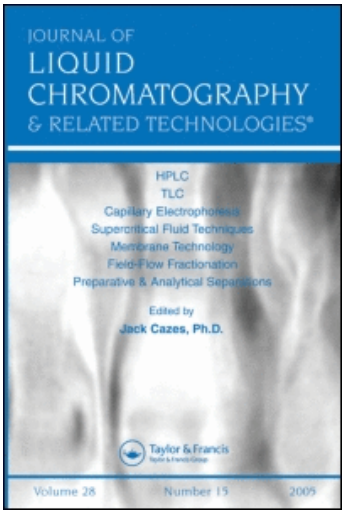

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Retention Phenomena Induced by Large Volume Injection of Solvents Non-Miscible with the Mobile Phase in Reversed-Phase Liquid Chromatography

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Abstract: Enhancement of sensitivity for spectrometric detection in liquid chromatography is obtained when increased sample volumes are loaded into the chromatographic column. A new approach of injecting large volumes of solvents non-miscible with the mobile phase is proposed. The paper focuses on the retention study of butylated hydroxyanisole (BHA) loaded into the chromatographic column dissolved in liquid alkanes (n-hexane, n-heptane, and iso-octane, respectively) at volumes up to 600 μL. A simple model based on adsorption for explaining the experimental results is proposed. A clear insight of the process was possible by studying the migration of the solvent zone within the column, at different mobile phase compositions and different loaded volumes, by means of refractive index detection. The model allowed calculation of the number of solvent molecules saturating a C_8 alkyl chain site within the stationary phase layer. Competition between the analyte and solvent molecules, together with the homogeneity of the "saturation" realized by solvent over the adsorption sites, could explain the experimental results. It was demonstrated that large volume injection of solvents non-miscible with the mobile phase is

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possible in RP-LC with a gradual reduction of retention and a relatively small loss in terms of peak efficiency.

Keywords: Large volume injection, Reversed phase liquid chromatography, Solvents non-miscible with the mobile phase, Adsorption model, Retention behavior, Butylated hydroxyanisole, Iso-octane, Liquid alkanes

INTRODUCTION

Injection volume is a major parameter in liquid chromatography (LC), directly influencing the limit of quantitation (LOQ) that can be obtained for analytes in different samples.[1,2] For this purpose, it is usually recommended that the sample composition injected into the analytical column to be as similar as possible to the mobile phase composition, in order to avoid uncontrolled influences on the retention process.[3] However, this condition can not be fulfilled in many cases, mainly when target analytes have low solubility in the mobile phase used at the beginning of the chromatographic separation. More often, to avoid incompatibilities between the sample solvent and the mobile phase, the injection volume is limited to a narrow interval (5–10 μL). In current practice, less attention is paid to the nature of the sample solvent and its role in the retention process. Old concepts, such as elutropic character or solvent strength are still being used in practice, but they do not offer a good insight of the complex process occurring during injection and retention processes within the chromatographic column. The role of sample solvent strength in fast-gradient LC has been emphasized recently by Layne et al,[4] demonstrating that earlier eluting analytes are much more affected by the sample solvent strength than the later eluting ones. Enhanced detection sensitivity, obtained by means of large volume injection (up to 50 μL) in reversed-phase micro-LC has been already reported with application to the assay of trace-level of pesticides.[5,6]

A method for determination of BHA as an antioxidant in pharmaceutical formulations containing statines has been previously developed.[7] This method is based on selective BHA extraction in iso-octane, followed by the direct injection of a large volume onto the chromatographic column, without any additional preparation. The aim of this work is to enlarge the concept of sample injection in reversed-phase LC using different organic solvents non-miscible with the mobile phase, and to evaluate the results of the experiments by means of the two theoretical models (partition and adsorption), generally accepted by literature, e.g.[8–12] Therefore, several solvents (lower aliphatic hydrocarbons) having increased similarities with the stationary phase were used for sample dissolution. A mandatory condition is that the analytes should be soluble in these solvents. The final advantage of large volume injection (up to 600 μL) involves not only increased sensitivity of the detection process, but also substantial reduction of the sample preparation

complexity and increased accuracy of the operations. For this reason, several stages in sample preparation procedures can be eliminated, such as sample concentration by evaporation, and/or back extraction techniques.

EXPERIMENTAL

Reagents and Column

All solvents (acetonitrile, methanol, n-hexane, n-heptane, iso-octane, chloroform, ethyl acetate, diethyl ether) were HPLC grade from Merck KgaA (Darmstadt, Germany). Phosphoric acid was p.a. grade from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany).

All experiments were carried out using Zorbax Eclipse XDB-C8, 150 mm length, 4.6 mm i.d. and 5 μm particle size (Agilent Technologies) as the chromatographic column(s). Column validation before starting the experiments revealed a reduced plate height ($\hat{h}$) of 2.19, using fluoranthene as target analyte and the negative peak as dead time indicator (t_0).

Target analytes in this study were: butylated hydroxyanisole (BHA). Stock solutions of BHA in the above solvents, with the concentration of 500 μg/mL, were used, and in some circumstances, diluted to lower concentrations according to the requirements of the experimental setup. For increasing injected volumes, concentrations of the target compound were progressively reduced, in order to keep almost constant its absolute amount loaded onto the column.

Mobile Phase

The mobile phase composition was given by acetonitrile and an aqueous component (0.1% phosphoric acid, v/v). The study was carried out in isocratic conditions (40% organic solvent and 60% aqueous 0.1% phosphoric acid). A gradient step profile (up to 100% organic solvent in 0.05 minutes) after elution of the target compound was often used for fast elimination of i-octane loaded onto the column (as can be further observed in Results and Discussion). A flow rate of 1.5 mL/minute was used if not otherwise stated in text. The mobile phase and column were maintained at 25°C using the Agilent column thermostat Peltier based heater (G1316A).

Instrumentation

Experiments were performed on an Agilent 1100 series liquid chromatograph containing degasser, quaternary pump, autosampler (with large volume injection option), column thermostat, diode-array detector (DAD), and refractive index detector (RID). Detection was made at 291 nm (± 2 nm), with a

reference wavelength of 480 nm (±5 nm). Chromatographic data were acquired by means of the Chemstation software (Agilent Technologies, Waldbronn, Germany).

Premises

Different injection volumes were applied, in accordance with the purpose of the work. The negative injection peak was used as t_0 (dead time) indicator. Calculation of the capacity factors was made according to the relation $k' = (t_R - t_0)/t_0$, where t_R (minutes) is the retention time for the target analyte.

Some of the characteristics related to solvents and target compound used for calculations were taken from The Merck Index, vers. 12.3. (Merck & Co. Inc. Whitehouse Station, NJ, U.S.A., or computed via Kowwin ™ vers. 1.67 (copyright © 2000, U.S. Environmental Protection Agency) as follows: n-hexane, CAS no. 110-54-3, $M_w = 86.18$ g/mol, $\rho_{4_4}^{20} = 0.660$ g/mL, solubility in water (20°C) = 9.5 mg/L, calculated log $K_{ow} = 3.28$, experimental $\log(K_{ow}) = 3.9$; n-heptane, CAS no. 142-82-5, $M_w = 100.2$ g/mol, $\rho_{4_4}^{20} = 0.684$ g/mL, solubility in water (20°C) – almost insoluble, calculated log $K_{ow} = 3.78$, experimental log $K_{ow} = 4.66$; n-octane, CAS no. 111-65-9, $M_w = 114.23$ g/mol, $\rho_{4_4}^{20} = 0.7028$ g/mL, solubility in water (20°C) = 0.7 mg/L, calculated $\log K_{ow} = 4.27$, experimental $\log K_{ow} = 5.18$; i-octane, CAS no. 540-84-1, $M_w = 114.23$ g/mol, $\rho_{4_4}^{20} = 0.69194$ g/mL, solubility in water (20°C) = 0.56 mg/L, calculated $\log K_{ow} = 4.09$; butylated hydroxyanisole—(1,1-dimethylethyl)-4-methoxyphenol; CAS no. 25013-16-5, $C_{11}H_{16}O_2$, calculated log $K_{ow} = 3.5$.

According to information kindly offered by the manufacturer, an XDB C_8 column (150 mm length × 4.6 mm i.d.) contains about 2 g of solid material, has a void volume of about 1.52 mL, a bulk bonded C_8 layer of about 0.12 mL, and consequently, a phase ratio (β) of 12.7.

The following notations have been used in the text: MP denotes the mobile phase; SP denotes the stationary phase; S denotes the injection solvent non-miscible with the mobile phase; A denotes the target compound; L denotes the C_8 chain bound to the silicagel surface; K is the partition constant; k is the association constant; k' is the capacity factor; K_{ow} is the octanol/water partition coefficient of an analyte; ρ is the specific density; V is used to define volumes.

Functional fitting and representation were made with Origin® vers. 7.0 (OriginLab Corporation, Northampton, MA, U.S.A.).

RESULTS AND DISCUSSIONS

Beyond the importance of large volume sample injections in analytical practice, the process is deeply involved in the theoretical treatment of the

retention process in RP-LC. Two points of view are usually taken into account when discussing the RP separation mechanism: the liquid–liquid partition and the adsorption models. The partition model relies on explanation of the influence of various parameters (pH, organic modifier nature/content, salting effects) on the retention parameters (retention time, capacity factors, peak symmetry). However, the assumption that the retention takes place mainly as an adsorption process has found agreement in many experiments, and, for instance, Guiochon and coworkers successfully developed many theoretic approaches on the topic.[12–15]

The present study, based on the two retention models, tentatively looks for an explanation of the experimental data issued from an analytical application (assay of BHA antioxydant in pharmaceutical formulations containing statines).[7] The most convenient way to explain the succes of large volume injections of analyte solutions in iso-octane (injection volumes up to 600 μL), should be the distribution of the target compound between the mobile phase and a solvent saturated stationary phase (over a precised volume within the chromatographic column). However, the possibility of transferring the entire volume of iso-octane (V_S) into the film of octyl chains covering the surface of the silicagel based material after injection results in creation of a new stationary phase, with a higher carbon content, available for the analyte *x*. In such a case, the volume of the stationary phase ($V_{SP.}$) would be increased from an initial value of $V_{initial}$ to ($V_{initial} + V_S$) and, consequently, the fundamental relationship in LC:

$$k'_X = K_X \cdot \frac{V_{SP}}{V_{MP}} \tag{1}$$

would lead to a higher retention (a higher capacity factor – k'_x), while the equilibrium constant K_x could be considered fairly constant. The stationary phase ratio V_{SP}/V_{MP} would increase with the increase of iso-octane volume loaded onto the column. Nevertheless, the experiments revealed the opposite situation, when k'_x decreases with the increase of iso-octane volume injected onto the column, as illustrated in Figure 1.

It is worthwhile to note, that the retention behavior of the target compound is not affected by the flow rate of the mobile phase (experiments achieved at flow rates of 1 and 2 mL/min., respectively, lead to slopes and intercepts of the linear regression equations within their normal variation intervals resulting from data in Figure 1). Good peak shapes are obtained up to 600 μL injection. At higher injection volumes the retention process is drastically disturbed, as the mobile phase emulsifies the sample solvent. As expected, the processes controlling interactions between injection solvent and the mobile phase are flow dependent. A flow rate of 1.5 mL/min seems the most permissive condition with respect to injection volumes. At flow rates of 1 and 2 mL/min, respectively, the highest injected volume avoiding emulsification and peak shape alteration is 500 μL.

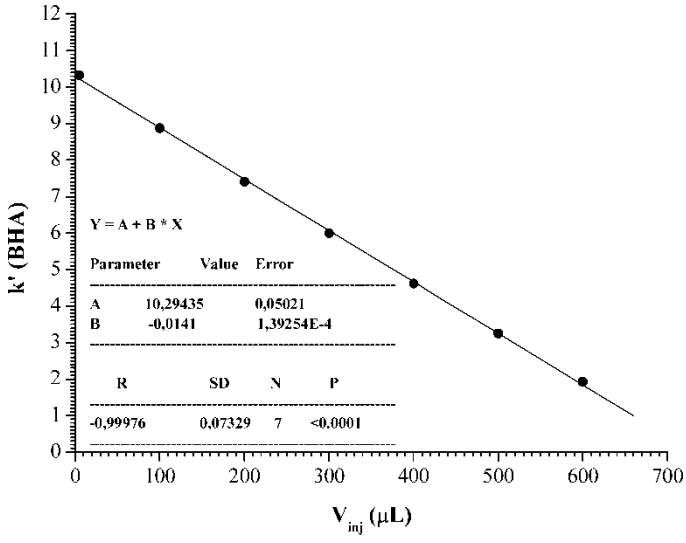

Figure 1. Reduction of the retention of BHA in the chromatographic column with the increment of the injected sample volume (sample solvent is iso-octane).

The adsorption mechanism starts from the equilibria involving interactions between the hydrocarbonaceus ligand, denoted L (octyl chain bound on the silicagel surface), the molecules of the sample solvent (S), and the target analyte (BHA, denoted by A). Two competitive equilibria characterized by the corresponding constants, describe the adsorption process, as following:

$$L + iS \rightleftarrows LS_i \tag{2}$$

$$k_1 = \frac{[LS_i]}{[L] \cdot [S]^i} \tag{3}$$

$$L + jA \rightleftarrows LA_j \tag{4}$$

$$k_2 = \frac{[LA_j]}{[L] \cdot [A]^j} \tag{5}$$

To compare the retention of S and A, given by their concentrations at equilibrium, $[LS_i]$ and $[LA_j]$, respectively, the following ratio (Φ) can be obtained from (3) and (5):

$$\Phi = \frac{[LA_j]}{[LS_i]} = \frac{k_2}{k_1} \cdot \frac{[A]^j}{[S]^i} \tag{6}$$

According to the eq. (6), the ratio Φ can be related to the separation selectivity between the two species, S and A: the more different from the unit is the ratio Φ, the more selective would be the chromatographic separation between

species A and S. The similarity between the two phases, i.e., mobile phase containing a major aqueous component and the stationary phase containing octyl chains bound on silica matrix, makes possible the assumption of proportionality by means of constants δ_1 and δ_2 between the above equilibria constants (k_1 and k_2) and the hydrophobicity descriptors given by n-octanol/water partition coefficients ($K_{o,w}$, or more conveniently, its ten base logarithm, log $K_{o,w}$):

$$k_1 = \delta_1 \cdot K_{ow(S)} \tag{7}$$

$$k_2 = \delta_2 \cdot K_{ow(A)} \tag{8}$$

Therefore, for evaluation of this ratio and its role, we must take into consideration, simultaneously, the concentration and hydrophobicity of different species taking part in the chromatographic process. Assuming comparable proportionality constants ($\delta_1 \approx \delta_2$) for relations (7) and (8), Φ becomes:

$$\Phi = \frac{K_{ow(S)}}{K_{ow(A)}} \cdot \frac{[A]^j}{[S]^i} \tag{9}$$

Obviously, for low concentration of A ([S] >> [A]) and more hydrophobic solvent S compared to the analyte A (log $K_{ow(S)}$ > log $K_{ow(A)}$, and consequently, $K_{ow(S)} >> K_{ow(A)}$), the ratio Φ tends to zero. In such a way, the analyte A has no retention as long as the solvent is not consumed by transfer onto the stationary phase. After a complete trasfer of solvent S from the mobile phase to the stationary phase, the analyte A can start its retention process.

In order to develop a mathematical model for retention in the case of two competitive adsorption equilibria, it must be taken into account that the value of [A] is much lower than the value of [S]. Therefore, it can be expected that the number of analyte molecules A interacting with one chain of octadecyl from stationary phase (j) to be 1 (Langmuir isotherm); a number higher than 1 occurs only under saturation conditions, such as for solvent. In this way, the expression of k_2 becomes simpler ($k_2 = [LA]/[L] \times [A]$) and can be easily modified in order to find out the expression of the capacity factor for the analyte A (k'_A) in dependence on several other experimental parameters.

For this purpose, the number of all octyl chains in the column packing material is denoted by N_L, and is given by the following relationship:

$$N_L = ([L] + [LA] + [LS_i]) \cdot V_{SP} \tag{10}$$

As mentioned above, [LA] << [L] + [LS_i], thus the value [L] can be related to the volume of the stationary phase ($V_{SP.}$) in the following manner:

$$[L] = \frac{N_L}{V_{SP}} - [LS_i] \tag{11}$$

$[LS_i]$ can be expressed as a function of i, the volume of S loaded to column (V_S), its density (ρ_S) and molecular weight ($M_{w(S)}$) as follows:

$$[LS_i] = \frac{[S]}{i} = \frac{V_S \cdot \rho_S}{i \cdot M_{w(S)} \cdot V_{SP}} \tag{12}$$

Finally, the expression of [L]:

$$[L] = \frac{1}{V_{SP}} \cdot \left[N_L - \frac{V_S \cdot \rho_S}{i \cdot M_{w(S)}} \right] \tag{13}$$

is introduced in the formula of K_A, where

$$K_A = \frac{[LA]}{[A]} = k'_A \cdot \frac{V_{MP}}{V_{SP}} \tag{14}$$

Thus, the capacity factor for the analyte A depends linearly on the volume of solvent injected into the column, V_S, according to the relationship:

$$k'_A = \frac{[LA]}{[A]} \cdot \frac{V_{SP}}{V_{MP}} = k_2 \cdot [L] \cdot \frac{V_{SP}}{V_{MP}} = \delta_2 \cdot K_{ow(A)} \cdot [L] \cdot \frac{V_{SP}}{V_{MP}}$$

$$k'_A = \frac{\delta_2 \cdot K_{ow(A)} \cdot N_L}{V_{MP}} - \frac{\delta_2 \cdot \rho_S \cdot K_{ow(A)}}{i \cdot V_{MP} \cdot M_{w(S)}} \cdot V_S$$

or,

$$k'_A = \alpha - \varphi \cdot V_S \tag{15}$$

In conclusion, the dependence of the capacity factor k'_A on the volume of solvent loaded to the column (V_S) should be linear, with the regression parameters α (intercept) and φ (slope), according to notations mentioned in eq. 15. If the target compound has no retention in the chromatographic column ($k'_A = 0$) that means that the solvent loaded during injection of a volume V_S of sample saturates all adsorption sites in the stationary phase ($V_S = \alpha/\varphi$).

Assuming that the number of the adsorption sites (N_L) can be calculated according to the relation:

$$N_L = \frac{V_{SP} \cdot \rho_{C8}}{M_{w(C8)}} \tag{16}$$

where ρ_{C8} and $M_{w(C8)}$ can be easily considered as the density and molecular mass of n-octane, it is possible to approximate the number i of solvent molecules saturating an adsorption site as follows:

$$i = \frac{\rho_S}{\rho_{C8}} \cdot \frac{M_{w(C8)}}{M_{w(S)}} \cdot \frac{V_S^{k'_A=0}}{V_{SP}} \tag{17}$$

From experimental data presented in Figure 1, V_S corresponding to $k'_A = 0$ is 0.73 mL, meaning that $i = 6$ (six molecules of iso-octane saturate an adsorption site).

To obtain an experimental confirmation on the model described above, the sample solvent has been switched from iso-octane to n-heptane and n-hexane, respectively. It seems logical that, due to sterical effects, the saturation number per adsorption site differs. The use of solvents having unbranched cathenes should also increase the capacity of the adsorption center. Graphic representation of the capacity factors as a function of the injected volumes leads to linear dependences characterized by the following parameters of the regressions: the intercepts (α) are 10.907 for n-hexane and 10.839 for n-heptane, the slopes (φ) are -0.0164 and -0.0159, respectively. Injection volume corresponding to $k'_A = 0$ is 0.665 mL for n-hexane and 0.681 mL for n-heptane. According to relation (17), such experimental data confirm a mean saturation number per adsorption site of 7 for n-hexane and 6.3 for n-heptane.

The refractive index detection (RID) has been used for better insight about the migration of the sample solvent within the chromatographic column, at different mobile phase compositions and injected volumes. As the absolute amount of iso-octane varies from 3.46 mg (corresponding to an injection volume of 5 μL) to 346 mg (corresponding to an injection volume of 500 μL), generating local column overloading, the profile of the elution zone is a frontal displacement rather than a Gauss shape. When retention within the column exceeded 300 minutes, data were generated by extrapolation.

The migration behavior of the iso-octane zone is given in Figure 2. Due to the specific profile, the plots are made for the retention times corresponding to the front (Figure 2A) and the end (Figure 2B) of the zone, as detected in the flow cell. It is important to note that the shape of iso-octane zone in the column is reversed compared to the detected trace. The icon in Figure 2 illustrates the profile of the eluting zone as it was detected.

Another point of interest relies on the possibility of adsorption of both analyte and solvent molecules on the same site, as follows:

$$L + (i-1)S + A \rightleftarrows LS_{i-1}A \tag{18}$$

Repetitive injections of 100 μL of BHA in iso-octane were made, at 12 minutes interval. Elution was isocratic, with 40% ACN in the mobile phase. In such conditions, one chromatographic run allows elution of the target compound, while the injection solvent front spreads slowly and accumulates within the column, injection after injection. Migration of the solvent zone should be associated to its diffusion, and consequently, a larger part of the column will be blocked by iso-octane. Experiments with multiple solvent injections in moderate portions at discrete intervals compared to a single injection of a very large volume, is illustrated in Figure 3 A and B. Mean speeds of the iso-octane zone edges can be roughly assimilated to the ratio between the column length and the retention times characterizing the front and the end of the solvent zone in the column, according to data in

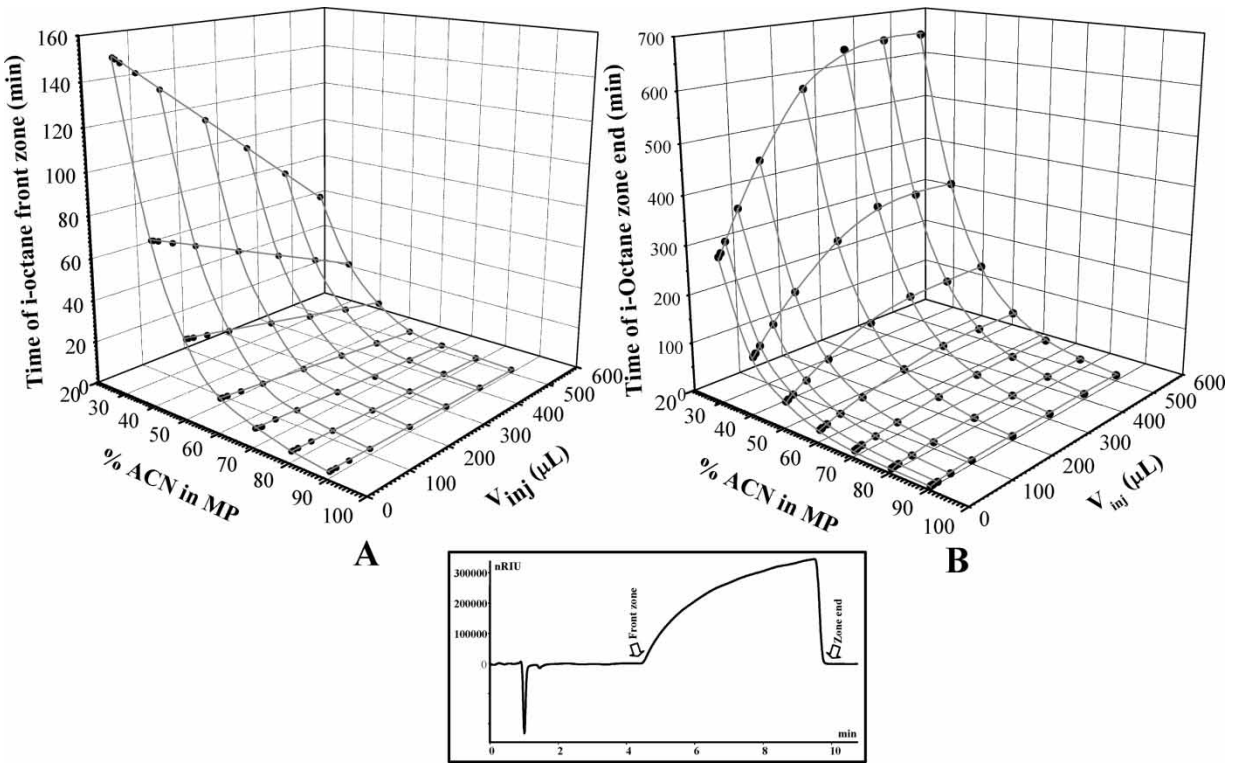

Figure 2. Retention times corresponding to the front and the end of the iso-octane zone within the chromatographic column, according to the mobile phase composition and the injected volume. Chromatogram in the icon illustrate the elution shape of the zone, as it was produced by the RID.

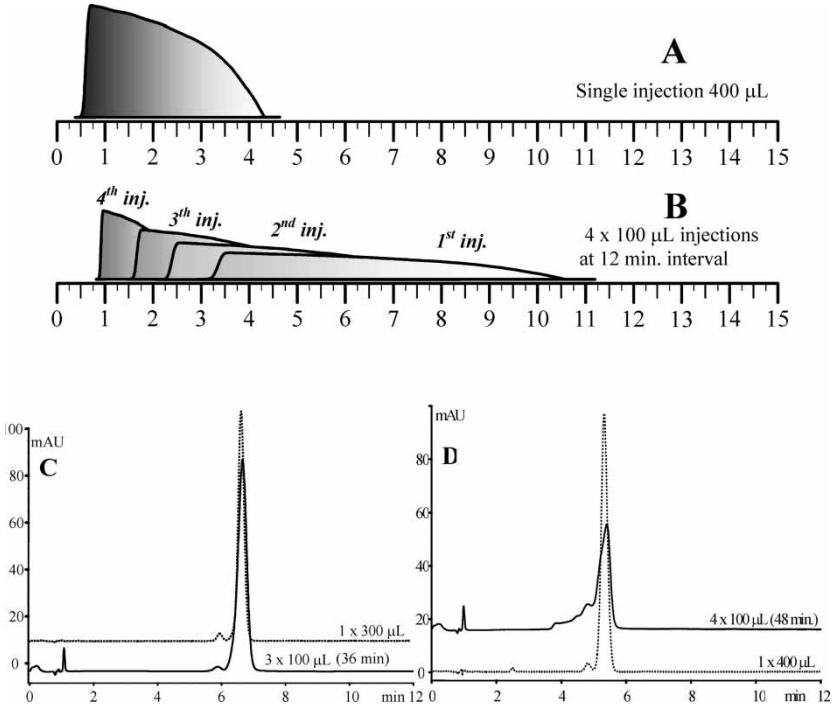

Figure 3. Graphic simulation of the solvent zone spreading process within the chromatographic column after a 400 μL injection volume (A) and four succesive injections of discrete volumes (100 μL) (B); Comparision of chromatograms resulting after a single large volume injection and multiple discrete injections repeated at precise intervals (C – total solvent volume 300 μL; D – total solvent volume 400 μL).

Figure 2, at different MP compositions and different injected volumes. The mean speed of the front zone of the solvent, in the conditions of the experiment, is about 0.22 cm/min, while the zone end moves with about 0.07 cm/min.

The decay of the retention time of the BHA peak in the successive chromatograms is observed, which perfectly corresponds to the developed model. Linear regression obtained for the plot of the capacity factor corresponding to the target compound in each consecutive chromatogram ($n = 6$) and the accumulated volume of iso-octane within the column (100 μL × no. of the injection) is characterized by a slope of -0.0143. It can be observed that the calculated value falls within the normal variation interval of the slope characterizing retention time decay as function of the injected volume (see Figure 1). One can conclude that retention of the analyte is similar at different spreading extents of the solvent zone within the column. The phenomenological meaning of the experimental results validates the occurrence of the interaction described in eq. 18.

However, the peak shape of the analyte is strongly affected after the fourth injection of 100 μL volume of solvent, as it results from chromatograms C and D in Figure 3. Emulsification effects observed by injecting a single 700 μL volume of sample does not occur after the fourth repetitive injection of 100 μL portion each and consequently, can not explain the peak distortion in this case. It is likely that the iso-octane zones overlapping in the chromatographic column after multiple injections lead to different occupation grades of the adsorption sites with the solvent molecules ($LS_{(i-q)}$, $q < i$) along column length. Thus, the following equilibrium should be dependent on q, which consequently affects the partition constant K_A of the analyte and generates the observed peak shape distortion:

$$LS_{(i-q)}A \rightleftarrows LS_{(i-q)} + A \tag{19}$$

The previous conclusion is sustained by the following experiment: large volume sample injections were achieved when doubling the volume of the stationary phase by coupling two Eclipse XDB C8 columns serially. In such conditions, emulsification of mobile phase with iso-octane was avoided, because the adsorption capacity of the stationary phase was increased, even at injected volumes up to 900 μL. However, BHA peak splitting effects were observed when injecting volumes higher than 650 μL. The explanation relates to the fact that the increase of the adsorption capacity (by increasing V_{SP}) avoids the emulsifying process of the solvent in the MP, but generates a non-homogenous coverage of the adsorption sites, allowing partition processes as given in eq. 19, which are dependent upon the "saturation degree" of the site L.

When repeating the experiment on coupled columns, with a modified composition of the MP in order to obtain almost the same retention data as on a single column (the percentage of the organic solvent was raised up to 50%), the ability of the system to sustain higher injection volumes without peak splitting effects was enhanced up to 800 μL. Increasing the percentage of the organic solvent in MP prevents the formation of the emulsion, allowing a more homogeneous coverage/saturation of the adsorption sites, and consequently inducing a constant value of K_A during the retention process.

As the physical properties of the mobile phase in relation to those characterizing the sample solvent can deeply affect experimental results, the organic component in the MP was switched from acetonitrile to methanol. The ratio between the aqueous and the organic components of MP was fixed in order to obtain comparable retention data for BHA with respect to experiments using ACN (aq. 0.1% H_3PO_4/MeOH = 1/1, v/v). Retention decay is linearly related to the injected sample volume, and the parameters of the regression equation are −0.0119 for the slope and 8.756 for the intercept. Calculation of the injection volume corresponding to $k'_A = 0$ leads

to a value of 736 μL, allowing determination of a capacity of adsorption at saturation (i) of 6. The maximum column loading with iso-octane is around 350 μL without observable BHA peak splitting effects. Such a relative reduced loading capacity (compared to mobile phases containing acetonitrile) may be explained again by the increased solubility of iso-octane in methanol, resulting in a higher zone spreading within the column, together with a less homogeneous coverage of the adsorption sites and an irreproducible K_A.

For a better understanding of the role played by the flow rate during experiments, Golay – Van Deemter curves were constructed for BHA when injecting 5 μL of a solution in ACN or 100 μL solution in iso-octane. Results are given in Figure 4.

It can be observed that injections in iso-octane are less tolerant to the increase of the speed of MP through the column (linear right parts of the curves make with the *Ox* axes angles of 15.3° and 10.6°, respectively), due to the problems raised by BHA mass transfer between phases. Over the whole interval of MP speeds, large volume injections of samples dissolved in iso-octane lead to a small/moderate loss in terms of peak efficiency.

The use of other solvents, such as chloroform, ethyl acetate, diethyl ether, as sample solvents for large volume injection processes has completely failed. Injection of large volumes of samples induced so called focusing effects, due to the low interacting capacity of these solvents with the SP. Therefore, it is expected to apply injection of large volumes of samples in non-miscible MP solvents only if they strongly interact by means of hydrophobic forces with ligands bound to silica gel surfaces.

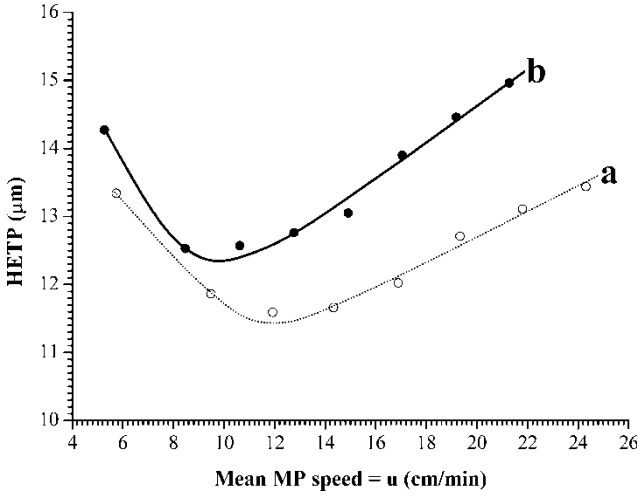

Figure 4. Golay–Van Deemter curves obtained for BHA when loading 5 μL of a sample solution in ACN (a) or 100 μL of a sample solution in iso-octane (b).

CONCLUSIONS

Injection of samples dissolved in solvents non-miscible with the mobile phase is feasible in RP-LC chromatographic separations if some conditions are fulfilled. The first condition is related to the solubility of the analyte in the organic solvent. The second condition relates to the use of a solvent strongly interacting with the hydrophobic stationary phase. Limitations arise when the stationary phase is entirely overloaded or the formation of an emulsion between the solvent and the mobile phase is observed. Sometimes, peak splitting effects appear owing to a non-homogeneous coverage of SP by solvent spreading in the column, resulting in irreproducible partition of the target compounds between phases. The adsorption mechanism explains the major phenomena experimentally observed. A simple model was developed, allowing calculation of the number of solvent molecules adsorbed on a single site. Large volume injections may induce a moderate loss in terms of separation efficiency and require optimization of the flow rate. Migration of the solvent zone within the column has to be carefully studied, as it can strongly influence the experimental results.

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