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Synthesis of micron diameter polybutadiene-encapsulated non-porous zirconia particles for ultrahigh pressure liquid chromatography

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Abstract

In this study, 1-μm diameter polybutadiene-encapsulated non-porous zirconia particles were synthesized, slurry packed into 50-μm I.D. fused-silica capillary columns, and evaluated using ultrahigh pressure liquid chromatography. The dependencies of column efficiency and solute retention factor on pressure were investigated. Efficiencies as high as 280 000 plates per meter were obtained for the separation of anti-inflammatory drugs at a pressure of 1351 MPa. Comparing the reversed-phase behavior of the polybutadiene-encapsulated non-porous zirconia with octadecylsilane bonded non-porous silica, greater selectivity was found using the zirconia-based material for the applications reported in this study. The encapsulated non-porous zirconia particles demonstrated excellent thermal stability in the separation of polycyclic aromatic hydrocarbons at a temperature of 100 °C and a pressure of 1351 MPa.

Keywords: Polybutadiene-encapsulated non-porous zirconia particles; Stationary phases, LC; Zirconia particles, polybutadiene-encapsulated; Column efficiency

1. Introduction

Reducing the diameter of spherical packing materials (<3 μm) can facilitate fast and highly efficient separations in liquid chromatography (LC) [1]. However, it is fundamentally impossible to significantly improve separation speed and maintain efficiency with conventional porous particles [2–4]. Recently, small non-porous particles have been introduced for liquid chromatography to overcome this limitation of porous particles for fast separations [5,6]. The particle diameter of non-porous particles can be reduced below 2 μm while still retaining their mechanical strength. Columns containing small non-porous particles have exhibited excellent ultrafast separations of both macromolecular compounds and small molecules. The most widely used non-porous particles have been silica-based particles [5–8]. Recently, there has been increasing interest in zirconia-based stationary phases because they are me-

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chanically and chemically stable over relatively wide pressure and pH ranges. In order to provide selectivity, these new zirconia particles have been encapsulated with polybutadiene [9,10], polystyrene (PS) [11], or carbon (C) [12] and have been successfully used for different applications such as protein separations and high temperature ultrafast separations [10,13]. Carr and co-workers recently developed a small diameter non-porous zirconia material [14].

Since the pressure drop along the length of a packed column is inversely proportional to the square of the particle diameter, higher pressures are required when using very small particles [15]. Therefore, ultrahigh pressure liquid chromatography (UHPLC) instrumentation has been developed, which can provide pressures over 1351 MPa. UHPLC has been used for high resolution separation of complex peptide mixtures [16–18] and high speed analysis of combinatorial chemistry samples, pharmaceutical compounds and chiral compounds [19–23].

In this study, synthesis and applications of polybutadiene-encapsulated non-porous zirconia particles are reported. UHPLC was utilized to evaluate capillary columns packed with these particles.

2. Experimental

2.1. Materials

Reagent grade zirconium *n*-propoxide ($Zr(OPr^n)_4$, 70% (w/w) solution in *n*-propanol), polybutadiene of molecular weight 5000 (20% vinyl, 80% *cis*- and *trans*-1,4-polybutadiene), dicumyl peroxide and acetone (analytical grade) were purchased from Aldrich (Milwaukee, WI, USA). Reagent grade (ACS grade) *n*-butanol, toluene, cyclohexanol and stearic acid (>95%), and HPLC grade acetonitrile (ACN), chloroform, and water were obtained from Fisher (Fair Lawn, NJ, USA). HPLC grade isopropanol was purchased from Mallinckrodt (Phillipsburg, NJ, USA) and HPLC grade hexane was purchased from EM Sciences (Gibbstown, NJ, USA). Deionized water was used for the preparation of the zirconia particles. All benzodiazepines, barbitals and anti-inflammatory pharmaceuticals used in this study were obtained from Sigma (St. Louis, MO). All buffers and solvents for chromatographic use were filtered through 0.22-μm pore Durapore® membrane filters (Millipore, Bedford, MA). Similarly, samples were filtered through 0.2-μm pore polytetrafluoroethylene syringe filters (Chromacol, Trumbull, CT). Zeflor™ membrane filters with pore size of 3.0 μm were supplied by Gelman (Ann Arbor, MI) and used to filter the particle slurry before packing.

2.2. Preparation of monodisperse zirconia microspheres

A 7.68-g portion of stearic acid was weighed into a 2000-ml polyethylene wide-mouth bottle, 644.6 g of butanol was added, and the bottle was tightly closed. The solution was stirred for 30 min to allow full dissolution. Then, 97.7 g of zirconium propoxide solution was slowly added with stirring, and the mixture was stirred for an additional 30 min.

A fresh solution consisting of 557.4 g of butanol and 14.6 g of deionized water was slowly added with stirring to the zirconium-containing solution over a period of 1 min. The bottle was tightly closed and the mixture was vigorously stirred using a magnetic stirrer until the solution became cloudy (~8 min). The stirring was then stopped and the suspension was allowed to age without agitation (~3 min). The reaction was quenched by dilution, by gently mixing in 1600 ml of butanol. The suspension was vacuum-filtered using four 0.45-μm pore nylon filter membranes. The microspheres were washed three times, each time gently resuspending in 50 ml of anhydrous butanol and refiltering. The microspheres were finally washed directly on the filter with three 50-ml aliquots of acetone.

After washing, the microspheres were transferred to a crucible with a spatula and dried in a vacuum oven at 120 °C for 3 h. The crucible was then transferred to a combustion oven, the temperature was ramped at 5 °C min^{-1} to 450 °C, and the microspheres were kept at 450 °C for 3 h to burn off any remaining carbon. Then the temperature was raised to 750 °C at 5 °C min^{-1} and held at 750 °C for 5 h to completely remove all carbon and to allow densification. Finally, the temperature was decreased at 5 °C min^{-1} to room temperature.

2.3. Encapsulation of non-porous zirconia in polybutadiene

A 50-ml volume of 0.3% (w/v) solution of polybutadiene (5000 average molecular weight) in hexane was added to 3 g of non-porous zirconia particles synthesized as described above. The slurry was sonicated under vacuum for 5 min to suspend the particles in the solution. Dicumyl peroxide crosslinking agent (2.5 mg) was added to the slurry. The mixture was sonicated for 5 min and then swirled for at least 2 h, after which the solvent was evaporated by applying low vacuum at 55 °C for 15 min. Then the coated particles were dried at this temperature for an additional 15 min. The polymer was thermally crosslinked in a vacuum oven at 120 °C for 5 h.

2.4. Instrumentation

The UHPLC system used in this study was previously described in detail [19]. Briefly, a double-head air-driven liquid pump (Model DSHF-32, Haskel, Burbank, CA, USA) was used to generate the necessary liquid pressures. A cylinder containing compressed nitrogen was used to drive the pump. The outlet of this pump was connected to a home-built injection system. A static-split injection technique was employed for sample introduction [16,19]. A Model UV3000 scanning detector from Thermo Separations (Sunol, CA, USA) was used to monitor UV absorbance on-column. Data were acquired with ChromQuest 2.5.1 (ThermoQuest, Sunol, CA, USA).

Water-resistant, flexible heater tape (Watlow Heatcon, Seattle, WA, USA) was used to provide the desired heat for elevated temperature operation. Due to differences in their thermal conductivities and masses, the tubing between the pump and the injector, ultrahigh pressure injector, and the column were heated separately [24]. Each heater cover was connected to a temperature controller (Omega, Stamford, CT, USA) which thermostated the whole system to ±0.2 °C at temperatures up to 150 °C. A linear restrictor (1 m×30 μm I.D.) was attached to the end of the column to prevent bubble formation, especially at temperatures higher than the boiling points of water and acetonitrile.

Scanning electron microscopy (JEOL 8401, JEOL, Peabody, MA, USA) was used to determine the microsphere size, shape, and state of aggregation. Particle size distributions of the final product were determined by images of hundreds of particles. All figures shown are representative of the entire sample collected. To verify that there were no internal pores, the microspheres were dispersed in epoxy resin (# 8778, Cole-Parmer, Chicago, IL, USA), polished to expose the interior of some microspheres, sputter-coated with a 50-Å layer of platinum and then viewed in the microscope.

2.5. Column preparation

The packing apparatus was described in detail in a previous paper [19]. A Model DSF-150-C1 air-driven pneumatic amplifier pump (Haskel, Burbank, CA, USA) was used for packing the capillary columns. Fused silica capillaries (50 μm I.D. and 370 μm O.D.) were packed with non-porous C_{18} particles strictly following the steps described previously. For packing 1.0-μm non-porous polybutadiene-encapsulated zirconia, a different procedure was used as described below. Instead of carbon dioxide, isopropanol was used to push the slurry into the column. Slurries of the particles in chloroform–cyclohexanol (50:50, v/v) were sonicated for more than 1 h before being transfered to the packing reservoir. A syringe pump was used to fill the solvent reservoir of the pneumatic amplifier pump with isopropanol, which was subsequently driven with nitrogen gas pressure. The initial packing pressure was 30 MPa, which was raised periodically to maintain a nearly constant packing rate until the column was completely filled. The final pressure was 1351 MPa. The column was left to depressurize overnight. Since it was not possible to sinter the zirconia particles to make frits, 1.5-μm bare silica particles were introduced into the capillary for several centimeters both before and after packing with zirconia particles. Internal frits were made by sintering the silica particles at a distance of 3 mm from the beginning of the zirconia particle bed while water flowed through the column under a pressure of 1013 MPa. The loose non-sintered silica particles were flushed away, and a detection window was made immediately after the outlet frit.

2.6. Chromatographic conditions

For column evaluation, a mobile phase of ACN–water (20:80, v/v) containing 40 m*M* NaH_2PO_4 at pH 7.0 was used. Test compounds included 1,4-dihydroxybenzene (hydroquinone), 1,3-dihydroxybenzene (resorcinol), phenol and ascorbic acid, the latter being used as a dead-time marker for the determination of linear velocity. All test compounds were dissolved in the mobile phase. The concentration of each solute was 5 mg ml^{-1}. Other chromatographic conditions are given in the figure legends.

3. Results and discussion

3.1. Characterization of 1-μm encapsulated zirconia particles

The best chromatographic efficiencies are obtained with well-packed columns, which significantly depends on particle uniformity [25]. Fig. 1A shows a scanning electron micrograph of a representative batch of non-porous zirconia particles prepared in this study. Nitrogen adsorption, confocal fluorescence microscopy and fluoride adsorption uptake measurements demonstrated that the synthesized particles were non-porous [14]. The particle size distribution for this batch of particles is given in Fig. 1B. It can be seen that these particles are non-aggregated, spherical, and monodisperse. The mean diameter is 1.14 μm and the standard deviation is ±0.22 μm.

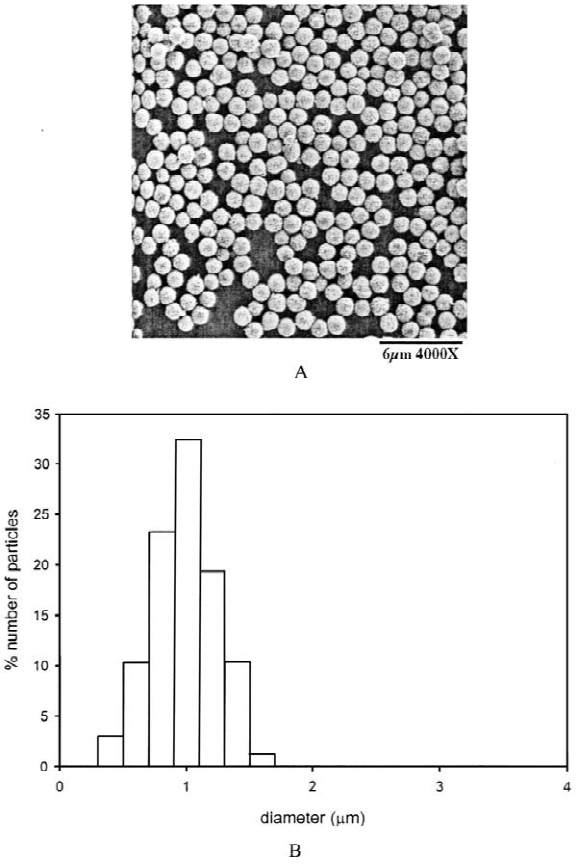

Fig. 1. (A) Scanning electron micrograph of zirconia microspheres. (B) Size distribution of the sample shown in (A) determined by analysis of 356 particles.

3.2. Column evaluation

The dependence of plate height (*H*) on mobile phase linear velocity (*u*) was plotted as shown in Fig. 2. The data were fit to the van Deemter equation as shown by the solid line. The minimum plate height (~3.5 μm) was obtained at a linear velocity of 0.5 mm s^{-1}. Previous work using well-packed columns containing 1.5-μm non-porous silica showed little change in column performance with increase in flow rate [16,17,19,20]. As is seen in Fig. 2, the van Deemter curve obtained using the zirconia column was also relatively flat at linear velocities much higher than u_{opt}. However, the efficiencies obtained using the zirconia packed columns are not as good as those obtained with silica packed columns, probably due to less favorable mass transfer characteristics of polymer-encapsulated particles compared to typical C_{18} bonded phases.

3.3. Effect of pressure on separation

Fig. 3 shows separations of four anti-inflammatory drugs using inlet pressures of 676 and 1351 MPa, respectively. The separation time was reduced in half when the pressure was raised from 676 to 1351 MPa. The average efficiency at 676 MPa was 250 000 plates m^{-1}, which represents a 21% loss compared to the average efficiency obtained at 676 MPa.

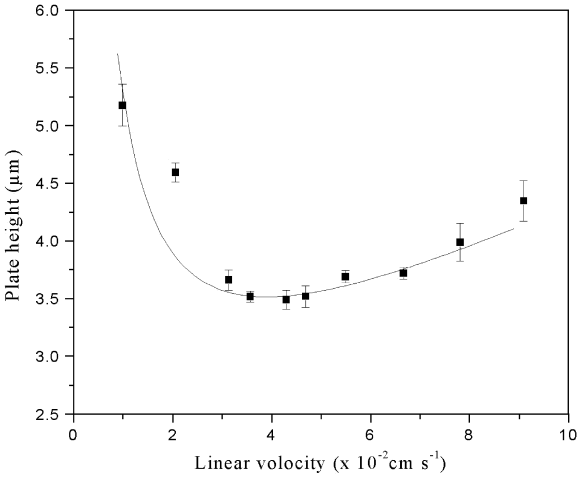

Fig. 2. UHPLC van Deemter plot for ascorbic acid. Conditions: 15 cm×50 μm I.D. fused-silica capillary column packed with 1.0-μm polybutadiene-encapsulated non-porous zirconia particles; water (40 m*M* NaH_2PO_4, pH 7.0)–acetonitrile (80:20, v/v); 254-nm UV detection.

However, all the drug compounds were still baseline resolved at 1351 MPa. The resolution values for peaks 2 and 3 were 2.01 and 2.34 at pressures of 676 and 1351 MPa, respectively. The increase in resolution is mainly due to an increase in retention factor with increase in pressure. The dependency of retention factor on pressure was investigated as shown in Fig. 4. An approximately linear relationship between retention factor and inlet pressure was observed. Similar observations were reported for silica packed columns [16,22,26].

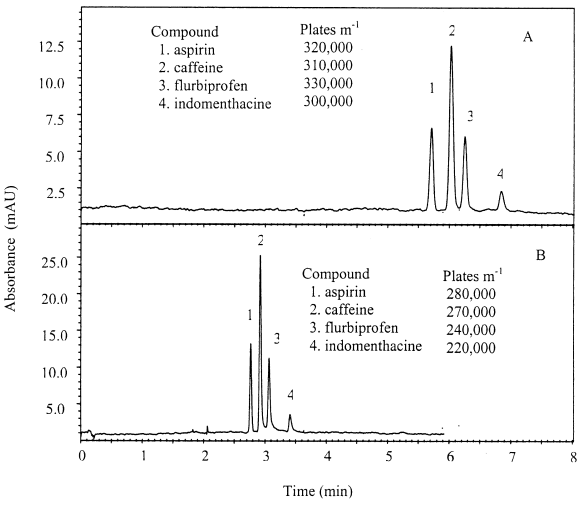

Fig. 3. UHPLC chromatograms showing the effect of pressure on separation of anti-inflammatory drugs. Conditions: (A) 676-MPa inlet pressure; 15 cm×50 μm I.D. capillary column packed with 1-μm polybutadiene-encapsulated non-porous zirconia particles; water (40 m*M* NaH_2PO_4, pH 7.0)–acetonitrile (80:20, v/v); 215-nm UV detection; (B) 1351-MPa inlet pressure; other conditions are the same as in (A). Peak identifications: (1) aspirin, (2) caffeine, (3) flurbiprofen, (4) indomenthacine.

3.4. Comparison of polybutadiene-encapsulated zirconia with C_{18} bonded silica

It is well known that in reversed-phase LC over a reasonable range in mobile phase composition, the logarithm of retention factor (k) is linearly proportional to the number of methylene groups in a homologous series of analyte molecules [9]. Four paraben standards were used to investigate the reversed-phase nature of the polybutadiene-encapsulated zirconia particles, and a good linear relationship between $\ln k$ and n_{CH2} was found. It was also found that as the organic modifier content in the mobile phase decreased, the retention of parabens on the encapsulated zirconia decreased as is generally observed for conventional ODS bonded phases. These results verify (as expected) that polybutadiene-encapsulated zirconia behaves similar to a C_{18} bonded reversed phase.

Fig. 5 shows chromatograms of barbitals using C_{18}

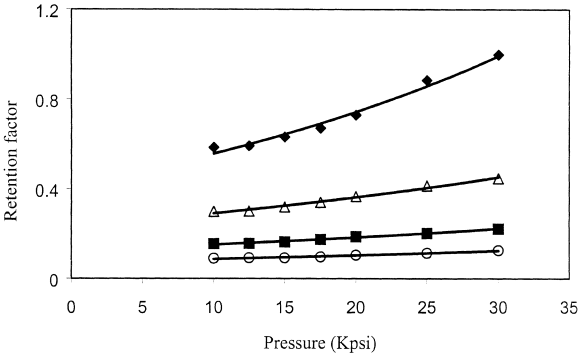

Fig. 4. Effect of pressure on retention factor. Conditions: 15 cm×50 μm I.D. fused-silica capillary column packed with 1.0-μm polybutadiene-encapsulated non-porous zirconia particles; water (40 m*M* NaH_2PO_4, pH 7.0)–acetonitrile (65:35, v/v); 215-nm UV detection; uracil as marker. Peak identifications: (○) methyl paraben, (■) ethyl paraben, (△) propyl paraben, (♦) butyl paraben.

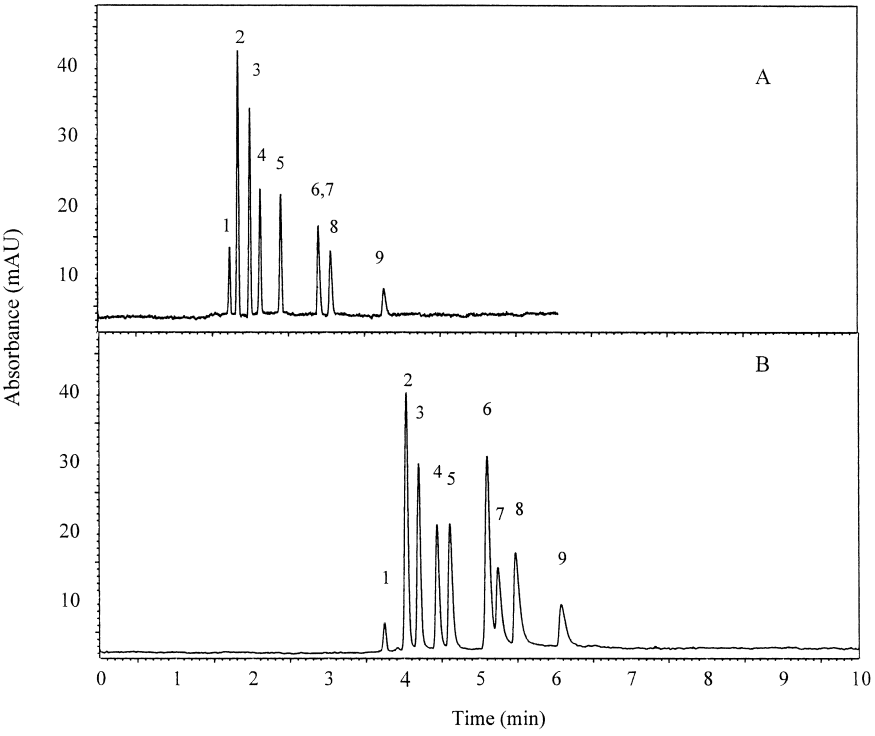

Fig. 5. UHPLC chromatograms of barbitals. Conditions: (A) 22 °C; 1013-MPa inlet pressure; 16 cm×50 μm I.D. fused-silica capillary column packed with 1.0-μm Kovasil C_{18} bonded non-porous particles; water (40 m*M* NaH_2PO_4, pH 7.0)–acetonitrile (80:20, v/v); 220-nm UV detection; uracil as marker; (B) 16 cm×50 μm I.D. fused-silica capillary column packed with 1.0-μm polybutadiene-encapsulated non-porous zirconia particles; water (40 m*M* NaH_2PO_4, pH 7.0)–acetonitrile (75:25, v/v); other conditions are the same as in (A). Peak identifications: (1) uracil, (2) allobarbital, (3) barbital, (4) phenobarbital, (5) butalbital, (6) hexabarbital, (7) amobarbital, (8) pentobarbitals, (9) secobarbital.

bonded non-porous silica particles (Fig. 5A) and PBD-encapsulated non-porous zirconia particles (Fig. 5B). It can be seen that most barbital standards are resolved on both stationary phases, and solute elution orders are the same. Obviously, a similar retention mechanism is involved in both separations. The average efficiency for the zirconia particles is more than 200 000 plates m^{-1}, which is comparable to that obtained using C_{18} bonded particles (i.e. 210 000 plates m^{-1}). However, *k* values for the zirconia column were lower than those for the C_{18} bonded silica except for the first peak. This could be due to differences in either partition coefficients or phase ratios, or both [9]. Peaks 6 and 7 are not resolved on the C_{18} bonded silica column, even with less organic modifier. This pair of peaks is resolved using the zirconia column with resolution of 1.2.

3.5. Separations using elevated temperature UHPLC

Temperature plays a significant role in all chromatographic techniques. The use of elevated temperature in LC has been advocated primarily as a means of decreasing the back pressure of the column, shortening the separation time and increasing the column efficiency [27]. It has been demonstrated that encapsulated zirconia particles are stable at a temperature of 100 °C for over 7000 column volumes [9]. The exceptional thermal stability of zirconia allows the use of elevated temperature UHPLC for fast separations [14,22]. Fig. 6B shows the separation of eight polycyclic aromatic hydrocarbons at a temperature of 100 °C using a pressure of 1351 MPa. Compared to Fig. 6A, the separation time was

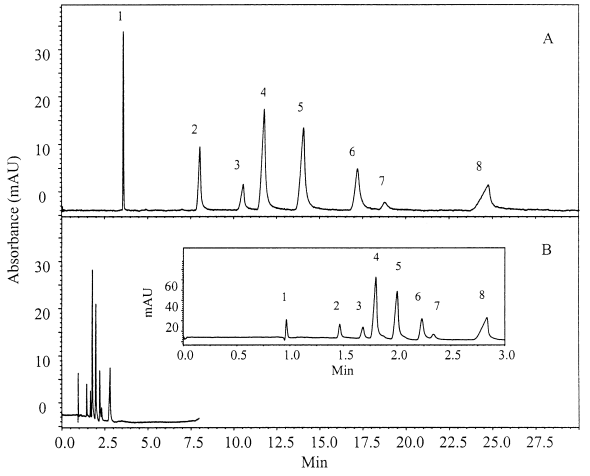

Fig. 6. UHPLC chromatograms of polycyclic aromatic hydrocarbons. Conditions: (A) 22 °C; 1013-MPa inlet pressure; 14.5 cm×50 μm I.D. fused-silica capillary column packed with 1.0-μm polybutadiene-encapsulated non-porous zirconia particles; water (40 m*M* NaH_2PO_4, pH 7.0)–acetonitrile (55:45, v/v); 254-nm UV detection. (B) 100 °C; 1351-MPa inlet pressure; 14.5 cm×50 μm I.D. fused-silica capillary column packed with 1.0-μm polybutadiene-encapsulated non-porous zirconia particles; water (40 m*M* NaH_2PO_4, pH 7.0)–acetonitrile (58:42, v/v); other conditions are the same as in (A). Peak identifications: (1) uracil, (2) naphthalene, (3) acenaphthylene, (4) biphenyl, (5) fluorine, (6) phenanthrene, (7) anthracene, (8) fluoranthene.

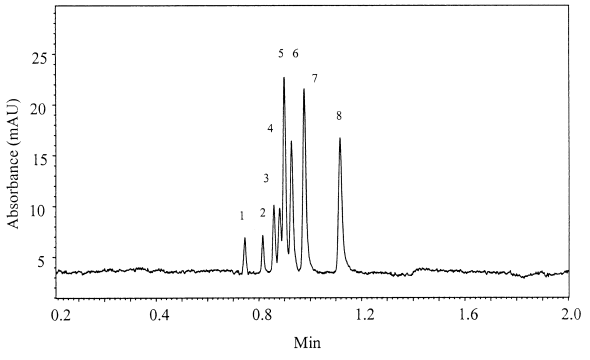

Fig. 7. UHPLC chromatogram of benzodiazepines. Conditions: 100 °C; 1486-MPa inlet pressure; 14.5 cm×50 μm I.D. fused-silica capillary column packed with 1.0-μm polybutadiene-encapsulated non-porous zirconia particles; water (40 m*M* NaH_2PO_4, pH 7.0)–acetonitrile (68:22, v/v); 215-nm UV detection. Peak identifications: (1) uracil, (2) clorazepate, (3) flunitrozepam, (4) clonazepam, (5) chlordiazepoxide, (6) oxazepam, (7) clorazepate, (8) diazepam.

reduced from 25 to 2.7 min, an ~10 times reduction. The overall quality of the resolution at elevated temperature and ultrahigh pressure is still quite acceptable, and peak shapes are improved significantly at elevated temperature. Separation of six benzodiazepines samples within 1.2 min is demonstrated in Fig. 7. Again this speed was possible because of the use of elevated pressure and temperature. The plate number for the last peak is ~26 000 plates.

4. Conclusions

Newly synthesized 1.0-μm polybutadiene-encapsulated non-porous zirconia particles were successfully packed into 50-μm I.D. capillary columns. The resultant columns showed comparable efficiency and better selectivity than C_{18} bonded silica particles for the separation of barbitals. These columns also demonstrated the potential for fast and highly efficient separations when using ultrahigh pressures and elevated temperatures with polycyclic aromatic hydrocarbons and benzodiazepines as examples.

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