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Reproducible preparative liquid chromatography columns☆

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Abstract

The level of reproducibility for sets of preparative liquid chromatography (prep-LC) columns was studied using a self-packing, axial compression system. Standard deviations of less than 5% of the mean of the column efficiency and less than 2% of the mean of the retention time are reported for three sets of packed, prep-LC columns.

Keywords: Stationary phases, LC; Preparative chromatography; Reproducibility; Efficiency; Retention times; Axial compression; Naphthalene

1. Introduction

The use of preparative liquid chromatography (prep-LC) for small-molecule and chiral separations is increasing in the industrial theatre (see a long list of special issues and proceedings issue in *J. Chromatogr. A* [1]). These modern separations require reliable tools that perform on a high level in terms of efficiency and reproducibility. At the preparative scale, ''fixed-bed'' columns that are packed with traditional techniques can fail to meet the needs of the prep-LC industry with respect to these parameters, especially column-to-column reproducibility. For example, it has been shown [2] that the closer to the optimum conditions under which a simulated moving bed (SMB) process is operated, the more the production rate and/or the product purity decrease with increasing fluctuations of the column characteristics. To meet purity requirements, operators often resort to selecting a well-matched set of columns from a larger pool of fixed-bed columns. This represents a practical problem that is increasing in importance as a result of the proliferation of the SMB for the preparation of compounds with high enantiomeric purities. Reports of the utility of the SMB for separating racemic mixtures [3–7], and statements issued by the US Food and Drug Administration (FDA) concerning the purity of chiral compounds to be used as pharmaceuticals [8] has increased the need for preparative-scale columns with highly reproducible efficiency and retention time.

Axial-compression systems represent an alternative to fixed bed prep-LC columns. These may be characterized by a movable piston that is situated within the chromatography column tube. The piston is employed both to slurry-pack the column and to maintain a compressive force on the bed during the chromatographic separation. It is well known [9–12] that modern high-performance prep-LC columns are

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typically slurry-packed with derivatized, spherical silicas. While this media delivers extremely high-performance on the analytical level, the achievability and reproducibility of the same level of performance becomes problematic at the prep-LC scale (I.D.>20 mm). While several reproducibility studies can been found for analytical-scale columns [13–17], little material [18] exists in the public domain that addresses the column-to-column chromatographic reproducibility that can be expected for prep-LC columns. Therefore, we are presenting this short communication to disseminate our laboratory information about column-to-column reproducibility on the preparative scale using a new axial-compression, self-packing system.

2. Experimental

All columns and packing instrument used in this study are available from MODcol (Morgan Hill, CA, USA). The packing instruments used were the Model IM2002-025IM1 and the IM2002-050IM1, the former packing the Model AS2002-025040 25 mm I.D. Spring column and the latter the Model AS2002-050040 50 mm I.D. Spring column. Slurries were prepared and packed using reagent-grade or better solvents and proprietary techniques at MODcol. The media used in this study includes MODsil C_8 10 μm, 100 Å (available from MODcol), Chiracel OD, 20 μm (available from Chiral Technologies, Exton, PA, USA) and Protein and Peptide C_{18}, 15–20 μm, 300 Å (available from Grace/Vydac, Hesperia, CA, USA).

The chromatographic system was comprised of a Varian (Walnut Creek, CA, USA) Model SD-1 pump, outfitted with 0.50 l/min heads, a Valco (Houston, TX, USA) Model E26-UW six-port injector valve having 0.030 in. (0.75 mm) bore ports outfitted with a sample loop of 100 μl volume. The injector was actuated remotely using the Star Workstation computer software available from Varian. Data collection was automatically begun at the time that the injector valve introduced the sample into the system. A different mobile phase mixture was used for each of the experiments presented here. Each mobile phase was made up using HPLC-grade solvents and filtered and degassed through a 0.45-μm filter using a vacuum pump prior to use. Distilled water for the mobile phases was obtained from tap water using a Barnstead Model MP-1 distillation unit (Barnstead Thermolyne, Dubuque, IA, USA). The MODcol media was tested using a mobile phase consisting of a mixture of acetonitrile (E.M. Science/Merck, Darmstadt, Germany)–distilled water (70:30, v/v). The mobile phase for testing the Grace/Vydac media was comprised of methanol (J.T. Baker, Phillipsburg, NJ, USA)–distilled water (60:40, v/v). The Chiral Tech media was tested using a mobile phase consisting of hexane (VWR Scientific, West Chester, PA, USA)–ethyl acetate (Burdick and Jackson, Muskegon, MI, USA) (85:15, v/v). The test mixture for the MODsil and Grace/Vydac media was made up of 100 mg uracil (Sigma–Aldrich, St. Louis, MO, USA), 1780 mg phenol (J.T. Baker), 14 ml toluene (J.T. Baker) and 500 mg naphthalene (Sigma–Aldrich, St. Louis, MO, USA) dissolved in 500 ml of mobile phase. The test mixture for the Chiral Tech media consisted of 100 mg *trans*-stilbene oxide (Aldrich, Milwakee, WI, USA) dissolved in 500 ml of the mobile phase. Three injections were performed on each column to insure that the results were stable and accurate.

3. Results

The presented data were not selected from a larger sample pool and constitute all of the columns packed in each production run. Five 50 mm I.D. Spring columns were packed to a bed height of 14.3 cm with MODsil C_8 media using the IM2002-050IM1 MultiPacker packing instrument. The data obtained on efficiency are shown in Fig. 1 and the data obtained on retention time in Fig. 2. Ten 25 mm I.D. Spring columns were packed to a bed height of 10.0 cm with Vydac C_{18} media using the IM2002-025IM1 MultiPacker packing instrument. The data obtained on efficiency are shown in Fig. 3 and the data for retention time is shown in Fig. 4. Twelve 50 mm I.D. Spring columns were packed to a bed height of 15.0 cm with Chiracel OD media using the IM2002-050IM1 MultiPacker packing instrument. The data obtained on efficiency are shown in Fig. 5 and the data for retention time is shown in Fig. 6.

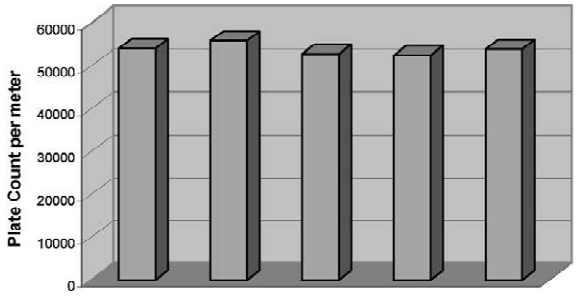

Fig. 1. Efficiency (calculated using the naphthalene peak) for five Spring columns packed with MODcol C_8 10 μm media.

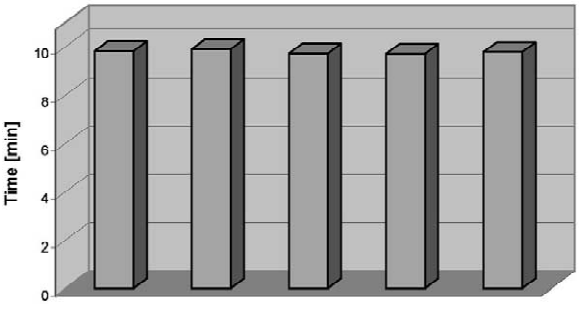

Fig. 2. Retention time of naphthalene peak for five Spring columns packed with MODcol C_8 10 μm media.

4. Discussion

Though published data for the chromatographic reproducibility of prep-LC columns is not available for comparison, the data shown in Figs. 1–6 compare favorably with the best level of reproducibility currently availably from modern preparative liquid chromatography column suppliers. To obtain more meaningful information on the level of reproducibility, a statistical analysis was performed on the retention time and efficiency data. Some selected statistics are shown in Table 1. The range (the difference between the maximum and minimum values) is reported to show the maximum variation that was encountered for this data set. The standard

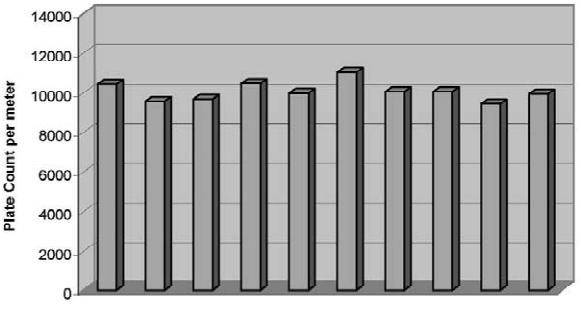

Fig. 3. Efficiency (calculated using the naphthalene peak) for 10 Spring columns packed with Grace/Vydac C_{18} 15–20 μm media.

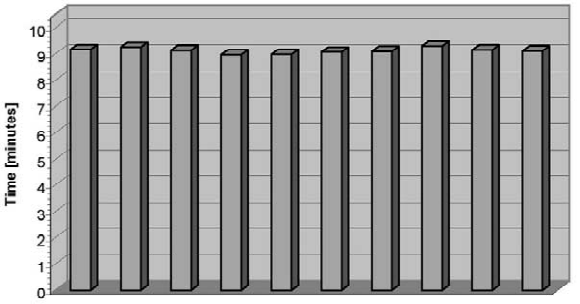

Fig. 4. Retention time of naphthalene peak for 10 Spring columns packed with Grace/Vydac C_{18} 15–20 μm media.

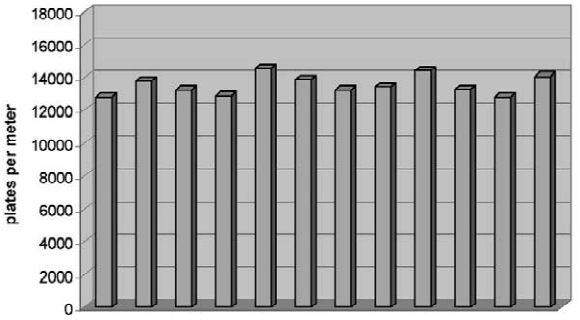

Fig. 5. Efficiency (calculated using the first peak) for 12 Spring columns packed Chiracel OD 20 μm media.

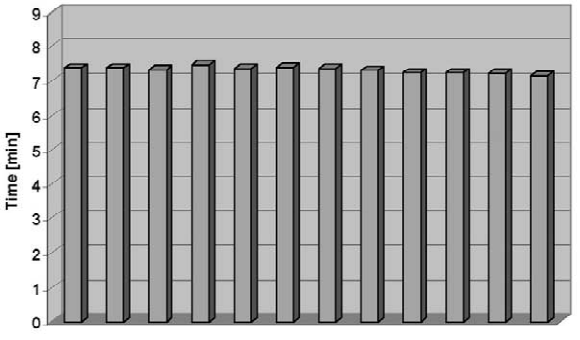

Fig. 6. Retention time of first peak for 12 Spring columns packed with Chiracel OD 20 μm media.

Table 1
Selected results of statistical data analyses performed on the efficiency and retention time data presented in Figs. 1–6

Data	Mean	Range	SD	Range/mean (%)	SD/mean (%)
MODsil C_8 efficiency (plates)	53 788	3350	1321	6.23	2.46
MODsil C_8 retention time (min)	9.78	0.20	0.082	2.04	0.83
Vydac C_{18} efficiency (plates)	10 065	1577	474	15.6	4.70
Vydac C_{18} retention time (min)	9.11	0.33	0.105	3.6	1.10
Chiracel OD efficiency (plates)	13 539	1737	593	12.8	4.38
Chiracel OD retention time (min)	7.32	0.31	0.090	4.23	1.23

deviation is presented to provide the distance from the mean that the majority of a population will be found for larger samples (approximately two-thirds of the population should lie within one standard deviation from the mean and approximately 95% of the population should lie within two standard deviations of the mean). The standard deviation can be used to estimate the spread of the values for larger numbers of columns.

Several observations can be made on the statistical data presented in Table 1. In general, excellent reproducibility is seen for both the efficiency and retention time data. The standard deviation of the mean is within 5% of the mean valve for the efficiency values while the standard deviation of the mean is within 1.25% of the mean for the retention time values. While the range of the data is somewhat greater, as would be expected, the overall level of deviation is still quite low. We attribute this high level of reproducibility to the use of an axial compression packing device.

Let us contrast axial compression and traditional methods for producing prep-LC columns by describing each, citing experience gained at MODcol through the use of fixed bed and axial compression products. When packing fixed-bed columns with traditional techniques, a slurry is pumped at a high flow-rate through the column and the solids are retained by a filter at the exit of the column tube. The initial volume of the slurry is typically several times that of the final bed volume and an extension, herein referred to as the reservoir, must be connected to the column to contain the entire volume. After the bed has been packed, the reservoir and the column must be separated and the open end closed. Typically, media in excess of what is required to complete the bed within the column is used so that the bed within the column is packed to a high density and is uniform. This extra media causes the bed to partially fill the reservoir so that when the two are separated a cut through the packed bed must be made. When this is done, the bed must be exposed and the combination of the cutting process and the exposure of the bed leads to performance irregularities. It is the opinion of the author that the need to open and cut the bed is the critical difference that causes the performance of columns produced by traditional techniques to be much more variable. In contrast, axial compression column packing systems force the liquid out of the slurry by forcing it through the outlet filter using a piston. The packing proceeds until all of the excess liquid is driven out of the solids so that all of the media that is introduced into the column via the slurry becomes part of the bed. Since the piston also serves as the inlet to the column, the bed can remain undisturbed after the packing has completed and the compression of the packed bed is not lost. When traditional techniques are employed to pack a fixed-bed column, much of the bed compression that is developed during the packing process is dissipated when the bed is exposed during the separation of the column and reservoir. For example, when a packed 25 cm×50 mm I.D. fixed-bed column is removed from the reservoir the bed can be seen to ‘‘grow’’ out of the open end to a height of approximately 1 cm in less than 30 s [19]. The significant increase in void fraction that results from the bed expansion decreases performance and, because it may not occur in a homogeneous way, might lead to instabilities within the bed that could lead to rapid failure of the column during use. Decades of experience in developing traditional packing techniques for preparative fixed bed columns [19] has not lead to the

development of methods that can match the level of reproducibility of which axial compression systems are capable. Axial compression packing systems that achieve high bed densities and preserve bed compression are more suited to producing the high level of reproducibility evident in the results presented in this work.

5. Conclusions

This communication has presented column-to-column reproducibility data obtained with a variety of stationary phases using a self-packing, prep-LC axial compression system. The level of reproducibility was seen to be better than 5% for efficiency and better than 2% for retention time. The storage of the compressive forces that are built up within the bed in a column packed using the axial compression technique was invoked to explain the data in a phenomenological way. A similar argument was used to highlight the dissipation of forces within the bed of a column packed using traditional techniques. These differences were cited in an effort to explain the level of reproducibility that was observed.

Examples of the importance of chromatographic reproducibility were given that highlight needs within the chromatographic community for highly reproducible and stable prep-LC columns. In order to maintain the momentum within the preparative-LC community that has been spurred by advances in the synthesis of small-particle and chiral stationary phases and the proliferation of novel separation processes, progress must be made in the column packing sector as well. With the onset of inexpensive and portable axial compression systems, the promise of a higher level of utility can be realized for prep-LC separations.

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