ELSEVIER

Journal of Chromatography A, 744 (1996) 55–61

JOURNAL OF CHROMATOGRAPHY A

Method for measuring very weak, residual electroosmotic flow in coated capillaries

Sergey V. Ermakov[1], Laura Capelli, Pier Giorgio Righetti*

Department of Cell Biology, University of Calabria, Arcavacata di Rende, I-87036 Cosenza, Italy

Abstract

Many different methods exist for measuring the velocity of electroosmotic flow in capillary electrophoresis (CE). The easiest and the most common one is photometric detection of a neutral marker. This method can be used on any CE unit, while other methods may require some modifications or special equipment. However, this method is not very suitable for measuring weak electroosmosis, since very long runs are necessary for the neutral marker to arrive at the detector. For this case, an alternative method is proposed here. It assumes that the neutral marker is injected by means of electroosmosis, then rinsed by applying a low pressure and recorded. The resulting peak is quantified and, according to the amount of sample injected, the velocity of electroosmosis can be estimated. The proposed method is much faster than the traditional one; its duration is determined mainly by the time of electroosmotic injection. It can be used for estimation of the quality of capillary coatings, for which the residual electroosmotic flow is one of the characteristics.

Keywords: Electroosmotic flow; Coating; Capillary columns

1. Introduction

The invention of the fused-silica capillary by Dandenau and Zerenner [1] in the late seventies, for gas chromatography, paved the way to modern capillary zone electrophoresis (CZE) as well. These capillaries have proved a mixed blessing in CZE: this material comprises a number of acidic surface silanols (isolated, vicinal, geminal), inert siloxane bridges and highly acidic hydrogen bonding sites [2]. At any pH above 2, silanol groups begin to ionize and produce a solvent flux, called electroosmotic flow (EOF). The electroosmotic force in a capillary column is generated by the electric field and transmitted by the drag of ions acting in a thin sheath of fluid adjacent to the silica wall column. The total density of ionogenic silanols on this surface is given as $8.31 \cdot 10^{-6}$ M/m^2, corresponding to ca. 5 silanols per nm^2. According to Salomon et al. [3], however, even at pH 9.55, only 0.3% of the total surface silanols are ionized, a statement which seems to contradict the notion that the average pK value of silanols is 6.3 [4]. While per se EOF is not dangerous in CZE, the mass of ionized negative charges bound to the wall is lethal in protein separations, since these macromolecules tend to bind cooperatively to ionized silanols and be tenaciously adsorbed into the Debye–Hückel layer. An impressive array of methods for partially or fully controlling EOF has been offered: they are extensively reviewed in [5]. One of the most generally accepted methods, especially in regard to protein separations, is to covalently affix to

*Corresponding author. Address for correspondence: L.I.T.A., Via Fratelli Cervi 93, Segrate 20090, Milan, Italy.

[1]Permanent address: Keldysh Institute of Applied Mathematics, Russian Academy of Sciences, Miusskaya Sq. 4, 125047, Russian Federation.

0021-9673/96/$15.00
PII S0021-9673(96)00174-4

such a surface soft polymeric material, neutral and highly hydrophilic, as epitomised by polyacrylamides [5]. Yet, even in the best coated surfaces, it has been recently shown that 'naked' patches can exists, interspersed among the covered silica regions [6,7]. A rapid and reliable method should thus be found for assessing with precision the extent of such residual EOF. A variety of methods have been already described for monitoring EOF. These include: (i) measurement of change in level of solution in the receiving vial [8]; (ii) taking mass measurements, an off-line method first described by Altria and Simson [8] and subsequently refined by Wanders et al. [9] and by Van de Goor et al. [10]; (iii) measuring the time required for the electrolyte level in one vial to reach a specific height [11]; (iv) measurement of streaming potential [12], later developed into an automated method for real-time measurements [10]; on-line measurements were also performed using a conductivity cell [13]; (v) monitoring colloidal particles of the same material as the capillary [14]; (vi) measuring a (fluorescent) marker downstream from the detector, a very fast method, which can measure changes as small as 1% in the flow-rate [15]; (vii) microscopic imaging onto a camera upon EOF flow across capillary [16,17]; (viii) 2-3-4 charged markers technique for high accuracy approximation of non-stationary EOF [18]; (ix) on-line photometric detection of neutral markers [9,11,19,20]. This is the easiest method and markers employed include benzyl alcohol [21,22], adenosine [23], mesityl oxide [24–28], iodine [29], acetophenone [30], formamide [31], 5-(hydroxymethyl)-2-furaldehyde [32], acetone [33], methanol [34], umbelliferon, riboflavin and 4-nitroaniline [35], pyridine [36], phenol [19], toluene [11], benzene and guanosine [37], acrylamide [5]. Even α-napthol has been used as a marker between pH 3.0 and 6.5, i.e. in the region where it does not ionize [38].

In the present report, we propose a simple, efficient method for measuring, in short analysis times, the velocity of weak EOF flows. In this case it could be a complementary addition to a traditional method [as listed above in (ix)] which would require very long electrophoretic runs. The advantage of the proposed method is that it could be done practically on every CZE unit without any modifications. The measurements of weak EOF flows could be important as one of the criteria for estimations of the quality of capillary coating.

2. Experimental

2.1. Materials and equipment

All chemicals were purchased from Merck (Darmstadt, Germany) and they were of analytical grade. The solutions were prepared from double distilled, filtered water bought from Nuova Idrochimica (Milano, Italy). Fused-silica capillaries of different diameters (100 and 50 μm) were obtained from Polymicro Technologies (Phoenix, AZ, USA). We tried both uncoated and coated capillaries, the coating being made by a modified Hjertén procedure described in [5]. Usually the length of capillaries was ca. 27 cm and the effective length was ca. 20 cm, as dictated by capillary cartridge of Beckman's (Palo Alto, CA, USA) P/ACE System 2100 on which all the experiments were performed. The following buffer solutions with different pH value were used: 20 m*M* formic acid titrated to pH 3, 3.5, 4 respectively with NaOH; 20 m*M* taurine+NaOH (pH 9); 20 m*M* glycine+NaOH (pH 10). Between experiments capillaries were always stored in the buffer solution; when the latter were changed, they were allowed to equilibrate in a new buffer for a night. Acrylamide (Pharmacia Biotech, Uppsala, Sweden) at 20 m*M* concentration was used as a neutral marker substance.

2.2. Method

The simplest and most natural method for measuring the EOF velocity is observing the migration of a neutral marker peak, (this method is referred to below as the traditional one). We used this method as a control in some of the experiments. However, for weak EOF, it is not suitable, since one might wait hours and hours with the risk (as it will be pointed out later) of seeing nothing. The method proposed here (referred to below as the present method) should improve the situation, offering much quicker results at reasonable accuracy.

The idea of this method of measuring the velocity of EOF is very simple. First, the electrokinetic

injection of a neutral marker is performed. Its duration depends on the magnitude of electroosmosis. Since the sample (marker) is neutral, i.e. electrically uncharged, the electrokinetic injection will correspond to the injection done by EOF and, thus, the amount of the marker injected should be proportional to the EOF velocity v_{eos}

$$\delta_{eos} = v_{eos} \cdot t_{eos} = \alpha \cdot A_{eos} \quad (1)$$

here δ_{eos} is the length of the sample plug and t_{eos} is injection time. Simultaneously, as follows from eq. 1, it is proportional to the sample peak area A_{eos}. After injection, the capillary inlet end is inserted back into the vial with a buffer solution and the capillary is rinsed by applying a low pressure. The sample plug is delivered to the detector and recorded. For the reference, the marker substance should be injected by an alternative method, e.g. low pressure injection, for which an accurate estimation of injected volume could be done:

$$\delta_{hydr} = \bar{v}_{hydr} \cdot t_{hydr} = \alpha \cdot A_{hydr}, \quad \text{where}$$

$$\bar{v}_{hydr} = \frac{\Delta p R^2}{8\eta L} \quad (2)$$

Here Δp, η, R, L are pressure differences applied to capillary, buffer viscosity, capillary radius and its total length respectively. As previously, the sample is rinsed and recorded. Comparing the area of the reference peak A_{hydr} and that of 'electroosmotic' injection, A_{eos} the amount of sample in the latter and, hence, the velocity of electroosmotic flow could be estimated:

$$\delta_{eos} = \frac{A_{eos}}{A_{hydr}} \delta_{hydr}, \quad v_{eos} = \frac{\delta_{eos}}{t_{eos}} = \frac{A_{eos}}{A_{hydr}} \cdot \frac{\delta_{hydr}}{t_{eos}} \quad (3)$$

In some cases it is more convenient to present the data in terms of retention time t_{det}, the time in which a neutral marker will reach a detector:

$$t_{det} = t_{eos} \frac{A_{hydr}}{A_{eos}} \cdot \frac{l}{\delta_{hydr}} \quad (4)$$

where l is the capillary effective length, i.e. the distance from the inlet end to the detection point.

The reference peak in our experiments was produced by 1 s or 2 s hydrodynamic injection at a pressure of 0.5 p.s.i. (1 p.s.i. = 6894.76 Pa). The rinsing of the capillary was always performed using the same pressure. The acrylamide peak was registered either at 254 nm or at 214 nm wavelength depending on whether high sensitivity conditions were necessary. The duration of electroosmotic injection varied from 1 min for uncoated capillaries to 10 min for coated. The lower was the electroosmotic flow the longer injection was necessary in order to load more material and, thus, to perform more accurate estimations.

3. Results

In order to verify the present method, the first set of experiments on measuring the velocity of EOF was performed for uncoated capillaries in an acidic pH range (pH 3–4). Here a moderate EOF was observed for which the retention time of a neutral marker (acrylamide) was of the order 8–14 min. The experiments were done in a series in which the velocity was measured by the traditional method and by the present one. Usually every series contained from 3 to 12 runs of each method. The runs were performed on different days. The results of these runs are summarized in Fig. 1 on which the average retention time measured by traditional method

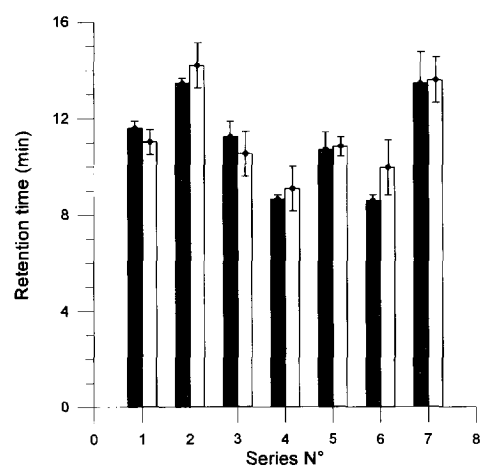

Fig. 1. Retention time corresponding to the EOF: measured by the traditional method (grey bars) and calculated by the present method (transparent bars) in different series of experiments. Error bars (standard deviation) are shown on top of the data bars. Capillary: 100 μm uncoated, effective length 20 cm. For further explanations see text.

(grayed bars), plotted together with its standard deviation, is compared with those calculated using the present method (transparent bars). Series 1–5 were performed in pH 3.5 formate buffer, series 6 in pH 4, while series 7 were performed in pH 3 buffer solutions. A rather good agreement between the two methods is observed with maximum deviation 14.95% for series 6, minimum 1.09% for series 7 and medium 5.63%. The deviation between the two methods changes quite randomly from series to series, so no systematic error is observed. The reproducibility of EOF was also subjected to quite arbitrary fluctuations as shown by the standard deviation. It is clear that the present method will show a higher standard deviation, since in this case the EOF is measured based on electroosmotic injection which lasts 1 min, while the traditional method is based on a direct measurement, the duration of which is 8–14 min, and thus relative error is higher in the first case. However the difference in standard deviations is not very large, the average value for the traditional method being 0.52 min while for the present one it is 0.776 min. The other set of experiments was performed in coated 50 μm capillaries. The experiments were initiated with buffer solutions having neutral pH values (pH 7), moving towards the higher ones. It was done in order to avoid subjecting the capillary immediately to highly alkaline conditions in which the coating degrades relatively fast. Until pH 10 was reached no significant EOF was observed (at least we failed to observe it by the traditional method) while at pH 10 it was possible to do so. Fig. 2 shows the retention time measured by traditional and present methods; the experiments were performed in pairs, one of each method. The electroosmotic injection used in the present method lasted 5 or 10 min. During the time that the capillary was stored in the alkaline buffer it was seen that the coating degraded with time and the EOF increased from day to day (the experiments were performed in one week). Here again rather good agreement is observed between the data obtained by traditional method and the present one. The maximum deviation was found 14.5% for the second experiment when the capillary wall had yet not been stabilized, while for the last experiments the error did not exceed 4.4%. It is worth noting that the present method was approximately 4 times faster

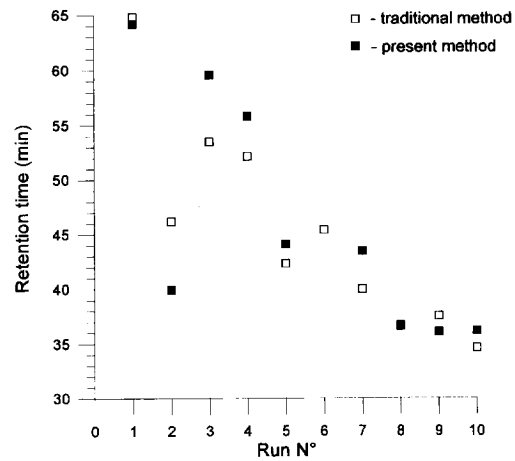

Fig. 2. Retention time corresponding to the EOF: measured by the traditional method (open squares) and calculated by the present method (filled squares) in different experiments in pH 10 buffer solution. Capillary: 50 μm coated with polyacrylamide, effective length 20 cm.

here, since the electroosmotic injection lasted 5 min followed by 4 min rinsing of the capillary during which the peak was recorded. With traditional method it was necessary to wait at least 40 min to see the marker peak. This advantage became more evident for weaker EOF when hours were required to observe the peak, while with the present method the experiment never exceeded 20 min, with a duration of electroosmotic injection no more than 10 min. As already mentioned, in experiments with coated capillaries for pH value 9 and lower we failed to observe marker peak in the normal runs continued for up to 3 h. At the same time the estimations done by the present method for buffer solutions with pH 9 gave retention times ranging from 2.3 to 5.2 hours.

4. Discussion

The measurement of EOF velocity by the present method has several restrictions. The sample volume loaded during electroosmotic injection is influenced by different physical factors resulting in variations of the volume. One of them, which in fact puts a limit to an accuracy of measurements, is an inadvertent hydrodynamic flow (IHF) caused by the difference in liquid layers between the capillary ends dipped in

the buffer solution and the surface of the liquid. This could be either due to the fact that different amount of liquid is contained in each vial (suppose that the vials are equal) or because the capillary ends are immersed to different depths. As a rule the buffer/sample vials used in capillary electrophoresis are rather small, hence capillary effects could play a significant role in making a liquid-air interface non-flat, so that equilibrating free surfaces between inlet and outlet vials without special precautions becomes more difficult. In our experiments we tried to have the liquid levels as similar as possible, however no special procedure or equipment was used.

Let us assume that in routine practice the difference in levels could be no more than $\Delta h = 0.5$ mm. It could be in both 'directions' i.e. the resulting IHF could be co- or counter-flow in respect with EOF. When its direction is opposite to that of EOF and their velocities are equal, the marker peak will never reach the detection point, being blocked at the inlet end. Such conditions present a limiting point in the EOF measurements. They depend on capillary diameter and its length, assuming that $\Delta h = \text{const}$. A series of curves for 5 different diameters corresponding to condition $v_{eos} = v_{hydr}$ for $\Delta h = 0.5$ mm are plotted by solid lines in Fig. 3. It is seen that the larger the capillary diameter and the shorter the capillary the more restrictive becomes the IHF. For EOF velocity lying below these lines, measurement becomes problematic. In practice if one wishes to distinguish EOF from IHF with accuracy, say 10%, it is necessary to demand that $v_{eos} = 10\ v_{hydr}$ which limits more the range of measurable EOFs. The last condition is presented in Fig. 3 by dashed lines. For a reference, the range of EOFs commonly met in practice are shown by a grey color. It was calculated assuming that the shortest retention time in a capillary with 20 cm total and 10 cm effective length is 30 s, while the longest time for the same capillary is 1 hr. Fortunately, only for rather short capillaries, of 30–40 cm total length with diameters 75–100 μm and weak EOF (below 10^{-4} m/s), is there a danger of deriving non-precise data on EOF velocity. For smaller diameters e.g. 25 μm the EOF will always overshoot IHF, provided that $\Delta h \leq 0.5$ mm and the EOF is not too weak. The magnitude of IHF may be estimated by performing two consecutive runs with and without applying the voltage and measuring

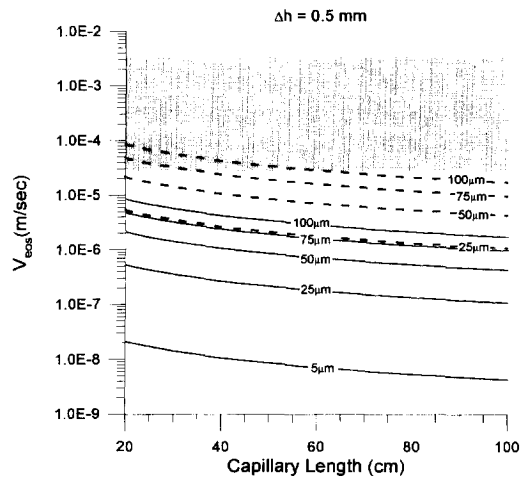

Fig. 3. Comparison of EOF with inadvertent hydrodynamic flow (IHF) for different capillary lengths and diameters. IHF corresponds to 0.5 mm difference in liquid layers between capillary ends. Solid lines show the parameters when the velocity of EOF is equal to that of IHF, dashed lines when the velocity of EOF is 10 times greater than that of IHF. The area of parameters shown in grey corresponds to the EOF conditions most frequently occurring in practice using uncoated capillaries.

respective peak areas. The difference between them (if the peaks are observed at all) should be caused by EOF, thus giving an idea of the magnitude of IHF as well. Two experiments are necessary for the case in which hydrodynamic flow is opposite to EOF. Thus, the present method could be used also for estimation of the magnitude of IHF. The above considerations on the influence of IHF on reproducibility and accuracy of measurements are valid not only for EOF but also for the electrophoretic velocity of any charged substance.

Other possible errors in the amount of sample loaded, intrinsic to any type of injection, originate in sample diffusion into and out of capillary [39] and in so-called 'extraneous' injection [40,41]. In practice it is very difficult (if possible at all) to separate them from each other as well as from IHF. In order to estimate their impact on the amount of sample loaded during electroosmotic injection, a series of experiments imitating a 5 min electroosmotic injection was performed. Imitating means that the voltage was not applied during such experiments, so that the sample penetrated into capillary by the same mechanisms excluding electroosmosis. These experi-

ments were performed simultaneously with those presented in Fig. 2. Comparing the areas of peaks injected it was found that diffusion, extraneous injection and IHF together can contribute from 1.9% to 17% of total sample injected with mean value of 7.4%. Subtracting this amount from the total sample mass we get the amount of sample injected solely by electroosmosis, which augments the calculated retention times in Fig. 2 by ca. 8%. The attempts to estimate separately the effect of extraneous injection, by immersing the capillary end in the sample vial for a short period of ca. 1 s, gave a contribution of about 1.5% in the total mass injected during a 5 min electroosmotic injection. It means that on average 7.4–1.5=5.9% of sample overloading could be attributed to diffusion and IHF.

5. Conclusions

A simple and fast method for measuring the velocity of electroosmotic flow especially in cases when it is weak, has been developed and tested. It could also be applied to the assessment of the magnitude of inadvertent hydrodynamic flow resulting from the difference in liquid levels between inlet and outlet vials. Its precision is limited only by physical effects influencing the process of injection, for instance, diffusion and extraneous injection, and by inadvertent hydrodynamic flow. This method could be applied to the assessment of the quality of capillary coating for which the residual electroosmosis is one of the characteristics.

Acknowledgments

P.G.R. is supported by grants from Telethon-Italy (No. E.153), by the Italian National Reasearch Council (Rome), Progetto Strategico ST-74 and by the European Community (Human Genome Project, Biomed 2, grant application No. PL-951158). S.V.E. is supported by the European Community.

References

[1] R.D. Dandenau and E.H. Zerenner, LC·GC Int., 4 (1991) 10–15.
[2] W.H. Wilson, H.M McNair and K.J. Hyver, J. Chromatogr., 540 (1991) 77–83.
[3] K. Salomon, D.S. Burgi and J.C. Helmer, J. Chromatogr., 559 (1991) 69–77.
[4] M.S. Bello, L. Capelli and P.G. Righetti, J. Chromatogr. A, 684 (1994) 311–322.
[5] M. Chiari, M. Nesi and P.G. Righetti, in P.G. Righetti (Editor) Capillary Electrophoresis: an Analytical Tool in Biotechnology, CRC Press, Boca Raton, FL, 1996, pp. 1–36.
[6] L. Capelli, S. Ermakov and P.G. Righetti, J. Biochem. Biophys. Methods, 32 (1996) 109–124.
[7] R. Barberi, J.J. Bonvent, R. Bartolino, J. Roeraade, L. Capelli and P.G. Righetti, J. Chromatogr. B, 683 (1996) 3–13.
[8] K.D. Altria and C.F. Simpson, Chromatographia, 24 (1987) 527–536.
[9] B.J. Wanders, A.A.A.M. van de Goor and F.M. Everaerts, J. Chromatogr., 470 (1989) 89–94.
[10] A.A.A.M. van de Goor, B.J. Wanders and F.M. Everaerts, J. Chromstogr., 470 (1989) 95–101.
[11] T.S. Stevens and H.J. Cortes, Anal. Chem., 55 (1983) 1365–1370.
[12] J.C. Reijenga, G.V.A. Aben, Th.P.E.M. Verheggen and F.M. Everaerts, J. Chromatogr., 260 (1983) 241–250.
[13] B.J. Wanders, A.A.A.M. van de Goor and F.M. Everaerts, J. Chromatogr. A, 652 (1993) 291–294.
[14] B.J. Herren, S.G. Shafer, J. van Alstine, J.M. Harris and R.S. Snyder, J. Colloid Inferface Sci., 115 (1987) 46–56.
[15] T.T. Lee, R. Dadoo and R.N. Zare, Anal. Chem., 66 (1994) 2694–2699.
[16] W.G. Kuhr, L. Licklider and L. Amankwa, Anal. Chem., 65 (1993) 277–282.
[17] J.A. Taylor and E. Yeung, Anal. Chem., 65 (1993) 2928–2932.
[18] J.H. Jumppanen and M.-L. Riekkola, Anal. Chem., 67 (1995) 1060–1066.
[19] K.D. Lukacs and J.W. Jorgenson, J. High Resolut. Chromatogr., 8 (1985) 407–412.
[20] H.H. Lauer and McManigill, Anal. Chem., 58 (1986) 166–172.
[21] M. Chen and R.M. Cassidy, J. Chromatogr., 602 (1992) 227–234.
[22] R.R. Chadwick and J.C. Hsieh, Anal. Chem., 63 (1991) 2380–2385.
[23] M. Aguilar, X. Huang and R.N. Zare, J. Chromatogr., 480 (1989) 427–433.
[24] P.D. Grossman and D.S. Soane, Anal. Chem., 62 (1990) 1592–1599.
[25] D.J. Rose and J.W. Jorgenson, Anal. Chem., 60 (1988) 642–646.
[26] G.M. Janini, K.C. Chan, J.A. Barnes, G.M. Muschik and J.H. Issaq, Chromatographia, 35 (1993) 497.
[27] S. Palmarsdottir and L.E. Edholm, J. Chromatogr. A, 693 (1995) 131–140.
[28] I.Z. Atamna, C.J. Metral, G.M. Muschik and H.J. Issaq, J. Liq. Chromatogr., 13 (1990) 3201–3205.
[29] H. Salimi-Moosavi and R.M. Cassidy, Anal. Chem., 67 (1995) 1067–1071.

[30] S. Terabe, Trends Anal. Chem., 8 (1989) 129–133.
[31] S.M. Masselter and A.J. Zemann, Anal. Chem., 67 (1995) 1047–1052.
[32] D. Corradini, G. Cannarsa, E. Fabbri and C. Corradini, J. Chromatogr. A, 709 (1995) 127–134.
[33] H.K. Jones and N.E. Ballou, Anal. Chem., 62 (1990) 2484–2488.
[34] C.P. Ong. C.L. Ng, H.K. Lee and S.F.Y. Li, J. Chromatogr., 588 (1991) 335–339.
[35] Ch. Schwer and E. Kenndler, Chromatographia, 30 (1990) 546–551.
[36] T. Tsuda, K. Nomura and G. Nakagawa, J. Chromatogr., 264 (1983) 385–391.
[37] I.Z. Atamna, C.J. Metral, G.M. Muschik and H.J. Issaq, J. Liq. Chromatogr., 13 (1990) 2517–2522.
[38] H.T. Chang and E.S. Yeung, J. Chromatogr., 608 (1992) 65–70.
[39] E.V. Dose and G. Guiochon, Anal. Chem., 64 (1992) 123–128.
[40] E. Grushka and R.M. McCormick, J. Chromatogr., 471 (1989) 421–428.
[41] H.A. Fishman, N.M. Amudi, T.T. Lee, R.H. Scheller and R.N. Zare, Anal. Chem., 66 (1994) 2318–2329.