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Original Paper

Effect of large volume injection of hydrophobic solvents on the retention of less hydrophobic pharmaceutical solutes in RP-LC

Injection of large volumes of samples in solvents other than mobile phase composition has been proved for some less hydrophobic compounds. Thus, the retention behavior of several compounds of pharmaceutical interest (isosorbide-2-nitrate, isosorbide-5-nitrate, tropicamide, pentoxifylline, and methyl *p*-hydroxybenzoate) was studied by using different hydrophobic solvents (*n*-hexane, *n*-heptane, or *i*-octane) as sample solvents. Two types of stationary phases were used: octyl and octadecyl modified silica (both of Zorbax Eclipse type). The experiments showed a linear dependence between capacity factor of each solute and sample injection volume, up to maximum volume values of about 680 μL for C8 stationary phase and 580 μL for C18 stationary phase, when the solutes are no longer retained in stationary phase. Injection of large volumes of these hydrophobic solvents is thus possible in RP-LC with a gradual reduction of retention and peak efficiency. Two major conditions are however necessary in order to apply such an injection approach: the solutes must have a proper solubility in hydrophobic solvents and meanwhile they have to be less hydrophobic than the sample solvent in order to avoid competition with solvent molecules in partitioning between mobile and stationary phases.

Keywords: Hydrophobic solvents / Large volume injection / Pharmaceutical compounds / Reversed-phase LC

Received: May 17, 2008; revised: June 24, 2008; accepted: June 24, 2008

DOI 10.1002/jssc.200800299

1 Introduction

Enhancement of sensitivity for spectrometric detection in LC is obtained when increased sample volumes are loaded into the chromatographic column [1]. For this purpose, it is usually recommended that the sample composition is injected into analytical column to have a similar composition with mobile phase.

Large volume injection in a solvent similar to the mobile phase composition in RP-LC has been applied to the determination of chiral pesticides [2], pyrethroids in soil samples [3], simazine, terbuthylazine, atrazine, and hydroxyatrazine in soil [4, 5], or naphthalene derivative pesticides [6]. Several utilizations of this approach have been reported to the determination of bisphenol A and 4-octylphenol [7], acetylcholine esterase (ACE) inhibiting peptides [8], perfluorooctane sulfonate and perfluorooctanoic acid [9], and fenofibric acid [10] in human plasma by LC with different detection techniques. Large volume injection has systematically been studied to improve LC-MS/MS sensitivity and LODs. This method can be combined with online SPE for the analysis of compounds directly in plasma samples [11]. Large volume injection up to 500 μL was used with no negative effect on the separation profile of trace carcinogenic polycyclic aromatic hydrocarbons in microcolumn LC [12]. Enhancement of the detection sensitivity in LC by large volume injection up to 50 μL by using packed capillary columns was also reported [13]. Separation of ceramides by temperature-programmed packed capillary LC has been performed using subambient temperature-assisted large volume injection [14].

Injection of large volume of solvents nonmiscible with mobile phase and having no affinity to the stationary phase induces a strong perturbation of the retention process, resulting in focusing effects of analytes in sample solvents [1]. Such solvents are chlorinated hydrocarbons, ethers, or esters. Recently, it has been shown that samples of solvents nonmiscible with mobile phase, but having affinity to the stationary phase, can be injected in large volumes (hundreds of microliters) in RP-LC if some

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