Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 64–71

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Shell and small particles; Evaluation of new column technology

Szabolcs Fekete[a,*], Jenő Fekete[b], Katalin Ganzler[a]

[a] Formulation Development, Gedeon Richter Plc, Gyömrői út 19-21, Budapest X., Hungary
[b] Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, Szt. Gellért tér 4, Budapest 1111, Hungary

ARTICLE INFO

Article history:
Received 11 August 2008
Received in revised form
29 September 2008
Accepted 6 October 2008
Available online 22 October 2008

Keywords:
Column efficiency
Shell particles
Peak capacity
Kinetic plot
Ascentis Express

ABSTRACT

The performance of 5 cm long columns packed with shell particles was compared to totally porous sub-2 μm particles in gradient and isocratic elution separations of hormones (dienogest, finasteride, gestodene, levonorgestrel, estradiol, ethinylestradiol, noretistherone acetate, bicalutamide and tibolone). Peak capacities around 140–150 could be achieved in 25 min with the 5 cm long columns. The Ascentis Express column (packed with 2.7 μm shell particles) showed similar efficiency to sub-2 μm particles under gradient conditions. Applying isocratic separation, the column of 2.7 μm shell particles had a reduced plate height minimum of approximately $h = 1.6$. It was much smaller than obtained with totally porous particles ($h \approx 2.8$). The impedance time also proved more favorable with 2.7 μm shell particles than with totally porous particles. The influence of extra-column volume on column efficiency was investigated. The extra-column dispersion of the chromatographic system may cause a shift of the HETP curves.

1. Introduction

In liquid chromatography a new age has started with using sub-2 μm particles, monolith columns and shell particles. The theory of Van Deemter and his well-known equation [1] paved the way to get higher plate numbers and better resolution in separation. On sub-2 μm particles, due to the narrow peaks, sensitivity and separation are improved albeit at the cost of pressure. Extra-column effects are more significant for scaled down separations, therefore it is essential to minimize extra-column dispersion. It took many years to introduce the theoretical approach into practice. The first fast and dedicated system for ultra-high-pressure separation was released in the year of 2004. The new hardware was able to work up to 1000 bar (15,000 psi) and the particle size of stationary phases was reduced down to 1.7 μm. To distinguish this new technology from conventional high-performance liquid chromatography (HPLC) a new name, ultra-performance liquid chromatography (UPLC) was introduced. It was proved that the analysis time could be reduced down to a 1- or 2-min interval without the loss of resolution and sensitivity [2,3].

One solution to moderate ultra-high pressure ($P > 400$ bar) is to elevate temperature. Analysis time can also be shortened without the loss of resolution through column heating [4–7]. Mobile phase viscosity decreases with increasing temperature and, thus, column back-pressure decreases. Systems with a maximum pressure capability of 400 bar can then be used with the sub-2 μm columns without over-pressuring the pump. Preheating of the mobile phase is essential to avoid brand broadening.

Monolithic columns are attractive alternatives to packed columns. Like other continuous media, monolithic columns approach fast analysis by bypassing the limitations imposed by pressure via through-pores, which allow higher flow rates than particulate columns at reasonable column back-pressure. Analyte retention is usually provided within the monolithic structure by smaller mesopores. This monolith approach, originally initiated by the work of Hjertén et al. [8], Svec and Frechet [9], Horvath and co-workers [10] and Tanaka and co-workers [11], which already lead to a number of well-performing, commercially available polymeric and silica monolith columns [12,13].

Another approach dates back to the early days of liquid chromatography. Horvath and co-workers [14,15] first demonstrated pellicular particles, made of a solid core surrounded by a layer of porous material. This medium became the first commercial HPLC packing that provided convincing results. Horvath at al. also suggested the pellicular type stationary phases for the separation of biopolymers. Later Kirkland [16] developed similar products that were useful in liquid–liquid chromatography and liquid–solid (adsorption) chromatography. The most recent introduction of a superficially porous particle is the so-called fused-core particle [17,18]. The outer shell is sufficiently thin (0.50 μm) to allow rapid mass transfer into and out of the stationary phase; because the inner core is solid, analytes cannot penetrate any further. This

* Corresponding author.
E-mail address: fekete.szabolcs1@chello.hu (S. Fekete).

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doi:10.1016/j.jpba.2008.10.009

diffusion path length is shorter than in the porous particles of approximately the same diameter and roughly equivalent to the sub-2 μm particles.

In this study the effectiveness of sub-2 μm totally porous particles and porous silica layered solid core type (2.7 μm) particles were compared under isocratic and gradient elution conditions. We also tried to compare the extra-column dispersion of dedicated UPLC and other fast and conventional HPLC systems when small columns packed with porous and shell particles are applied. The test analytes were steroids and a non-steroidal hormone (polar neutral compounds), which are used as a treatment in contraception, climax, prostatic hyperplasia, prostate cancer and hirsutism. Comparison of shell particles to other porous particles is a favorable topic [17,19] but according to our best knowledge there is no such study in which the peak capacity, Van Deemter plots, and other kinetic plots of 1.5, 1.7, 1.9 μm, totally porous particles and porous silica layered solid core type (2.7 μm) particles are compared.

2. Experimental

2.1. Chemicals, column

Acetonitrile and methanol (gradient grade) were purchased from Merck (Darmstadt, Germany). For measurements water was prepared freshly using Milli-Q equipment (Milli-Q gradient A10 by Millipore).

The reference materials and samples as dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9(10)-diene-3-one), finasteride (*N*-*tert*-butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide), gestodene (13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,15-dien-20-yn-3-one), levonorgestrel (13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one, (−)), estradiol (estra-1,3,5(10)-triene-3,17β-diol), ethinylestradiol (19-nor-17-pregn-1,3,5(10)-trien-20-yn-3,17-diol), noretistherone acetate (17-acetoxy-19-nor-17α-pregn-4-en-20-yn-3-one), bicalutamide (*N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenyl)sulfonyl)-2-hydroxy-2-methyl propanamide, (±)), and tibolone (17-hydroxy-7α-methyl-19-nor-17α-pregn-5(10)-en-20-yn-3-one) were produced by Gedeon Richter Plc (Budapest, Hungary).

Ascentis Express C18 column (Supelco) with a particle size of 2.7 μm (50 mm × 2.1 mm) was purchased from Sigma–Aldrich Ltd., Budapest. Waters UPLC™ BEH C18 column with a particle size of 1.7 μm (50 mm × 2.1 mm) was purchased from Waters Ltd., Budapest. Grace Vision HT C18 column with a particle size of 1.5 μm (50 mm × 2.0 mm) was purchased from Lab-Comp Ltd., Budapest. Hypersil Gold C18 column (Thermo) with a particle size of 1.9 μm (50 mm × 2.1 mm) was purchased from Lab-Comp Ltd., Budapest.

2.2. Equipment, softwares

Throughout the measurements a Waters Acquity UPLC™ (ultra-performance liquid chromatography) system with Empower software from Waters Ltd., Budapest, Hungary, a Shimadzu UFLC (ultra-fast liquid chromatography) Prominence system with Class VP software from Simkon Ltd., Budapest, Hungary and an Agilent 1200 RRLC (rapid resolution liquid chromatography) system with Chemstation software were employed. Calculation and data transferring to obtain the kinetic plots was achieved by using the Kinetic Method Plot Analyzer template (Gert Desmet, Vrije University Brussel, Belgium). Solvent optimization was performed using Dry Lab 2000 Plus chromatography optimization software (Molnar-Institute Berlin, Germany). Image-J (freeware image-processing software program developed at the National Institutes of Health) was used to determine the particle size distribution of column packing materials.

2.3. Apparatus and methodology

The mobile phases were prepared by mixing appropriate amount of HPLC gradient grade acetonitrile and Milli-Q water. The mixtures were degassed by sonication for 5 min.

The stock solutions of reference standards (dienogest, finasterid, gestodene, levonorgestrel, estradiol, ethinylestradiol, noretisterone acetate, bicalutamide and tibolone) were dissolved in methanol (1000 μg/ml). The solutions for the chromatographic runs were diluted from the stock solutions with acetonitrile/water 40/60 (v/v). The concentration of the test analytes was 10 μg/ml.

For the measurement of peak capacity, gradients with different time (5, 10, 15, 20 and 25 min) were run from 10% to 80% acetonitrile. For this study 35 and 60 °C column temperature, 0.5 ml/min flow rate, 0.5 μl injection and detection at 220 nm were applied.

The kinetic efficiency of the columns were determined with a mobile phase contained 40% acetonitrile in case of all columns, 35 °C column temperature, 0.5 μl injection and detection at 220 nm were applied. The flow rate was varied from 0.05 up to 1.0 ml/min.

2.4. Equations used for calculation

Peak capacity defines a measure of the column performance under gradient conditions. Several definitions and equations are used for the determination of peak capacity [20–22]. One of those is conditional peak capacity, which can be calculated with very simple formulas, which use the data of obtained chromatograms, such as retention times, average peak width and gradient duration time.

In this study we used the following equation to determine peak capacity:

$$n_c^* = 1 + \frac{t_G}{w}$$

where t_g is the gradient duration, and w is the average peak width.

The column efficiency is mostly illustrated by the Van Deemter curves. Previously Desmet et al. showed [23,24] that it is very straightforward to map the kinetic performance of a given chromatographic support type by taking a representative set of the Van Deemter curve data and re-plotting them as H^2/K_{V0} versus $K_{V0}/(uH)$ instead of H versus u. The minimal analysis time can be calculated by simple rearranging the data in a measured Van Deemter curve and the value of the column permeability (K_{V0}). The following equations transform the linear velocity–plate height data into t_0 time versus plate number (N).

$$N = \frac{\Delta P}{\eta}\left(\frac{K_{V0}}{u_0 H}\right)$$

$$t_0 = \frac{\Delta P}{\eta}\left(\frac{K_{V0}}{u_0^2}\right)$$

where N is the plate number, η mobile phase viscosity, ΔP available pressure drop, K_{V0} column permeability, u_0 linear velocity, and H is the plate height. The obtained values correspond directly to the minimal t_0 time needed in a column taken exactly long enough to yield a given number of theoretical plates. It is easy to combine the given N value with the corresponding plate height value to obtain the corresponding column length.