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Predictable and consistent performance of angular packing in axial compression columns

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ABSTRACT

Various silica-based angular materials were packed and tested in a dynamic axial compression (DAC) column. Effects of packing pressures and solvents on the performance of the angular packings in DAC columns were examined. Column performance (*i.e.*, column efficiency and bed pressure) was examined using environmentally friendly pure solvents as slurry solvents; performance was compared with theoretical predictions. Plots of the reduced plate height (*h*) *vs.* reduced velocity (*v*) for two silica-based packing materials, bonded-phase and bare silica, with a nominal pore diameter of 100 Å and nominal particle diameter of 10 μm were established to evaluate mass transfer effects on the column performance under different packing conditions. Results were in close agreement with theoretical predictions. Stability and repacking of these two packing materials in DAC columns were also investigated.

INTRODUCTION

Preparative high-performance liquid chromatography (HPLC) has proven itself to be a powerful technique for the pharmaceutical industry. This method aids in the development of chemical processes, quick purification of limited quantities of drugs for further investigation and clinical trials, as well as purification of impurities for characterization. There are several column hardware designs which are utilized in preparative HPLC [1,2]. Among these are dynamic axial compression (DAC) columns. Packing methods, and resulting performance of axial compression columns are not always predictable and consistent [3–6]. Many variables have to be explored to determine their effect on column performance. Expectations of axial compression columns in the industrial environment can be summarized as follows: (1) high column efficiency with low back-pressure, (2) minimum mass transfer limi-

tation, (3) long-term stability, (4) packing convenience in regard to procedure and solvents used [3,7] and (5) the ability to repack.

Only a homogeneously packed, tight and stable column bed can insure the plug flow, and meet these expectations of performance and stability. A non-homogeneous bed, on the other hand, will lead to channeling, ghost peaks, poor peak symmetry and efficiency [8]. It will also cause internal friction, thereby grinding the packing material, leading to fines, high back pressure and rapid decline of performance. A homogeneously packed bed depends on many packing parameters. Some of these parameters were investigated to determine their effects on column performance. The key parameters that resulted in a homogeneously packed and stable DAC column include: type of packing material, slurry solvents and concentrations, packing pressures utilized, and packing procedure used.

In this paper, we focused on investigating the effect of slurry solvents and packing pressures on the performance of angular material in axial compression columns. Angular silica-based ma-

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terials including bare and bonded-phases were utilized in a commercial (5 cm I.D.) axial compression column.

EXPERIMENTAL

Apparatus

Experiments were carried out on a preparative liquid chromatography system which included a Versa Prep pump from Varex (Rockville, MD, USA), a DAC LC 50 column (50 × 5 cm I.D.) from Prochrom (Indianapolis, IN, USA), a Spectro-Monitor D UV–Vis detector from LDC/ Milton Roy (Riviera Beach, FL, USA), and a HP 3396A integrator from Hewlett-Packard (Avondale, PA, USA). Nelson 2600 chromatography software from Perkin-Elmer (Cupertino, CA, USA) was utilized for data acquisition.

Chemicals

HPLC-grade acetone, methanol, acetonitrile, heptane and 2-propanol were obtained from Fisher Scientific (Malvern, PA, USA). Toluene was purchased form J.T. Baker (Phillipsburg, NJ, USA). Phenol, methyl benzoate, dimethyl phthalate, diethyl phthalate, dioctyl phthalate and cinnamyl alcohol were obtained from Aldrich (Milwaukee, WI, USA). Uracil and naphthalene were purchased from Sigma (St. Louis, MO, USA). A variety of angular IMPAQ® materials obtained from BTR Separations (Wilmington, DE, USA) were evaluated (the characteristics of these materials are listed in Table I).

Methods

Slurry solutions were prepared by mixing several IMPAQ materials in solvents with total volume to 980 ml. The concentrations of the slurry solutions were between 0.18 and 0.23 g/ ml. After a brief degassing, the slurry was poured onto the top of the DAC column. The packing process was carried out at the appropriate piston pressure by securing the top flange and activating the piston. Unless stated otherwise, the columns were compressed at the same piston pressures as the packing pressures.

Evaluation

C_{18}-silica and bare silica columns were equilibrated with acetonitrile–water (60:40) and heptane–2-propanol (97:3), respectively. A 200-μl volume of test mixture was injected to calculate column efficiency. These mixtures consisted of uracil, phenol, methyl benzoate and toluene for the reversed-phase systems, and naphthalene, dimethyl phthalate, diethyl phthalate, dioctyl phthalate and cinnamyl alcohol for the normal-phase, bare silica.

Unless otherwise stated, the column efficiencies were calculated using the conventional equation: $N = 5.54(t_R/w_{1/2})^2$ based on the toluene peak for the reversed phase and the cinnamyl alcohol peak for the bare silica at a flow-rate of 94 ml/min ($u = 0.14$ cm/s) and a wavelength of 254 nm. The experimental error of column efficiency (plates/m) was within 15%.

To establish the reduced plate height vs. reduced velocity plot, flow-rates were varied from 24 to 130 ml/min for silica with a nominal

TABLE I

IMPAQ MATERIAL PACKED IN DYNAMIC AXIAL COMPRESSION COLUMNS

Material[a]	Particle diameter (μm)	Pore diameter (Å)	Surface area (m^2/g)
RG1010Si	8.5	102	404
RG0610Si	8.1	52	638
RG1010C18	8.5	102[b]	404[b]
RG1020C18	16.7	100[b]	395[b]

[a] The nomenclature RGXXYYSi or RGXXYYC18 indicates a silica of nominal XX nm pore diameter and a nominal particle diameter of YY μm.

[b] These values are obtained by BET-N_2 measurement before bonding.

10 μm particle diameter bonded with C_{18} (RG1010C18) and from 14 to 180 ml/min for bare silica with a nominal 10 μm particle diameter (RG1010Si). The reduced plate height (h) is defined as H/d_p and the reduced velocity (v) as ud_p/D_m, where H, d_p, u and D_m are plate height (cm), particle diameter (cm), linear velocity (cm/s) and diffusion coefficient (cm^2/s), respectively. Diffusion coefficients, D_m, of the samples in the mobile phase was calculated using the Wilke and Chang equation [9]. In this study, h and v were calculated based on a particle size of 10 μm and with a D_m of the toluene peak ($1.53 \cdot 10^{-5}$ cm^2/s) for the reversed-phase and a D_m of dioctyl phthalate ($1.2 \cdot 10^{-5}$ cm^2/s) for the bare silica. Theoretical predictions of column efficiency were obtained using the Knox equation [10]: $h = v^{0.33} + 2/v + 0.05v$. Theoretical predictions of bed pressure drop were calculated using the formula [11]: $p = (6.894 \cdot 10^{-3} \cdot 15\,000)unL/d_p^2$ where p is pressure (MPa), n viscosity (cP) and L column length (cm).

To test the stability of the bed over time, the columns were compressed at their appropriate pressures. Throughout this stability testing, the effluent of acetonitrile–water (60:40) for reversed phase and heptane–2-propanol (97:3) for normal phase were constantly flowing through the system at a flow-rate of 94 ml/min.

RESULTS AND DISCUSSION

In preparative liquid chromatography, high column efficiency and low bed pressure are essential for the optimization of throughput. A minimum mass transfer limitation is required for the chromatography system which is usually operated at a relatively high flow-rate in order to obtain an improved production rate. A column operated in process-scale chromatography for preparative separation should have long-term stability, and the packing material should be able to withstand the stresses of unpacking and re-packing. To meet various regulatory requirements in process-scale liquid chromatography, it is important that more common and environmentally friendly solvents can be used during packing. The data presented below shows the

influence of packing solvents and packing pressures on column performance.

Silica with a nominal 100 Å pore diameter and 10 μm particle diameter bonded with C_{18} (RG1010C18)

A typical reversed-phase chromatogram of RG1010C18 in the DAC column is shown in Fig. 1, in which a high column efficiency, 44000 plates/m (based on the toluene peak), was obtained, and the bed pressure was close to theoretical prediction. All peaks showed good peak symmetry.

The effect of solvent choice is presented in Table II. Two slurry solvents were evaluated for packing the column (LC50) with RG1010C18. As is shown in Table II, both methanol and acetone resulted in similar column efficiencies; both were higher than theoretical predictions.

Acetone resulted in a lower bed pressure than methanol; however, since methanol is more environmentally friendly than acetone, we elected to use methanol to study the influence of packing pressures on column efficiency and bed pressure. Fig. 2 presents data indicating that although initially both column efficiency and bed pressure increased with packing pressure, the
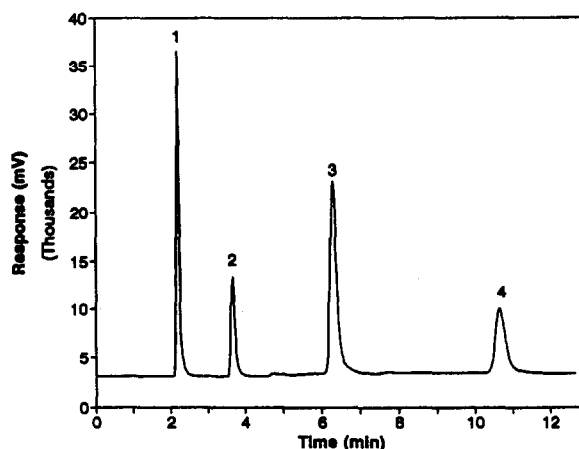

Fig. 1. Chromatogram of four compounds separated on RG1010C18 packed in a dynamic axial compression column (5 cm I.D.) with methanol. Peaks: 1 = uracil; 2 = phenol; 3 = methyl benzoate; 4 = toluene. The concentrations were between 0.1 and 5 mg/ml. Flow-rate: 94 ml/min; mobile phase: acetonitrile–water; sample size: 200 μl; wavelength: 254 nm.

TABLE II

EFFECT OF PACKING SOLVENT ON RG1010C18

Column efficiencies are calculated based on the toluene peak, and the conditions were as stated in the Experimental section.

Slurry solvent	Efficiency (N/m) (36 000[a])	Bed pressure (MPa)	Column dimensions (L × I.D.)
Methanol	44 000	2.52 (2.34[a])	20.2 × 5 cm
Acetone	43 000	1.83 (2.49[a])	21.5 × 5 cm

[a] Theoretical predictions were based on a diffusion coefficient, D_m of toluene = $1.53 \cdot 10^{-5}$ cm^2/s and the particle size of 10 μm.

column efficiency leveled off to 44 000 plates/m as pressures approached 6.2 MPa. The bed pressure however continued to increase with the piston packing pressure (possibly due to the more compressed bed).

A comparison of the theoretical and experimental values of h vs. v plot with acetone or methanol as slurry solvents was established to examine mass transfer effect on column behavior. Under all three packing conditions, the reduced height remained insensitive to the reduced velocity (Fig. 3), which indicated that the system was not controlled by the mass transfer

limitation. The experimental values were in close agreement with the theoretical values, which suggested that the columns were well packed.

The RG1010C18 material was unpacked and repacked six times into the DAC column using methanol as a slurry solvent. Throughout the entire unpacking and repacking procedure, the column efficiency did not change significantly (Fig. 4), and the bed pressures only increased slightly. Similar results were obtained using acetone (the results are not presented in this paper).

Stability studies of the RG1010C18 in the DAC column were carried out under two different slurry solvents and the results are shown in Table III. Final column efficiences and bed
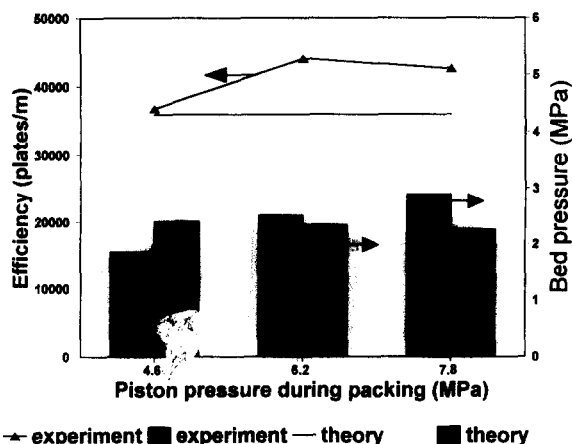

Fig. 2. Effect of piston packing pressure on the column efficiency and bed pressure of RG1010C18 in a DAC column packed with methanol. Comparison between experimental data and theoretical predictions. The column efficiencies were calculated based on the toluene peak. The conditions were as stated in Fig. 1 and the Experimental section.
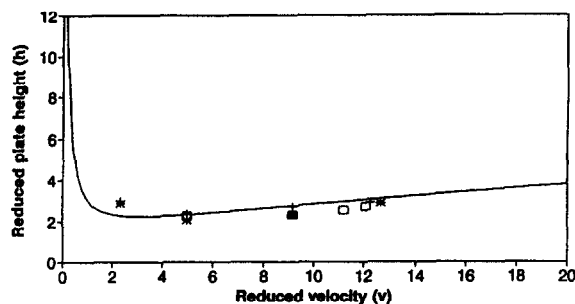

Fig. 3. The h vs. v plot for RG1010C18 in a DAC column with acetone and methanol. Comparison between experimental data and theoretical predictions. Points represent experimental values: + = methanol, 4.6 MPa; * = methanol, 6.2 MPa; □ = acetone, 6.2 MPa. The curve is a theoretical prediction ($h = v^{0.33} + 2/v + 0.05v$). For conditions, see Experimental section.
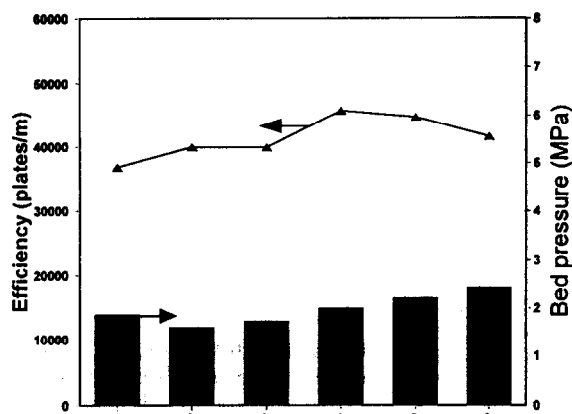
Fig. 4. Repacking of RG1010C18 in a DAC column with methanol. The column efficiencies are calculated based on the toluene peak. The conditions were as stated in Fig. 1 and the Experimental section.
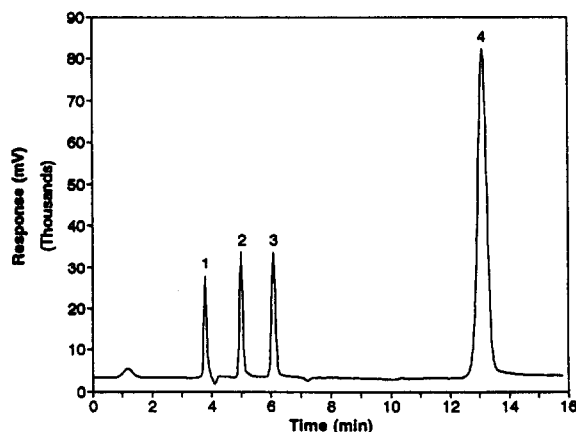

Fig. 5. Chromatogram of four compounds separated on RG1010Si packed with acetone on a dynamic axial compression column (5 cm I.D.). Peaks: 1 = dioctyl phthalate; 2 = diethyl phthalate; 3 = dimethyl phthalate; 4 = cinnamyl alcohol. The concentrations were between 0.5 and 2.5 mg/ml. Flow-rate: 94 ml/min; mobile phase: heptane–2-propanol; sample size: 200 μl; wavelength: 254 nm.

pressures did not change significantly under any of the three conditions, which indicated that the columns remained stable throughout testing.

Bare silica with a nominal 100 Å pore diameter and 10 μm particle diameter (RG1010Si)

A typical normal-phase chromatogram of RG1010Si in the DAC column is shown in Fig. 5, in which a high column efficiency, 43 000 plates/m (based on the cinnamyl alcohol peak), was obtained, and the bed pressure was lower than theoretical prediction. All peaks showed good peak symmetry.

The influence of four slurry solvents were

evaluated for packing the column (LC50) with the RG1010Si. The results are shown in Table IV. Although acetone results in a lower bed pressure than methanol, methanol resulted in a higher column efficiency than acetone. Methylene chloride produced the highest column efficiency, 50 000 plates/m, but the more environmentally friendly 2-propanol had a similar efficiency of 44 000 plates/m. In all the cases, the bed pressures were lower than the theoretical predictions. In summary, it appeared that under

TABLE III

STABILITY OF IMPAQ RG1010C18 PACKED IN DAC COLUMNS

Column efficiencies are calculated based on the toluene peak, and the conditions were as stated in the Experimental section. (Columns were packed at different piston pressures.) c.v. = Column volumes.

Slurry solvent	Initial efficiency (N/m)	Final efficiency (N/m)	Initial bed pressure (MPa)	Final bed pressure (MPa)	Column dimensions (L × I.D.)
Methanol	45 000	49 000 (after 15 h, 277 c.v.)	3.17	3.17 (after 15 h, 277 c.v.)	19.4 × 5 cm
Methanol	40 000	39 000 (after 65 h, 1080 c.v.)	1.58	1.86 (after 65 h, 1080 c.v.)	21.6 × 5 cm
Acetone	43 000	43 000 (after 15 h, 250 c.v.)	1.83	1.93 (after 15 h, 250 c.v.)	21.5 × 5 cm

TABLE IV

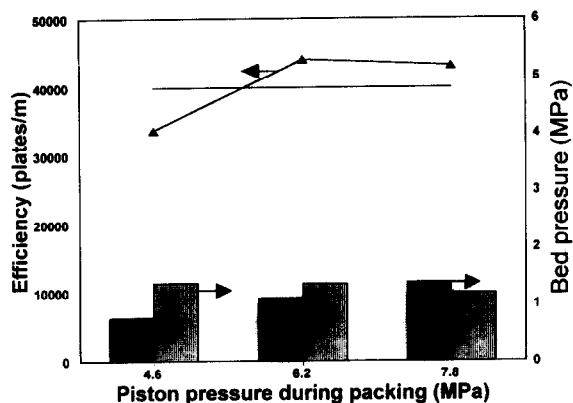EFFECT OF PACKING CONDITIONS ON IMPAQ RG1010Si PACKED IN DAC COLUMNS

Column efficiencies are calculated based on the cinnamyl alcohol peak and the conditions were as stated in the Experimental section. (The top two columns were packed at different piston pressures than the bottom two.)

Slurry solvent	Efficiency (plates/m) (40 000[a])	Bed pressure (MPa)	Column dimensions (L × I.D.)
Methanol	44 000	1.10 (1.35[a])	23.4 × 5 cm
Acetone	38 000	0.69 (1.43[a])	24.8 × 5 cm
2-Propanol	44 000	0.76 (1.43[a])	24.8 × 5 cm
Methylene chloride	50 000	0.69 (1.45[a])	25.2 × 5 cm

[a] Theoretical predictions are based on a diffusion coefficient, D_m of cinnamyl alcohol = $2.3 \cdot 10^{-5}$ cm^2/s and the particle size of 10 μm.

these experimental conditions, both methanol and 2-propanol could result in high column efficiencies.

Methanol was chosen to study the effect of

Fig. 6. Effect of piston packing pressure on column efficiency and bed pressure of RG1010Si in a DAC column packed with methanol. Comparison between experimental data and theoretical predictions. Column efficiencies are calculated based on the cinnamyl alcohol peak. The conditions were as stated in Fig. 5 and the Experimental section.
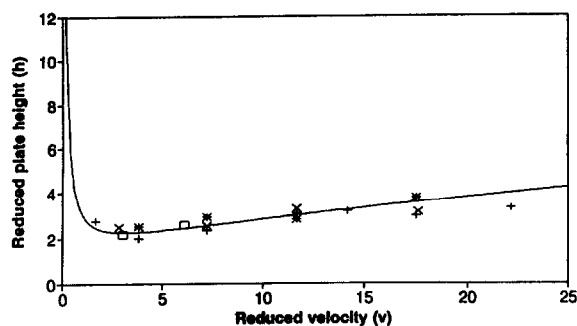

Fig. 7. The h vs. v plot for RG1010Si in a DAC column packed with either acetone or methanol. Comparison between experimental data and theoretical predictions. Points represent experimental values: + = methanol, 7.8 MPa; * = acetone, 6.2 MPa; □ = acetone, 7.8 MPa; × = methanol, 6.2 MPa. The curve is a theoretical prediction ($h = v^{0.33} + 2/v + 0.05v$). For conditions, see Experimental section.

packing pressures on column efficiency and bed pressure. Fig. 6 presents data that showed a similar trend to those in Fig. 2. It appeared that 6.2 MPa was the optimum piston pressure which resulted in a column efficiency higher than theoretical prediction, and a bed pressure in close agreement with theoretical value.

Theoretical and experimental values of the h vs. v plot with different packing solvents and piston pressures were compared to examine mass
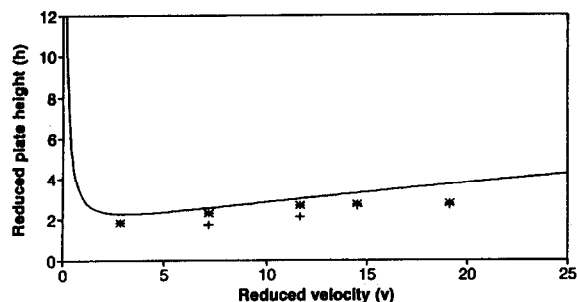

Fig. 8. The h vs. v plot for RG1010Si in a DAC column packed with 2-propanol or methylene chloride. Comparison between experimental data and theoretical predictions. Points represent experimental values: + = methylene chloride, 4.6 MPa; * = 2-propanol, 4.6 MPa. The curve is a theoretical prediction ($h = v^{0.33} + 2/v + 0.05v$). For conditions, see Experimental section.
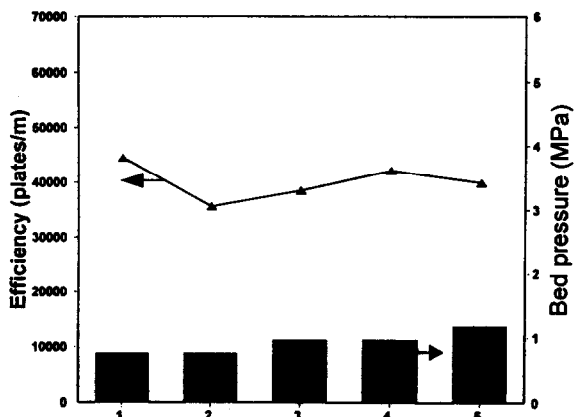
Fig. 9. Repacking of RG1010Si in a DAC column with 2-propanol. The column efficiencies are calculated based on the cinnamyl alcohol peak. The conditions were as stated in Fig. 5 and the Experimental section.

transfer effect on column behavior. Figs. 7 and 8 indicate that the reduced heights remained insensitive to the reduced velocities for all the packing conditions. The experimental values (in Fig. 8) were below the theoretical values, which suggested that the column performances were better than theoretical predictions.

The RG1010Si material was unpacked and repacked five times into the DAC column using 2-propanol. Throughout the entire unpacking and repacking procedure, the column efficiency did not change significantly (Fig. 9), and the bed pressures only increased slightly. Similar results were obtained using methylene chloride as a slurry solvent (the results are not presented in this paper).

Stability studies of the RG1010Si in DAC columns were carried out under two different slurry solvents (Table V). Final column efficiencies and bed pressures did not change significantly under any of the three conditions.

The influence of the compression piston pressure on column performance of the RG1010Si is shown in Table VI. In this study, the column was packed at 7.8 MPa and then the piston pressure was decreased. No effect of the compression piston pressure on the column efficiency and bed pressure was observed, which indicated that the bed was very tight and stable.

Bare silica with a nominal 60 Å pore diameter and 10 μm particle diameter (RG0610Si) and silica with a nominal 100 Å pore diameter and 20 μm particle diameter bonded with C_{18} (RG1020C18)

Typical chromatograms of RG0610Si and RG1020C18 in a DAC column were shown in Figs. 10 and 11, respectively. The column efficiencies were 45 000 plates/m for RG0610Si (based on the cinnamyl alcohol peak), and 18 000 plates/m for RG1020C18 (based on the toluene peak). Further work on these two ma-

TABLE V

STABILITY OF IMPAQ RG1010Si PACKED IN DAC COLUMNS

Column efficiencies are calculated based on the cinnamyl alcohol peak, and the conditions were as stated in the Experimental section. (All columns were packed at the same piston pressures.) c.v. = Column volumes.

Slurry solvent	Initial efficiency (N/m)	Final efficiency (N/m)	Initial bed pressure (MPa)	Final bed pressure (MPa)	Column dimensions (L × I.D.)
Methanol	43 000	38 000 (after 15 h, 263 c.v.)	1.38	1.38 (after 15 h, 263 c.v.)	20.5 × 5 cm
Acetone	43 000	39 000 (after 65 h, 108 c.v.)	0.76	0.90 (after 65 h, 108 c.v.)	23.0 × 5 cm
Acetone	35 000	32 000 (after 6 days, 1814 c.v.)	0.83	0.83 (after 6 days, 1814 c.v.)	22.8 × 5 cm

TABLE VI

INFLUENCE OF COMPRESSION PISTON PRESSURE ON COLUMN PERFORMANCE PACKED WITH RG1010Si

Bed pressure 0.76 MPa in all cases. The column was packed with acetone at a piston pressure of 7.8 MPa, and the piston pressure was decreased. Column efficiencies are calculated based on the cinnamyl alcohol peak, and the conditions were as stated in the experimental section.

Piston pressure (MPa)	Efficiency (plates/m)
7.8	38 000
6.2	39 000
4.6	38 000
3.0	38 000
1.8	38 000

terials will be needed to examine the effect of packing solvent and pressure on column performance, mass transfer effect, long-term stability, and repacking.

CONCLUSIONS

Packing of DAC columns with IMPAQ material can be conveniently achieved by using standardized methods and environmentally friendly

Fig. 11. Chromatogram of three compounds separated on RG1020C18 packed in a dynamic axial compression column (5 cm I.D.) with methanol. Peaks: 1 = uracil; 2 = phenol; 3 = toluene. The concentrations were between 0.1 and 5 mg/ml. The conditions were as stated in Fig. 1.

solvents such as methanol and 2-propanol. The column efficiencies and bed pressures were in good agreement with theoretical predictions. Mass transfer limitation did not play a significant role in either the RG1010C18 or RG1010Si systems. Column performance remained stable over long periods of time. IMPAQ material could be packed repeatedly while maintaining good column performance.
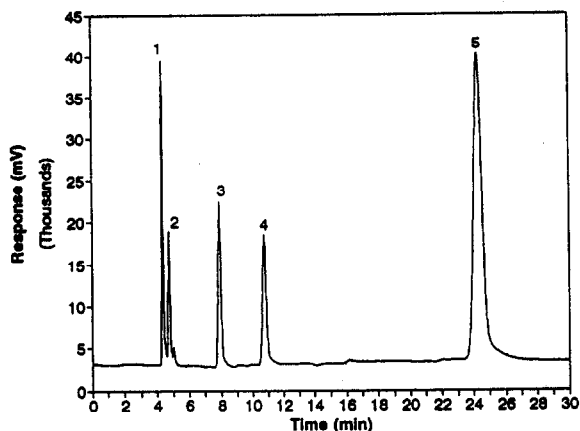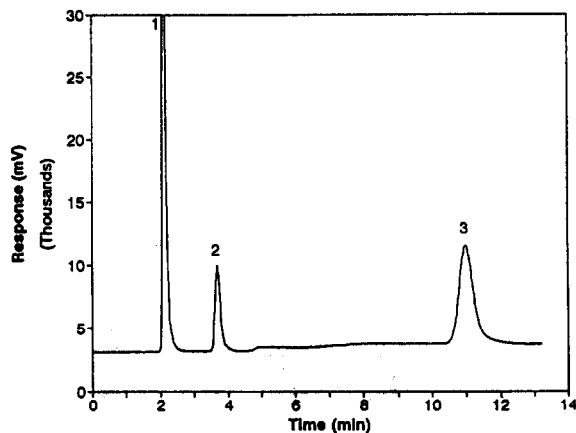
Fig. 10. Chromatogram of five compounds separated on RG0610Si packed in a dynamic axial compression column (5 cm I.D.) with acetone. Peaks: 1 = naphthalene; 2 = dioctyl phthalate; 3 = diethyl phthalate; 4 = dimethyl phthalate; 5 = cinnamyl alcohol. The concentrations were between 0.4 and 2.5 mg/ml. The conditions were as stated in Fig. 5.

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