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Effects of large volume injection of aliphatic alcohols as sample diluents on the retention of low hydrophobic solutes in reversed-phase liquid chromatography

Victor David [a], Toma Galaon [a], Hassan Y. Aboul-Enein [b,*]

[a] University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, Sos. Panduri, no 90, 050663 Bucharest, Romania
[b] Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Cairo 12311, Egypt

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ABSTRACT

Recent studies showed that injection of large volume of hydrophobic solvents used as sample diluents could be applied in reversed-phase liquid chromatography (RP-LC). This study reports a systematic research focused on the influence of a series of aliphatic alcohols (from methanol to 1-octanol) on the retention process in RP-LC, when large volumes of sample are injected on the column. Several model analytes with low hydrophobic character were studied by RP-LC process, for mobile phases containing methanol or acetonitrile as organic modifiers in different proportions with aqueous component. It was found that starting with 1-butanol, the aliphatic alcohols can be used as sample solvents and they can be injected in high volumes, but they may influence the retention factor and peak shape of the dissolved solutes. The dependence of the retention factor of the studied analytes on the injection volume of these alcohols is linear, with a decrease of its value as the sample volume is increased. The retention process in case of injecting up to 200 μL of upper alcohols is dependent also on the content of the organic modifier (methanol or acetonitrile) in mobile phase.

1. Introduction

Quantitation limit reached in liquid chromatography with different detection modes is directly proportional to the sample volumes that are loaded into chromatographic column. However, large volumes injected may influence the retention process and its parameters. Therefore, it is generally recommended in analytical reversed-phase liquid chromatography that injection volumes to be situated within the range 1–25 μL in order to keep acceptable chromatographic parameters, such as efficiency, retention factor, and peak shape of the chromatographic separation [1]. Injection volume depends on column characteristics, such as internal diameter, particle size and on hydrophobicity of compounds. In preparative liquid chromatography the sample solvent and sample nature can tremendously influence the column loadability, and some technical solutions, such as at-column dilution approach or using hybrid packings improved this parameter [2,3]. In case of dissociable compounds pH of mobile phase plays also a main role, as experiments have shown that loadability can be increased by 20 times when pH assures an almost entirely dissociated form of the analyte [4,5].

In analytical chromatography, by using the mobile phase as sample solvent, it is expected that even larger injection volumes will not represent a critical parameter for the separation process [6–8]. However, the target analytes are not always soluble or they are poorly soluble in mobile phase and for injecting moderate or large volumes of sample (V_{sample} > 25 μL) it is necessary to find out a proper solvent, which has to be also suitable with the retention process. Furthermore, sample preparation procedures based on liquid-liquid extraction with additional steps of prelevation of organic layer, solvent evaporation and residue redissolution in mobile phase solvent may be time consuming and may cause significant errors [9]. Therefore, this drawback can be avoided by direct injection of samples from organic solvent if it is of hydrophobic nature [10–12]. So far, such hydrophobic solvents proved their potential for analytical purposes, among them being aliphatic or aromatic hydrocarbons [13–15], or some aliphatic alcohols [10,11]. Moreover, injection of large volume of hydrophobic solvents has been used in estimating the lipophilicity for organic compounds, using hexane as sample diluent [16]. In conclusion, this approach is potentially useful in analytical studies based on several hundred complex samples that require liquid extraction as the main sample preparation step.

Starting from these few known applications, this paper is focused on a systematic study carried out on a series of eight

* Corresponding author. Tel.: +20 1003678948; fax: +20 2 33370931.
E-mail address: haboulenein@yahoo.com (H.Y. Aboul-Enein).

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Table 1
Mobile phase composition, detection wavelength and injected amount employed for the studied solutes.

Solute	Wavelength (nm)	Aqueous/organic ratio (ACN)	Aqueous/organic ratio (MeOH)	Injected amount (ng)
Pentoxifylline	273	80/20	70/30	200
Paracetamol	260	92/8	82/18	200
Caffeine	260	92/8	82/18	200
Codeine	235	88/12	73/27	400
Aspirin	230	80/20	85/15	200
Acetylcysteine	210	90/10	90/10	1000
Methylparaben	254	50/50	50/50	100
Ethylparaben	254	50/50	50/50	100
Propylparaben	254	50/50	50/50	100
Butylparaben	254	50/50	50/50	100

aliphatic alcohols (from methanol to 1-octanol) used as sample solvent (diluent), which are injected in different volumes (within the interval 10–200 μL) under RP-LC conditions. The influence of the injection volume on the retention factor and peak efficiency of some less hydrophobic analytes, when sample solvent is changed from methanol to upper alcohols, is systematically studied for different mobile phases that allow measurable peaks for the chosen analytes. It is also the aim of this paper to find out the influence of solvent hydrophobicity on the retention of dissolved analytes for large volume injection under RP condition, using methanol or acetonitrile as organic modifiers in mobile phase composition.

2. Experimental

2.1. Reagents

Methanol, acetonitrile, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol and 1-octanol of HPLC grade were from Sigma–Aldrich (Steinheim, Germany). Phosphoric acid pro-analysis grade was from Merck (Darmstadt, Germany), while triethylamine pro-analysis grade was from Fluka (Buchs, Switzerland). Water for chromatography was obtained within the laboratory by means of a TKA Lab HP 6UV/UF instrument. Reference standards of pentoxifylline (3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione), caffeine (1,3,7-trimethyl-3,7-dihydro-1*H*-purine-2,6-dione), paracetamol (*N*-(4-hydroxyphenyl) acetamide), codeine (7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol), aspirin (2-(acetyloxy)benzoic acid) and acetylcysteine ((2R)-2-(acetylamino)-3-sulfanylpropanoic acid) were obtained from European pharmacopoeia (Strasbourg, France). Four parabens (C1–C4) was also used, which were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Instrumentation and chromatographic conditions

Experiments were performed using an Agilent 1100 series LC system (Agilent, Waldbronn, Germany) consisting of: degasser (G1379A), binary pump (G1312A), autosampler (G1313A), column thermostat (G1316A) and variable wavelength detector (G1314A). Data acquisition and analysis were performed with Agilent Chemstation software, revision B.03.02.

Chromatographic runs were carried out on a single Zorbax XDB-C18 150 mm × 4.6 mm × 5 μm column from Agilent. Column temperature was kept at 25 °C. All experiments were performed using isocratic conditions and a constant flow-rate of 1.0 mL/min. Mobile phase composition was different for each solute depending on its hydrophobicity. Acetonitrile and methanol were both used as organic modifier for the mobile phase, one at a time. Aqueous component of the mobile phase was 0.1% H_3PO_4 for all solutes excepting codeine for which a buffer made of 0.2% triethylamine and phosphoric acid at pH = 6 was used. Detection wavelength, mobile phase composition and injected amount for each studied solute are given in Table 1.

The injected sample diluents were: methanol; ethanol; 1-propanol; 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol and 1-octanol. In order to dissolve the polar solutes in these hydrophobic solvents, concentrated stock solutions in methanol were prepared, followed by large dilutions in the chosen alcohols. Methanol content in the final solutions was less or equal to 2%. Injection volume range was between 1 and 200 μL. Solute samples in hydrophobic solvents were injected at 10, 20, 50, 75, 100 and 200 μL. To highlight the changes in solute retention, peak shape, symmetry or efficiency when injection from the above mentioned hydrophobic solvents were applied, a reference injection of 1 μL in methanol of the specified solutes was performed for comparison. Regardless of the injected volume, the absolute amount of solute loaded in the chromatographic column (Table 1) was kept constant by modifying accordingly the solute concentration. Further retention studies by changing simultaneously mobile phase organic modifier content and injection volume were achieved in the case of pentoxifylline. Ranges of 20–50% ACN and 30–60% MeOH were covered when this solute was injected in 1-octanol (20 μL). Also, pentoxifylline was injected from 1-pentanol and 1-octanol (10–100 μL) by changing also mobile phase composition in the range 15–25% ACN. After each injection of hydrophobic solvent, the chromatographic column was washed for 10 min using 100% ACN or MeOH and then the column was re-equilibrated to the initial elution conditions. Retention factor was calculated by known formula ($k = (t_R - t_0)/t_0$), where the dead time t_0 was measured from negative peak observed in the chromatograms (average value of t_0 was 1.451 min, and a relative standard deviation below 1%), which was the most convenient approach in comparison with that based on using potassium nitrate or uracyl that are less soluble in the used sample diluents [17].

3. Results and discussion

3.1. Dependence of the retention factor on the injection volume

Injections of different volumes of samples (V_{inj} from 10 to 200 μL) containing as sample diluent the alcohols from series methanol to 1-octanol were performed in order to see the effect of these sample diluents on the retention of chosen analytes, under the elution conditions detailed in Section 2. Expectedly, injection of more than 10 μL of methanol, ethanol or 1-propanol as sample diluent produced the perturbation of the retention process, with chromatograms containing distorted peaks for the analytes used in this study. Injection of samples containing upper alcohols produced acceptable peak shapes for all studied compounds, but the retention factor k was influenced by the sample volume injected into the column: the value of k decreased almost linearly with the injection volume of samples containing 1-butanol, or upper alcohols as sample solvent. Some examples of the decrease of retention taking place