Journal of Chromatography A, 1383 (2015) 47–57

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Efficiency of short, small-diameter columns for reversed-phase liquid chromatography under practical operating conditions☆

Yan Ma[a], Alexander W. Chassy[a], Shota Miyazaki[b], Masanori Motokawa[b], Kei Morisato[b,c], Hideyuki Uzu[b], Masayoshi Ohira[b], Masahiro Furuno[b], Kazuki Nakanishi[c], Hiroyoshi Minakuchi[d], Khaled Mriziq[e], Tivadar Farkas[f], Oliver Fiehn[a,g], Nobuo Tanaka[a,b,*]

[a] University of California, Davis, 451 Health Sciences Drive, Davis, CA 95616, USA
[b] GL Sciences Inc., 237-2 Sayamagahara, Iruma, Saitama 358-0032, Japan
[c] Kyoto University, Department of Chemistry, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan
[d] Kyoto Monotech Co., 1095 Shuzei-cho, Kamigyo-ku, Kyoto 602-8155, Japan
[e] AB SCIEX, 1201 Radio Road, Redwood City, CA 94065, USA
[f] Phenomenex, Inc., 411 Madrid Avenue, Torrance, CA 90501, USA
[g] University of Jeddah, Biochemistry Department, Saudi Arabia

ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form 5 January 2015
Accepted 7 January 2015
Available online 14 January 2015

Keywords:
Column efficiency
1 mm I.D. column
Monolithic silica
Core–shell particles
Extra-column effect
Weak-wash solvent

ABSTRACT

Prototype small-size (1.0 mm I.D., 5 cm long) columns for reversed-phase HPLC were evaluated in relation to instrument requirements. The performance of three types of columns, monolithic silica and particulate silica (2 μm, totally porous and 2.6 μm, core–shell particles) was studied in the presence of considerable or minimal extra-column effects, while the detector contribution to band broadening was minimized by employing a small size UV-detector cell (6- or 90 nL). A micro-LC instrument having small system volume (<1 μL) provided extra-column band variance of only 0.01–0.02 μL^2. The three columns generated about 8500 theoretical plates for solutes with retention factor, $k > 1$–3 (depending on the column), in acetonitrile/water mobile phase (65/35 = vol/vol) at 0.05 mL/min, with the instrument specified above. The column efficiency was lower by up to 30% than that observed with a 2.1 mm I.D. commercial column. The small-size columns also provided 8000–8500 theoretical plates for well retained solutes with a commercial ultrahigh-pressure liquid chromatography (UHPLC) instrument when extra-column contributions were minimized. While a significant extra-column effect was observed for early eluting solutes ($k < 2$–4, depending on column) with methanol/water (20/80 = vol/vol) as weak-wash solvent, the use of methanol/water = 50/50 as wash solvent affected the column efficiency for most analytes. The results suggest that the band compression effect by the weak-wash solvent associated with partial-loop injection may provide a practical means to reducing the extra-column effect for small-size columns, while the use of an instrument with minimum extra-column effect is highly desirable.

1. Introduction

Small-size columns for high performance liquid chromatography (HPLC) attracted considerable attention in 1970s and 1980s [1–13]. Some groups attempted column miniaturization by adopting a capillary format [1,3–5], while others tried to reduce the size of conventional columns. The importance of minimizing extra-column band broadening was described in the reports. Because of the practical limitations in minimizing the instrumental contribution to analyte band broadening for small-volume columns, and because low-dispersion liquid chromatographs were not commercially available, it was more advantageous to explore the performance of small-diameter columns in longer formats leading to extensive research aiming at generating ultrahigh numbers of theoretical plates in the early stage of size reduction of packing materials [4–6,8,9].

During the past decades, the dimensions of high-speed, high-efficiency columns have been reduced from 4 to 4.6 mm I.D. and 15–30 cm length to 2.1 mm I.D. and 5–10 cm length. This reduction

☆ Presented at the 30th International Symposium on Chromatography (ISC 2014), Salzburg, Austria, 14 - 18 September 2014.

* Corresponding author at: GL Sciences Inc., Iruma, Saitama 358-0032, Japan. Tel.: +81 4 2934 2123; fax: +81 4 2934 3412.
E-mail address: n-tanaka@gls.co.jp (N. Tanaka).

http://dx.doi.org/10.1016/j.chroma.2015.01.014
0021-9673/

in column dimensions was made possible by the development of advanced packing materials (sub-2 μm totally porous particles and sub-3 μm core–shell particles) that maintained high plate counts and allowed for high-speed separations. All along, the difficulties associated with the use of small-size columns in HPLC in providing high column efficiency have been recognized and emphasized [14–20]. The reduction of extra-column effects has become even more desirable than before, actually a necessity, because solute bands eluted from small-size columns packed with very small, high-efficiency materials could be less than a few μL in volume, corresponding to a band variance less than a few μL^2 [14–19]. Therefore, peaks eluted from small-size columns are very sensitive to extra-column band broadening.

The reduction in column diameter from 4.6 mm to 3 mm, and from 3 mm to 2.1 mm is accompanied by a reduction in cross section by a factor of 2.35 and 2.04, respectively. However, a further reduction from 2.1 to 1 mm I.D. results in a more significant decrease in cross sectional area by a factor of 4.41. While most UHPLC instruments with system-caused band variance of several μL^2 allow for adequate operation of short, 2.1 mm I.D. columns maintaining high chromatographic efficiency, such instruments are inadequate for the operation of short 1 mm I.D. columns [15,16]. In spite of this severe practical limitation, analysts are still interested in using columns of very small volume due to their potential in terms of significant reduction in solvent consumption, reduction in sample size or improvement in detection sensitivity both with UV/vis and MS detectors, along with effective dissipation of the heat generated by the high velocity mobile phase percolating through such columns at high linear velocity.

This study on the performance of small volume columns had two objectives: first, to evaluate the current limits in chromatographic efficiency of such columns, and second, to analyze the major factors imposing these limits. In these terms, the intrinsic efficiency of small volume columns is of interest. Can such columns be prepared today to provide the same performance as larger size columns (which are less affected by the current instrument limitations)? Should the focus be on making better columns or have they already reached their limit of performance? Relatively low column efficiency has been reported for a column of small dimensions [15,16]. In early days, electrochemical detection was sometimes employed to reduce extra-column volume (V_{extra}) and extra-column band variance (σ^2_{extra}) when using columns of small size [10,12]. The use of popular and widely applicable UV detection is of much practical importance with small-size columns, although sensitivity can be an issue [1,4]. The studies referenced above reported observed column efficiency ($N_{observed}$ in Eq. (1), where V_R and σ^2_{total} stand for retention volume and band variance of a solute observed, respectively) (as opposed to true column efficiency) while recognizing the negative effect of extra-column band variance (σ^2_{extra} in Eq. (2), where σ^2_{column} describes band variance acquired in a column) and the need for reducing it.

$$N_{observed} = \frac{V_R^2}{\sigma^2_{total}} = \frac{[V_0(1+k)]^2}{\sigma^2_{total}} \quad (1)$$

$$\sigma^2_{total} = \sigma^2_{column} + \sigma^2_{extra} \quad (2)$$

$$N_{intrinsic} = \frac{V_R^2}{\sigma^2_{column}} \quad (3)$$

Intrinsic column efficiency ($N_{intrinsic}$ in Eq. (3)) can be estimated based on observed efficiency and extra-column variance following Eqs. (1)–(3), in principle. Extra-column band broadening has been studied based on the peak variance observed when the chromatographic column is replaced with a zero-dead-volume union. A noninvasive method for the determination of extra-column variance and intrinsic plate height has been proposed for the measurement of extra-column effect [19,20]. Clearly, the current limitations in HPLC system performance need to be overcome by reducing both system volume and the extra-column variance associated with it. This need is very much the focus of HPLC instrument manufacturers. In the meantime, analysts have only limited choices to improve the performance of a column-instrument combination. Numerous reports have proposed practical solutions to reducing extra-column variance such as shortening connecting tubing, using small volume injectors and/or detector cells, and implementing on-column detection. Alternatively, injection techniques associated with compression of the injected band taking place in weak sample solvent have been reported [21–24]. In this report the performance of three prototype columns of 1 mm I.D., 5 cm length packed with sub-2 μm particles, 2.6 μm core–shell particles, or monolithic silica, will be discussed along with the practical evaluation conditions.

2. Experimental

2.1. Instrument and columns

An Eksigent ExpressLC®-Ultra (AB SCIEX, Redwood City, CA, USA) was equipped with a 0.2 μL sample loop and a UV detector with a chip cell (volume: 90 nL, path length: 5 mm). An Acquity UPLC (Waters, Milford, MA, USA) with a 2 μL loop was used in combination with a UV detector (MU701, GL Sciences, Tokyo, Japan) having a capillary cell (volume: 6 nL, path length: 3 mm). Three prototype columns of 1 mm I.D., 5 cm length were examined. They were packed with (1) Kinetex C18, 2.6 μm core–shell particles (Phenomenex, Torrance, CA, USA), (2) InertSustain C18, 2 μm particles (GL Sciences), and (3) glass-clad monolithic silica column modified with dimethyloctadecylsilyl moieties (MonoTower, GL Sciences). The monolithic silica was prepared and glass-clad according to the procedure described previously [25]. Two columns were supplied for each particulate packing material which showed very similar results in a preliminary test.

2.2. Chemicals

Acetonitrile of LC–MS grade was purchased from Fisher Scientific (Hampton, NH, USA). Water was purified with a Milli-Q Quantum EX from Millipore (Bedford, MA, USA). Reversed-phase column performance test mixture containing (1) acetanilide, (2) acetophenone, (3) propiophenone, (4) butyrophenone, (5) benzophenone, (6) valerophenone, (7) hexanophenne, (8) heptanophenone, and (9) octanophenone at 100 μg/mL each, in acetonitrile/water = 65/35 was obtained from Agilent (Little Fall, DE, USA). A triazine pesticides standard mixture containing ametryn, atrazine, prometon, prometryn, propazine, simazine, and terbutryn in methanol at a concentration of 100 μg/mL (Supelco, Bellefonte, PA, USA) was also employed. Thiourea and uracil were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Chromatographic measurements

Columns were connected to the injector and to the detector of Express-LC with 0.025 mm I.D., 10 cm long PEEKSil tubing (SGE, Austin, TX, USA). In the case of the Acquity instrument, columns were connected either directly to the injector, or to a column port via a Viper tube (0.13 mm I.D., 6.5 cm, Thermo Fisher Scientific, Germering, Germany). In the latter case, a heat exchange tube was also a part of the chromatographic system. On the detector side PEEK tubing of 0.0635 mm I.D., 20–90 cm length (IDEX Health & Science, Oak Harbor, WA, USA), was used to connect the column to the detector. The longer PEEK tubing was necessary to suppress air gaps from reaching the UV detector cell when partial-loop injection was applied in the absence of a column with the Acquity instrument.