80% porogens to 20% monomers (Table 3, column 1). However, the GMA/ β -CD monolith possesses a permeability that is an order of magnitude higher than the GMA/ β -CD-VBTA materials (Table 3, column 2). This is most likely because the smaller well defined particles in the GMA/ β -CD provide more flow paths for the mobile phase both through and around the edges of the clusters of the monolithic materials. In contrast, the more globular less well defined structure of the GMA/ β -CD-VBTA monolithic material may only allow the mobile phase to flow around but not through the different particle clusters. Additionally, the data for BET surface area measurements show that the GMA/ β -CD-VBTA monolith has almost half the surface area of the GMA/ β -CD monolith (Table 3, column 3). Note that GMA/ β -CD-VBTA monolith have a unique combination of globular (Fig. 2A) and less porous structure (Table 3, column 1). The more globular nature of the monolith could have caused the GMA/ β -CD-VBTA material to have the lowest total surface area, being the least exposed. These preliminary SEM studies with dry-state methods can be further elucidated with the use of solvent interaction studies recently proposed by Nischang [31,32].

The overlay plots of the back pressure versus the volumetric flow rate on the two monolithic columns are shown in Fig. 3. Both columns showed good stability over the pressure ranges as demonstrated by the excellent linearity in the range of 1.5–25 MPa (217–3625 psi) in the split flow mode of capillary HPLC, where all R^2 values are 0.9999. In addition, it is noted that the GMA/ β -CD (Fig. 3▲) column could achieve the highest flow rates, as high as 0.27 $\mu\text{L}/\text{min}$ at 25 MPa (3625 psi) and the smaller slope as would be expected for the monolithic column with higher permeability. This trend is consistent with the theory that permeability being directly proportional to the ratio of flow rate to pressure change as shown in Eq. (2) in Section 2. The GMA/ β -CD-VBTA (Fig. 3◆) column had relatively slower flow rates in the range of 0.010–0.061 $\mu\text{L}/\text{min}$ over the same pressure range with correspondingly higher slope compared to GMA/ β -CD column consistent with the permeability data. Note, that the regression line of both columns do not converge at zero flow rate but both provided negative intercept (GMA/ β -CD-VBTA, -3.1 ; GMA/ β -CD, -3.4). Considering the fluid mechanics, it is possible that some elastic compression or liquid perfusion might occur at low flow rate under low column pressure [33].

3.2.4. Optimization of the mobile phase composition—The next part of the separation system to be analyzed was the mobile composition (concentration of buffer, pH of the buffer, type of buffer, volume fraction of organic solvents) and the applied voltage. For example, the type of buffer chosen can have a large effect on the retention mechanisms taking place inside the monolithic column. The pH of the buffer will affect the extent to which the negatively charged analytes (being studied in this work) are ionized based on their different pK_a values, which in turn will affect the charge of the monolithic column (if the monomers are weakly basic or acidic). Although the VBTA co-monomer possesses quaternary ammonium groups, the residual underivatized hydroxyl groups of the GMA- β -CD monomer are very weakly acidic. Thus, the GMA- β -CD-VBTA based monolithic material should remain cationic at all relevant pHs.

The concentration of the formate buffer was varied while measuring the retention, resolution and efficiency of eleven negatively charged model test compounds at fixed voltage of -15 kV and pH 3.0. With the change in concentration of the ammonium formate there was very little change in the chiral R_s (Table S1, SI) of the compounds, but a large drop in the t_R of the analytes was observed by simply doubling the concentration of formate buffer from 5 to 10 mM. The increase in electrolyte concentration provides more formate ions, which compete with the analytes for the anion-exchange sites on the monolithic material leading to a decrease in t_R but no substantial change in R_s .

The effect of pH on retention time and enantiomeric resolution of eleven model acidic compounds were studied in 5 mM NH_4COOH . The results are summarized in Table S2, SI. Furthermore, as the pH increases the analytes become more negatively charged and retain longer on the cationic monolith. With the increase in pH from 3.0 to 6.5 there is an increase in the t_R of all acidic analytes (due to decrease in reversed EOF) and increase in electrostatic interactions, but unexpectedly there was a concomitant decrease in the chiral R_s . This is because most of the compounds (except BNP) have $\text{p}K_a$ values in the range of 2.8–3.8 (see Figure S1, SI). It appears that the best R_s is obtained close to the $\text{p}K_a$ of these compounds. Moreover, note that pH 6.5 seemed to destabilize the monolithic material in the column and only few compounds were detected within two hours of chromatographic run. When using the same column and switching from pH 6.5 back to pH 3.0, the original current and separations observed previously at pH 3.0 could not be obtained. Therefore, a new column had to be fabricated, which verified that pH 3.0 is indeed the best condition for optimum chiral R_s .

Next, using another monolithic column, the voltage was raised to -30 kV as shown in Table S3, column 1–2, SI). With an increase in voltage we see the expected further decrease in t_R , there was slight loss in R_s for most compounds (most likely due to decrease in dramatic drop in retention at -30 kV). The only one exception was 2-(3-chlorophenoxy)-propionic acid, where we actually see increase in chiral R_s from 1.0 to 1.5, possibly due to increase in efficiency. The -30 kV voltage was chosen due to shortest analysis time.

Comparing formate versus acetate based buffers the latter buffer provided comparable retention times to that of the formate buffer, but the chiral resolution of nearly all of the compounds was worse (Table S3, column 3, SI). It appears that the low concentration of ammonium formate would be appropriate to provide a reasonable trade-off between R_s and analysis time. Therefore, 5 mM ammonium formate buffer at pH 3.0 with an applied -30 kV voltage was chosen as the optimum conditions.

The final step in optimization was variation in organic solvent to affect the hydrophobicity of the monolithic column. For this study the amount of ACN added to the mobile phase was 55, 75 and 90% (v/v) (Table 4). Upon increasing from 55% to 75% ACN the mobile phase becomes more nonpolar and there was an expected decrease in retention time for all negatively charged compounds. In addition, resolution was increased slightly due to increase in N . One interesting retention trend was seen for the same negatively charged compounds with increasing % of ACN from 75 to 90. For example, longer retention times and lower chiral resolutions values were seen, which was not expected. We hypothesize that this is most likely because increasing ACN resulted in electrostatic ion-pairing of the negatively charged analytes with the positively charge VBTA co-monomer of the monolith. To test this idea, four nonionic and hydrophobic compounds [benzene, anthracene, naphthalene, and benzo[a]pyrene] were injected using 75% (v/v) and 90% (v/v) ACN in mobile phase to see if the expected trend in hydrophobicity based interactions would be seen. The retention time of the four compounds decreased, as expected, as the mobile phase became more non-polar. This suggest that the presence of mixed-mode both electrostatic and hydrophobic retention on the GMA/ β CD-VBTA monolithic column is responsible for the retention mechanism of negatively charged solutes. Therefore, based on shortest retention time and best resolution, 75% (v/v) ACN in the mobile phase was used for the screening of additional acidic chiral compounds on the same column.

3.3. Compound screening using GMA/ β -CD-VBTA and GMA/ β -CD columns

After the mobile phase conditions was optimized, 30 additional negatively charged compounds (for a total of 41, see Figure S1, SI) for structures, were screened on the optimized GMA/ β -CD-VBTA column. In addition, a monolithic column with no ionic co-

monomer (GMA/ β -CD) was also compared. The results of the resolutions obtained for these two monolithic columns are summarized in Table 5. Looking at the bar graph in Fig. 4, it can be seen that the VBTA based monolithic column was able to separate 27 of the 41 anionic compounds, with 9 providing resolution values > 1.5 , another 9 with resolution between 1.0 and 1.5, and the remaining 9 compounds with resolutions between 0.1 and 1.0. In general, two classes of anionic compounds that showed better resolution were the dansylated amino acids and the dinitro amino acids (Table 5). The GMA/ β -CD with no co-monomer was only able to show very slight resolution for only one negatively charged compound (α -bromophenyl acetic acid $R_s = 0.1$, Table 5) out of the 41 compounds screened.

The significant difference in separating capability between the two monolithic stationary is explained by the type of ionic sites present on the polymer. The GMA/ β -CD VBTA monolithic material is covered with positively charged moieties, which can help to attract the anionic chiral analytes facilitating complex formation with the β -CD monolithic material. On the other hand, GMA/ β -CD column lacks any ionization sites. Although the GMA/ β -CD column may allow inclusion complexation for some hydrophobic one or two ring compounds, the complexation does not seem to cause the anionic analytes to allow enantioselective interactions. This is surprising as β -CD chiral selector have been shown to be broadly applicable to separate a wide variety of enantiomers as stationary phase in HPLC or as buffer additive in EKC.

Representative chromatographic separation for three classes of acidic analytes screened on the two monolithic stationary phase are shown in Fig. 5A–C. The compounds selected show good contrast between the different separating abilities of the two monolithic columns.

For the separation of the 2-(3-chlorophenoxy)propionic acid (2-(3-CPPA) in Fig. 5A, the GMA/ β -CD-VBTA monolithic column shows baseline resolution in just less than 50 min. The reversal of the EOF and the presence of the positively charged VBTA sites on the monolithic column allow for the baseline resolution but some peak tailing was noted due to electrostatic ionic interactions, and possible mixed-mode interactions as well. In contrast, monoliths made from the GMA/ β -CD material showed no separation due to the lack of any ionic interaction sites like those found in the GMA/ β -CD-VBTA monolith.

When chiral separations of 2,4-dinitrophenylthreonine (2,4-DNPT, Fig. 5B and atrolactic acid (ALA), Fig. 5C) are profiled it is seen that the GMA/ β -CD-VBTA monolithic column was able to baseline resolve the two negatively charged analytes in just under 50 min and 70 min, respectively. The GMA/ β -CD-VBTA monolithic column has a reversed EOF and positively charged sites allowing for the effective separation of the 2,4-DNPT and ALA, though with a slightly longer retention times. Again, the GMA/ β -CD monolithic column showed very little retention and no chiral resolution, as it lacks the ability to interact with the analyte in any significant way due to no charged groups being present in the monolith.

3.4. Column-to-column reproducibility

In addition, column-to-column and operator-to-operator repeatability skills were tested with the separation of the 2,4-DNPT using GMA/ β -CD-VBTA monolith examining retention time, resolution, and efficiency. The data tabulated in Table 6 demonstrates that the monolithic column is stable and robust with respect to repeatability of retention time, resolution, and peak efficiency.

3.5. Utility of GMA/ β -CD-VBTA monolith column in CEC-ESI-MS and CEC-ESI-MS/MS

The final portion of this work was to show the potential of GMA/ β -CD-VBTA monolith column for its compatibility in CEC-MS mode for select negatively charged compounds.

Hence, the three best resolved compounds in CEC-UV were run on an Agilent CE-ESI-MS instrument (Fig. 6). The CEC-MS experiments were hampered by the low flow rates and the anodic electroosmotic flow (EOF) produced by the GMA/ β -CD-VBTA monolithic column. Two modifications to the system were used to counteract this problem. First, a 150 μ m i.d. capillary was used to increase the flow rate. Second, for these experiments it was necessary to place the segment of the capillary containing monolith in the inlet CE side of the capillary cassette, and the open segment of the capillary at the MS outlet end of the CE instrument. This flip of open segment and monolithic segment of the column from inlet to outlet end was necessary to keep the monolithic phase to remain wetted by keeping it closest to the only source of mobile phase, which is the inlet vial. Note that in CE-MS, the outlet portion of the capillary is inserted through the nebulizer and is directly sprayed into in the mass spectrometer. Hence, there is no outlet vial used. Reversal of this set up led to current breakdowns and sometime poor reproducibility of migration time. Although this set-up led some increase in band broadening, the enantiomeric resolution was still achieved. For example, in case of ($\pm$)-ALA and ($\pm$)-2-(3-CPPA) the R_s dropped slightly in CEC-MS when comparing the enantioseparations of the same two compounds in CEC-UV. On the other hand, in case of ($\pm$)-2,4-DNPT higher resolution value ($R_s = 2.3$) was observed in CEC-MS compared to CEC-UV ($R_s = 1.5$) (Fig. 5B versus Fig. 6C). However, for all three chiral compounds there was 2-4 folds improvement in the S/N in CEC-MS. For example, the ALA, 2,3-CPPA and 2,4-DNPT had respective S/N of 96, 150, and 215 in CEC-UV as compared to a S/N of 378, 465 and 550 in CEC-MS. Thus, to counteract the extra dead volume created by the empty segment of the GMA/ β -CD-VBTA monolithic bed, it is important to carefully evaluate a range of positively charged monomer. Perhaps, the best co-monomer combination and a porous polymerization mixture will generate a chiral column to be easily operated in CEC-MS, provided the column retains its high enough enantioselectivity with R_s values greater than 2.0 in CEC-UV.

The ($\pm$)-2,4-DNPT, which was one of the best resolved compounds with the shortest retention time, was tested using CEC-ESI-MS/MS. The use of a triple quadrupole MS/MS allows for the use of multiple reaction monitoring (MRM). One of the major advantages of triple quadrupole MS/MS over single quadrupole is the improvement in S/N when operated in the MRM mode of MS/MS. As demonstrated, the S/N of the SIM run of ($\pm$)-2,4-DNPT on the single quadrupole is 550 (Fig. 7A), while that in MRM of the triple quadrupole was 1200 (Fig. 7B) for the same test analyte. This is essentially because the triple quadrupole “filters” the analyte of interest twice (compared to once in a single quadrupole) before detection occurs. This removes any interfering background signals that may be present in the sample, reducing the noise level and therefore increasing the S/N ratio. This improvement of S/N in CEC-MS/MS is aided by the optimizing in a flow injection analysis mode of HPLC/MS/MS using optimizer software, which allows identification of the product ion, fragmentor voltage and collision energy to obtain the most abundant signal in MRM mode. In addition, this optimization provides the most abundant product ion (e.g. m/z 178) generated in the collision cell. Though one would expect that there is a corresponding drop in signal strength because of the extra fragmentation of the analytes. However, the signal loss is essentially offset by a greater decrease in noise by the triple quadrupole mass spectrometer when operated in MRM mode. As shown in Fig. 7C and D, the MS/MS allows for much lower limit-of-detection (LOD) as compared to the single quadrupole MS. The single quadrupole has an LOD of 500 ng/mL for ($\pm$)-2,4-DNPT with a S/N of 2.8 (Fig. 7C), while the MS/MS with its triple quadrupole has a LOD two orders of magnitude lower at 5 ng/mL with a S/N of 2.3 (Fig. 7D).

4. Conclusions

The incorporation of achiral positively charged, VBTA as a co-monomer into a β -CD based monolith, which has been modified with GMA, allows the development of a more selective CSP providing anodic EOF and anion exchange sites. The morphology of this hybrid GMA- β -CD-VBTA column was characterized with optical microscopy. To characterize the differences between the GMA- β -CD-VBTA and GMA/ β -CD columns, SEM images, the flow characteristics (porosity, permeability) and BET surface area of the two columns were compared.

After synthesis and characterization of the monolithic column, the polymerization of this GMA/ β -CD-VBTA monolith was studied by optimizing the concentration of γ -MAPS and VBTA co-monomer. The mobile phase was optimized in terms of pH, buffer type and concentration as well as voltage, and finally percent organic modifier (ACN). The best compromise between retention time and resolution was found when using a monolithic column containing (% w/w): GMA/ β -CD: 14.6%; VBTA: 0.4%; EDMA: 5%; 1,4-butanediol: 30%; DMSO: 30%; 1-propanol: 20%), which was injected into a capillary pretreated with 50% γ -MAPS. The best mobile phase conditions were 75/25 ACN/5 mM NH_4COOH pH 3.0 and a voltage of -30 kV. The mobile phase optimization suggests that both electrostatic and hydrophobic interactions are important to the retention of the tested acidic compounds.

After stationary phase and mobile phase optimization, a compound library of 41 anionic chiral analytes was screened using the GMA/ β -CD-VBTA and GMA/ β -CD monolithic columns. The comparison suggests that the charged co-monomers were important for control of the EOF and providing appropriate charged sites for chiral interactions. The GMA/ β -CD-VBTA monolith separated negatively charged compounds with the highest success compared to the other GMA/ β -CD monolithic column. The electrostatic attractive interaction of achiral VBTA comonomer seems to be a major reason for improved separation of partially anionic acidic enantiomers using GMA/ β -CD-VBTA column.

The GMA/ β -CD-VBTA monolithic column presented here showed good separation for a wide range of negatively charged chiral compounds. The column has applicability not only in CEC-UV but also in CEC-ESI-MS mode. This was demonstrated for the separation of four different negatively charged enantiomeric compounds. Although there was some loss of R_s , a gain in S/N is seen when switching from CEC-UV to CEC-ESI-MS mode. The baseline separation of the enantiomers of $(\pm)$ -2,4-DNP demonstrated the ability to apply this type of chiral monolithic material to high sensitivity detection by use of a triple quadrupole MS/MS instrument. The application of this monolithic column in CEC-MS suggests potential benefits for future application of pharmaceuticals and other chiral compounds at very low concentrations in biological samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2012.08.002>

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Fig. 1. Polymerization scheme for the formation of the GMA/β-CD-VBTA monolithic columns. The subunits a, b, and c presented in the final structure of macroporous polymeric monolith is a random arrangement with multiple variations in number of subunits and ordering of subunits.

Fig. 2. Scanning electron micrographs of the magnified center sections of the monoliths. A1: GMA/ β -CD-VBTA; B1: GMA/ β -CD. For conditions see Section 2. Inset green bar represents scale of 2 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 3. Pressure versus flow rate for the permeability study of the two monolithic materials GMA/β-CD (▲) and GMA/β-CD-VBTA (◆). Inset R^2 values represent the linearity of the best fit line.

Fig. 4. Bar plots representing the number of acidic compounds separated individually on GMA/β-CD and GMA/β-CD-VBTA monolithic columns out of 41 compounds screened. See Section 2 for conditions.

Fig. 5. Examples of CEC-UV separations of (A) 2-(3-chlorophenoxy)propionic acid [2-(3-CPPA); (B) 2,4-dinitrophenylthreonine [2,4-DNPT], and (C) atrolactic acid (ALA) on the optimized GMA/β-CD-VBTA and the GMA/β-CD monolithic columns. Mobile phase: 75/25 ACN/H₂O, 5 mM NH₄COOH pH 3.0; Capillary 35 cm × 100 μm i.d.; 25 °C at −30 kV.

Fig. 6.

CEC-MS of (A) ALA, (B) 2-(3-CPPA) and (C) 2,4-DNPT on the optimized VBTA co-monomer monolithic column. Mobile phase: 75/25 ACN/5 mM NH_4COOH pH 3.0. Capillary 50 cm $\times$ 150 μm i.d. with 25 cm of the monolithic portion in the inlet side is followed by 25 cm of empty portion of capillary entering the MS outlet end. CEC runs were set at 25 $^\circ\text{C}$, -30 kV; MS conditions: Select ion monitoring of the $[\text{M}-\text{H}]^- = 165$ m/z (AA); 199 m/z (2-(3-CPPA)); 284 m/z (2,4-DNPT); drying gas temperature 150 $^\circ\text{C}$; drying gas flow rate 3 L/min; nebulizer pressure 20 psi; capillary voltage -3500 V; fragmentor voltage 84 V; gain 3.

Fig. 7. Representative chromatograms of 2,4-dinitrophenylthreonine separations using SIM on a single quadrupole CEC-MS (A), and MRM on a triple quadrupole CEC-MS/MS (B). Mobile phase: 75/25 ACN/5 mM NH_4COOH pH 3.0. Capillary 50 cm $\times$ 150 μm i.d. with 25 cm of the monolithic portion in the inlet side is followed by 25 cm of empty portion of capillary entering the MS outlet end; 25 $^\circ\text{C}$ at -30 kV; MS conditions: For SIM: $[\text{M}-\text{H}]^- = 284$ m/z ion used for single quadrupole; For MRM: 284 m/z precursor ion and 178 m/z product ion monitored; drying gas temperature 150 $^\circ\text{C}$; drying gas flow rate 3 L/min; nebulizer pressure 20 psi; capillary voltage -3500 V; fragmentor voltage 83 V; gain 3.

Effects of varying the concentration of 3-(trimethoxysilyl)propyl methacrylate (γ -MAPS) on the separation of 11 select negatively charged compounds.

Table 1

	30% γ -MAPS			50% γ -MAPS			70% γ -MAPS		
	tr_2 (min)	R_s	N (plates/m)	tr_2 (min)	R_s	N (plates/m)	tr_2 (min)	R_s	N (plates/m)
2-Phenoxypropionic Acid	44.9	1.0	10,000	35.1	1.5	16,000	33.2	0.0	2,000
2,2-(Chlorophenoxy)propionic acid	24.1	0.0	9,000	15.2	0.0	17,000	12.5	0.0	1,000
2,3-(Chlorophenoxy)propionic acid	56.1	0.5	24,000	47.5	1.5	32,000	46.1	0.5	1,000
2,4-(Chlorophenoxy)propionic acid	67.3	0.7	8,000	60.3	1.3	8,100	56.3	0.0	2,000
2,2,4-(Dichlorophenoxy)propionic acid	40.9	0.3	6,200	32.0	0.4	8,300	30.2	0.0	1,500
2,2,4,5-(Trichlorophenoxy)propionic acid	84.6	0.2	5,900	76.9	0.3	8,200	72.4	0.0	2,000
Mandelic acid	34.8	0.1	2,400	25.7	0.1	8,000	22.9	0.0	1,500
Acetylmandelic Acid	45.2	0.3	2,000	34.2	0.5	9,000	27.1	0.0	2,600
2,5-(Dinitrobenzoyl)- α -phenylglycine	39.1	0.3	2,300	29.0	0.5	7,400	27.7	0.0	4,000
BNP	83.5	0.2	4,000	73.4	0.5	7,000	26.7	0.0	2,000
γ -Bromophenyl acetic acid	79.2	0.6	5,300	36.7	0.5	6,500	33.9	0.0	1,000

[†] Monolith (% w/w): GMA/ β -CD: 14.8%; VBTA: 0.2%; EDMA: 5%, 1,4-Butanediol: 30%; DMSO: 30%; 1-Propanol: 20%; -30 kV, 25 °C; injected electrokinetically -6 kV, 6 s. Inset digital images show the homogeneity and conditions of each type of monolith. See Section 2 for all other conditions. At 10% and 90% γ -MAPS unstable current was observed even after repeated reconditioning.

Table 2

Effect of VBTA co-monomer in the polymerization mixture on the separation of 11 select negatively charged compounds.^a

	VBTA: 1 mg				VBTA: 2 mg				VBTA: 4 mg				VBTA: 6 mg			
	<i>tr</i> ₂ (min)	<i>Rs</i>	<i>N</i> ^b (plates/m)		<i>tr</i> ₂ (min)	<i>Rs</i>	<i>N</i> ^b (plates/m)		<i>tr</i> ₂ (min)	<i>Rs</i>	<i>N</i> ^b (plates/m)		<i>tr</i> ₂ (min)	<i>Rs</i>	<i>N</i> ^b (plates/m)	
2-Phenoxypropionic Acid	10.8	0.0	2900		35.1	1.5	16,000		51.0	0.0	7000		71.9	0.0	6500	
2,2-(Chlorophenoxy)propionic acid	15.1	0.0	1700		15.2	0.0	17,000		17.2	0.0	4000		33.9	0.2	4700	
2,3-(Chlorophenoxy)propionic acid	28.8	0.5	3200		47.5	1.5	32,000		57.0	1.3	8000		59.7	1.0	7100	
2,4-(Chlorophenoxy)propionic acid	40.9	1.2	8000		60.3	1.3	8100		63.4	1.4	1,100		78.4	1.7	1700	
2,2,4-(Dichlorophenoxy)propionic acid	21.7	0.6	2700		32.0	0.4	8300		39.1	0.4	3200		63.8	0.2	3200	
2,2,4,5-(Trichlorophenoxy)propionic acid	40.5	0.3	5000		76.9	0.3	8200		79.5	0.3	4500		86.1	0.3	1400	
Mandelic acid	22.2	0.1	21,000		25.7	0.1	8000		34.9	0.0	4000		41.5	0.0	2400	
Acetylmandelic Acid	15.1	0.0	1100		34.2	0.5	9000		37.7	0.4	2000		42.4	0.0	5800	
2,5-(Dinitrobenzoyl)-α-phenylglycine	15.3	0.4	4100		29.0	0.5	7400		55.9	0.5	5900		60.4	0.6	4000	
BNP	10.1	0.3	11,000		73.4	0.5	7000		89.1	0.4	6900		96.1	0.4	5000	
γ-Bromophenyl acetic acid	22.7	0.4	24,000		36.7	0.5	6500		43.6	0.4	4700		47.4	0.1	1600	

^a All other conditions the same as Table 1 except the capillary was treated 50% γ-MAPS.

^b *N* was calculated by measuring the width at half-height by the ChemStation software using the following equation: $N = 5.55 (R)^2 / (w_1/2)^2$.

Table 3

Porosity and permeability as determined by the micro HPLC flow method and BET surface area measurement as determined by nitrogen adsorption experiments.

	Porosity ^a (%)	Permeability ^a (m ²)	BET surface area (m ² /g)
GMA/β-CD-VBTA	83 ± 2	$3.9 \times 10^{-16} \pm 2.1 \times 10^{-17}$	16 ± 1
GMA/β-CD	86 ± 3	$1.6 \times 10^{-15} \pm 3.1 \times 10^{-16}$	33 ± 1

^aStandard Deviation of porosity and permeability was obtained using three replicate measurements at each flow rate. BET surface area was obtained as three replicate measurements from the adsorption/desorption isotherm.

Table 4

Effect of organic modifier concentration on the separation of select negatively charged compounds and non-ionic hydrophobic compounds using the GMA/β-CD-VBTA monolithic column.^a

	50% ACN			75% ACN			90% ACN		
	<i>t_r</i> (min)	<i>R_s</i>	<i>N^b</i> (plates/m)	<i>t_r</i> (min)	<i>R_s</i>	<i>N^b</i> (plates/m)	<i>t_r</i> (min)	<i>R_s</i>	<i>N^b</i> (plates/m)
2-Phenoxypropionic Acid	39.4	0.8	10,400	35.1	1.5	16,000	53.5	0.6	11,200
2,2-(Chlorophenoxy)propionic acid	28.4	0.0	12,900	15.2	0.0	17,000	28.8	0.0	10,800
2,3-(Chlorophenoxy)propionic acid	58.1	0.6	20,100	47.5	1.5	32,000	51.8	0.5	16,700
2,4-(Chlorophenoxy)propionic acid	73.8	0.9	7900	60.3	1.3	8100	69.2	0.7	7800
2,2,4-(Dichlorophenoxy)propionic acid	50.9	0.4	7500	32.0	0.4	8300	33.5	0.0	6900
2,2,4,5-(Trichlorophenoxy)propionic acid	81.2	0.5	7500	76.9	0.3	8200	65.5	0.2	7600
Mandelic acid	24.8	0.0	9600	25.7	0.1	8000	53.9	0.2	7900
Acetylmandelic Acid	27.4	0.0	1700	34.2	0.5	9000	36.1	0.0	2100
2,5-(Dinitrobenzoyl)-α-phenylglycine	32.1	0.4	6500	29.0	0.5	7400	22.9	0.4	6100
BNP	83.3	0.4	7300	73.4	0.5	7000	20.9	0.4	6800
γ-Bromophenyl acetic acid	59.5	0.4	6200	36.7	0.5	6500	46.1	0.4	6600
Anthracene	*	*	*	9.7	*	15,000	5.7	*	19,000
Benzene	*	*	*	13.2	*	40,000	7.2	*	44,000
Naphthalene	*	*	*	18.6	*	8000	9.1	*	9000
Benzo[a]pyrene	*	*	*	8.7	*	8500	5.2	*	9400

^a All other conditions are the same as Table 1 except the capillary was treated 50% γ-MAPS and 2 mg/mL of VBTA was added to the polymerization mixture. Mobile phase: 5 mM NH₄COOH, 75/25% (v/v) ACN/H₂O, pH 3.0, -30 kV.

^b *N* was calculated by measuring the width at half-height by the ChemStation software using the following equation: $N = 5.55 (R)^2 / (w)^2$.

Table 5

Resolution values of the screening of 41 acidic compounds on GMA/ β -CD-VBTA and GMA/ β -CD monolithic columns. Conditions presented in Section 2.^a

	GMA/ β -CD-VBTA	GMA/ β -CD Resolution
Propionic acids and derivatives		
2-Phenylpropionic acid	0.0	0.0
2-Phenoxypropionic acid	1.5	0.0
2,2-(Chlorophenoxy)propionic acid	0.0	0.0
2,3-(Chlorophenoxy)propionic acid	1.5	0.0
2,4-(Chlorophenoxy)propionic acid	1.3	0.0
2,2,4-(Dichlorophenoxy)propionic acid	0.5	0.0
2,2,4,5-(Trichlorophenoxy)propionic acid	0.5	0.0
2-Phenylbutyric acid	1.2	0.0
Dansyl amino acids		
DNS-Valine	2.0	0.0
DNS-Leucine	1.3	0.0
DNS-Threonine	2.9	0.0
DNS-Serine	2.2	0.0
DNS-Phenylalanine	0.0	0.0
PTH-amino acids		
PTH-Glutamic acid	0.0	0.0
PTH-Aspartic acid	0.0	0.0
Dinitro amino acids		
2,4-DNP-Methionine sulfone	1.1	0.0
2,4-DNP-Norleucine	0.9	0.0
2,4-DNP-Norvaline	2.4	0.0
2,4-DNP-Threonine	2.4	0.0
3,5-(Dinitrobenzoyl)phenylglycine	0.5	0.0
3,5-(Dinitrobenzoyl)leucine	1.5	0.0
α -Hydroxyl acids and derivatives		
Mandelic acid	0.1	0.0
Acetylmandelic acid	0.5	0.0
Atrolactic acid	2.1	0.0
4-Chloromandelic acid	1.0	0.0
4-Bromomandelic acid	0.0	0.0
3,4-(Methylenedioxy)mandelic acid	1.1	0.0
β -Phenyl lactic acid	0.0	0.0
α -Bromophenyl acetic acid	0.8	0.1
α -Methoxyphenyl acetic acid	1.3	0.0
Profens		
Carprofen	0.0	0.0
Ibuprofen	0.5	0.0

	GMA/ β -CD-VBTA	GMA/ β -CD Resolution
Indoprofen	0.0	0.0
Suprofen	0.0	0.0
Flurbiprofen	0.0	0.0
Fenoprofen	0.0	0.0
Ketoprofen	0.0	0.0
Miscellaneous		
Warfarin	1.4	0.0
Coumachlor	1.4	0.0
Malic acid	0.0	0.0
BNP	0.5	0.0

^aOptimized composition of polymerization mixture was used for each monolith column as mentioned in Table 1. Mobile phase: 5 mM NH₄COOH, 75/25% (v/v) ACN/H₂O, pH 3.0, -30 kV.

Table 6

Intra- and Intercolumn repeatability of the monolithic columns comparing retention time, resolution, and efficiency of 2,4-dinitrophenylthreonine on GMA/β-CD-VBTA column.

	<i>t_r</i> , min (%RSD)		<i>R_s</i> (%RSD)	Efficiency (<i>N</i> ^a , plates/m (%RSD))	
	Peak 1	Peak 2		Peak 1	Peak 2
Column 1 <i>n</i> = 15	41.1 (2.4)	44.8 (2.7)	2.2 (4.2)	15,300 (6.2)	14,300 (5.4)
Column 2 <i>n</i> = 15	40.9 (3.8)	45.1 (4.0)	2.4 (5.1)	16,100 (6.8)	15,400 (5.1)
Column 3 <i>n</i> = 15	41.3 (2.8)	44.5 (3.3)	2.3 (4.8)	15,600 (7.4)	14,200 (6.2)
	<i>t_r</i> , min (%RSD)		<i>R_s</i> (%RSD)	Efficiency (<i>N</i> ^a , plates/m (%RSD))	
	Peak 1	Peak 2		Peak 1	Peak 2
<i>n</i> = 45	41.3 (3.6)	44.6 (4.3)	2.4 (5.3)	15,300 (8.2)	14,300 (7.4)

^a *N* was calculated by measuring the width at half-height by the ChemStation software using the following equation: $N = 5.55 (R)^2 / (w_1/2)^2$.