Journal of Chromatography A, 1218 (2011) 6302–6307

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A test to determine the nature and presence of the memory effect columns packed with the amylose tris(3,5-dimethylphenylcarbamate) stationary phase

Joel Putnam, Georges Guiochon*

Department of Chemistry, University of Tennessee Knoxville, TN 37996-1600, USA

ARTICLE INFO

Article history:
Received 24 March 2011
Received in revised form 3 June 2011
Accepted 9 June 2011
Available online 1 July 2011

Keywords:
CHIRALPAK AD
Memory effect
Ethanesulfonic acid
Reproducibility
Normal phase chromatography

ABSTRACT

Acid/base mobile phase modifiers affect enantioseparations in ways that are not fully understood yet, for the lack of systematic studies. This makes chiral analysis of some pharmaceuticals difficult to reproduce. Once a column has been exposed to a modifier, the selectivity of certain pairs of enantiomers may change, for the better or the worse. We study the behavior of five enantiomeric pairs, three which are highly sensitive to the addition of certain modifiers and two that have little sensitivity to these modifiers. Their use permits the determination of the extent of the memory effect response on individual columns. The selectivity of 4-chlorophenylalanine methyl and ethyl ester, and of ketoprofen improve as a solution of ethanesulfonic acid is percolated through the column. As a result, these pairs are most useful for the determination of the extent of acid memory effect on a column. The selectivity of propranolol HCl and, to a lesser degree, Tröger's base increases as a solution of diisopropylethylamine is percolated through the column. The separation of each one of these five pairs is inversely affected by the percolation of the opposite acid/base solution. We used *trans*-stilbene oxide (TSO) as a 'standard' to determine the column stability because no memory effect is observed for it (its retention, enantioselectivity, and resolution remain constant). Understanding whether a column is under the influence of the memory effect is critical to both the analysis of pharmaceutical ingredients and to the development of preparative purification techniques for racemic mixtures. Thus, columns that were unreliable for method development and method transfer, due to the memory effect and a lack of proper solvent exposure records, can now be used.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

The pharmaceutical industry relies on HPLC analysis as one of the most suitable systems for quantitative analysis [1]. Numerous laboratories are involved with bringing new active pharmaceutical ingredients (API) to the market place, which requires the transfer of methods between columns, instrumentation, and laboratories [2]. The transfer and scaling of a method developed on one column to a second column depends on the proper reproducibility of the mobile phase composition, the flow rate, the mass of stationary phase, and the sample injection mass. Additionally, the variability in manufacturing batches of stationary phase can influence the separation when transferring a method to a second column. In this research, the problem of batch variability was minimized by using one column from each batch of stationary phase. The most significant consideration when transferring a separation method developed on the amylose tris(3,5-dimethylphenylcarbamate) (CHIRALPAK® AD®, Diacel Industries, Osaka, Japan) column to other columns of the same stationary phase is a phenomenon called the memory effect, first studied by Ye and Stringham in 2001 [3,4]. Once a column with this stationary phase has been exposed to an acid or a base mobile phase modifier, the separation of certain, but not all, racemic mixtures will change. After removing the mobile phase modifier, the change in separation capacity is retained during the percolation of the mobile phase through the column for thousands of column volumes [5].

In this research, the mobile phase composition was kept constant and the flow rate for each column was adjusted to have identical retention times of the solvent peak, even on columns with different dimensions. The injection mass was adjusted to give the same ratio of enantiomeric mass to stationary phase mass for each column. For example, the amount of *trans*-stilbene oxide (TSO) enantiomers injected on a 4.6 × 150 mm analytical column (labeled 4019) was 10 μL of a 1 mg/mL solution, giving an injection mass of 10 μg/1.55 g of stationary phase. To provide the same injection mass to stationary phase mass ratio, the injected mass on the 10 × 100 mm SMB columns (columns labeled SMB-C and SMB-E) requires injecting 2.6 times as much material on the SMB columns, due to the extra stationary phase in the larger SMB columns. As a

* Corresponding author. Fax: +1 865 974 2667.
E-mail addresses: guiochon@utk.edu, guiochon@ion.chem.utk.edu (G. Guiochon).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.06.060

result, the injection volume on the SMB columns was 26 μL at a concentration of 1 mg/mL.

A better understanding of the memory effect, as well as efficient tests for the detection of this phenomenon, are crucial to the separation of racemic mixtures. By focusing on a stationary phase that clearly exhibits this phenomenon, three objectives can be accomplished. First, understanding the correct method for detecting the memory effect is critical for developing methods for controlling the phenomenon, thus allowing the separation of additional racemic mixtures on this stationary phase. Second, properly determining if this phenomenon exists on other carbamate stationary phases will expand the number of racemic mixtures separated by chromatographic methods. Third, using a column exhibiting a known memory effect in preparative separations can be combined with partial asymmetric synthesis and/or enantiomeric enrichment crystallization to improve success in purifying new APIs.

In order to apply the memory effect properly to the development of analytical and preparative methods, a decisive test must be developed to determine whether a column has been exposed to mobile phase modifiers. Determining if a column has been exposed to a modifier and whether that column is still under the influence of the same modifier can make the difference between success and failure in separating a racemic mixture. The goal of this research was to determine which racemic mixtures are good test probes for the memory effect and to determine if one or more steady-state conditions exist within the memory effect phenomenon.

2. Experimental

2.1. Chemicals

The mobile phase used in the following experiments consisted of hexanes obtained from Fisher Scientific (Pittsburgh, PA, USA) and manufactured by JT Baker (Phillipsburg, NJ, USA). This product contains more than 85% *n*-hexane, with less than 2% methyl-cyclopentane and small amounts of branched hexanes. The alcohol modifier of the hexanes was ACS reagent grade alcohol containing 90% ethyl alcohol, 5% isopropyl alcohol, and 5% methyl alcohol. Chemicals obtained from Sigma–Aldrich (St. Louis, MO, USA) included 4-chlorophenylalanine methyl ester (4CPME), 4-chlorophenylalanine ethyl ester – 97% (4CPEE), 1,3,5-tri-*tert*-butylbenzene – 97% (TTBB) used as a column void marker, ethanesulfonic acid – 95% (ESA), propranolol hydrochloride – 99%, and Tröger's base. The ketoprofen – 99% was obtained from Spectrum Chemicals (New Brunswick, NJ, USA), and the *trans*-stilbene oxide – 97% was obtained from Acros Organics. The *N*,*N*-diisopropylethylamine (DIPEA) was obtained from Alfa Aesar (Ward Hill, MA, USA).

2.2. Equipment

An HP 1100 (Agilent, Santa Clara, CA US) was used to carry out all the experiments and to collect all the measurements reported. A single pump and a single batch of prepared mobile phase were used to eliminate possible variations of the ethanol concentration during individual tests. A column heater was used to control the separation temperature at 40 °C. An autosampler was used to allow for repetitive injections over the entire data collection period. A single wavelength detector was used, all the racemic mixtures tested providing an excellent signal to noise ratio at 210 nm.

2.3. Columns and stationary phase

The only analytical 4.6 × 150 mm column used for these studies was packed by Chiral Technologies (West Chester, PA, USA) and was labeled 4019. This column had been used in previous studies of the memory effect but had not been exposed to any mobile phase or additive other than those which were documented in a previous publications [5,6]. Specifically, this column was exposed to ESA, ethanol, DIPEA, and hexanes as mobile phases and/or additives and also to the racemic mixtures of TSO, 4CPEE, 4CPME, ketoprofen, propranolol, and Tröger's base. Prior to exposing this column to mobile phase modifiers the column was tested with the six racemic mixtures. The selectivity and resolution data collected from these initial tests have been recorded and used as a control value labeled '4019 – original'.

Two preparative 10 × 100 mm columns were also packed by Chiral Technologies, these columns were labeled SMB-C and SMB-E. These two columns had been used previously for the preparative separation of Tröger's base. All solvents used in these columns had been reported by Mihlbachler et al. [7]. In particular, the columns were exposed to methanol, isopropyl alcohol, and Tröger's base. Additionally one of these columns (labeled SMB-C) had been used for the preparative separation of 4CPEE, 4CPME, ketoprofen, Tröger's base, propranolol, and TSO. The SMB-C column was additionally exposed to both ESA and DIPEA.

Two additional 4.6 × 250 mm columns were obtained from Chiral Technologies and labeled ID006 and FB001. These columns had been used by numerous groups and laboratories. Due to their unknown solvent history, these columns were excellent for comparisons to the previous columns listed. By comparing the separation of different racemic mixtures on these columns with unknown solvent histories, we could determine whether they had been exposed to additives inducing the acid memory effect (AME) or base memory effect (BME).

3. Procedures

The mobile phase was made as 4 L of 90/10 (v/v) hexanes/ethanol, to ensure that all columns were exposed to the same mobile phase and that all separations would use the same mobile phase. All samples (4CPEE, 4CPME, ketoprofen, propranolol, Tröger's base, and TSO) were made at a concentration of approximately 1 mg/mL in a solution of 90/10 (v/v) hexanes/ethanol. Each column was kept at a temperature of 40 °C when in use. The flow rate was controlled for each column to ensure that the TTBB, used as a column void marker, eluted at the same time from each column.

Before any samples were injected on to a column, the column was flushed with mobile phase for at least twenty column volumes. In the case of the analytical 4.6 × 150 mm column (4019), this mobile phase volume was 30 mL. For the preparative 10 × 100 mm columns (SMB-C and SMB-E) this mobile phase volume was 80 mL. For the two 4.6 × 250 mm columns with unknown history (ID006 and FB001), an additional step of flushing with isopropyl alcohol was carried out prior to the hexanes/ethanol flush. This additional flush was to ensure that the hexanes/ethanol mobile phase was compatible and miscible with the previous (unknown) mobile phase held within the column when received.

The injection sequence followed for each column was: 4CPEE, 4CPME, ketoprofen, propranolol, Tröger's base, and then TSO. This sequence was repeated three times for each column.

The determination of whether a column had been exposed to the additives inducing AME or BME was done by comparing the selectivity of all six racemic mixtures to data collected previously [5]. An example of these data and the concept is presented in Fig. 1 These data were collected by exposing the analytical 4.6 × 150 mm column (4019) to a maximum load of ESA, followed by the continuous injections of the six racemic mixtures made until the selectivity of the 4CPEE and ketoprofen reached a value of one. Then, the column were exposed to DIPEA, and the injections of the six racemic mixtures was continued until the selectivity of Tröger's base and

Download English Version:

https://daneshyari.com/en/article/1202471

Download Persian Version:

https://daneshyari.com/article/1202471

Daneshyari.com