Journal of Chromatography A, 1216 (2009) 3409–3412

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Short communication

Water-immiscible solvents as diluents in reversed-phase liquid chromatography

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ARTICLE INFO

Article history:
Received 30 October 2008
Received in revised form 30 January 2009
Accepted 3 February 2009
Available online 11 February 2009

Keywords:
Injection solvent
Band broadening
Diluent
Solvent strength
Immiscible

ABSTRACT

It has recently been shown that the use of strong organic solvents as diluent is possible in RPLC, provided that the solvent used as diluent is retained more strongly by the column than the analytes in the sample. In this study, the phenomenon was further studied experimentally using several water-immiscible solvents (ethyl acetate, isopropyl acetate, and methyl isobutyl ketone) and several model analyte compounds. In all cases, analyte peak distortion was minimal provided the analyte eluted earlier than the diluting solvent, in contrast to substantial broadening and distortion when the analyte eluted after the diluting solvent. The potential analytical utility of this approach is discussed, and an example of a practical application is also presented.

1. Introduction

Reversed-phase liquid chromatography (RPLC) is typically used to analyze the purity of active pharmaceutical ingredients (APIs) and synthetic intermediates (SIs) in the pharmaceutical industry. The purity analysis of APIs and SIs is a challenging problem, because it is often necessary to detect impurities which are smaller in chemical structure and significantly more polar than the API or SI itself. The smaller more polar impurities will frequently require a weaker, more water-rich mobile phase than the larger, less polar API or SI, an example of the so-called "general elution" problem [1]. Unfortunately, in many cases the solubility of the API or SI is very low in aqueous solution, and the solubility becomes adequate only when large amounts of the organic solvent are added. This is a serious problem, because when the injection solvent becomes stronger than the mobile phase, the impurity peaks at the beginning of the chromatogram will be broadened or distorted, making the method poorly suited for quantitative analysis [2].

Recently, a different approach to solving this problem has been explored. It is possible, when mobile phase is extremely weak (such as 100% aqueous), for the diluting solvent to elute after the analytes. This "retained-diluent" injection approach was recently demonstrated for polar analytes, using the solvents isopropanol and tetrahydrofuran as diluents [3]. The analyte peaks showed minimum band broadening whenever the injection solvent was more strongly retained by the column than the analyte. Other studies have shown that extremely hydrophobic solvents such as alkanes can also be successfully used as injection solvents in RPLC [4,5]. The alkanes were so strongly retained by the column that they eluted significantly later than all of the model analytes. It was shown that when large injection volumes were used, analyte retention factors decreased proportionately to the injection volume, but the analyte peaks were not seriously distorted or broadened. However, because the polarity of alkanes is the opposite extreme as pure water, the solubility of many APIs or SIs as well as many polar impurities will likely be very limited in pure alkane solvents, although these investigators found that adding small amounts of a co-solvent such as methanol helped solubilize APIs in the alkane.

All of these previous studies suggest that, as long as the injection solvent elutes after the analyte of interest, distortion or broadening of the analyte peak will be minimal. The present study explored this phenomenon further, testing several solvents of intermediate polarity as diluents to see if this trend was consistent, and a practical application of this approach towards trace analysis of a polar impurity in a relatively non-polar SI is also presented.

2. Experimental

Experiments were conducted using an Agilent 1100 series HPLC system with photodiode array detection (DAD; Agilent Technologies, Palo Alto, CA, USA). Data was collected and analyzed using Empower Chromatography Software (Waters, Milford, MA, USA). Solvents were all HPLC grade. Unless stated otherwise, experiments were conducted using a SymmetryShield RP18 column (Waters, 100 mm × 4.6 mm, d_p = 3.5 μm) and a gradient of 10–100% acetonitrile (MeCN) in 9 min (balance water) at a flow rate of 1 mL/min.

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doi:10.1016/j.chroma.2009.02.012

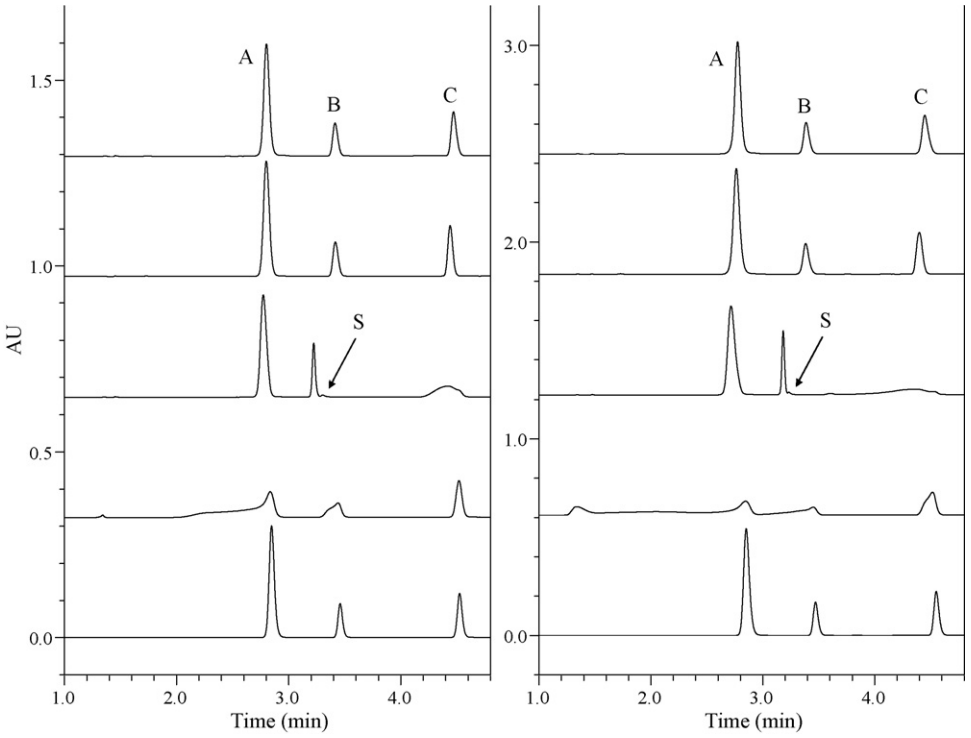

Fig. 1. Effect of different diluents on peak shapes of analytes A, B, and C. In all cases chromatography conditions are the same except for composition of diluent. Diluents are (from bottom to top) 10:90 (v/v) MeCN/water, MeCN, EtOAc, i-PrOAc, MIBK. Left panel shows 10 μL injections, right panel 20 μL injections. (S) indicates position of leading edge of diluent peak for EtOAc (~3.2 min). Leading edges of other diluent peaks were beyond the region of chromatogram shown (i-PrOAc ~ 5 min, MIBK ~ 5.3 min).

Test analytes were (A) 4-acetylaminophenol, (B) benzamide, and (C) acetanilide, dissolved at a nominal concentration of 1 mg/mL in the various diluents.

3. Results and discussion

Fig. 1 shows chromatograms obtained for a test mixture of three model analytes A, B, and C, injected under typical RPLC conditions. The only variation was the solvent used as diluent. The control sample (bottom trace) was dissolved in the initial mobile phase, and shows three sharp peaks for model analytes A, B, and C. Note that, when diluent was 100% MeCN, the peaks were significantly broadened or distorted. The broadening was worst for the earliest eluting peaks, which has been shown to be typical in gradient RPLC when the diluent is richer in organic solvent compared to the mobile phase [2]. On the other hand, when the diluent was a more strongly retained water-immiscible solvent such as isopropyl acetate (i-PrOAc) or methyl isobutyl ketone (MIBK) which eluted after analytes A, B, and C, all three analyte peaks remained relatively sharp, very similar in appearance to the chromatogram for the control sample (Fig. 1).

Ethyl acetate (EtOAc, middle trace in Fig. 1) was a unique case where the diluent peak eluted at about the same time when analyte B would normally elute. In this case, analyte A, which eluted before

Table 1
Effect of diluent on retention time (t_R), mean of two injections.

Diluent	Analyte A, t_R (min)		Analyte B, t_R (min)		Analyte C, t_R (min)	
	10 μL[a]	20 μL[a]	10 μL[a]	20 μL[a]	10 μL[a]	20 μL[a]
10% MeCN	2.85	2.85	3.46	3.47	4.53	4.55
100% MeCN	*	*	3.45	3.45	4.52	4.52
EtOAc	2.78	2.72	3.23	3.18	4.43	4.36
i-PrOAc	2.80	2.76	3.42	3.39	4.45	4.40
MIBK	2.80	2.77	3.42	3.39	4.48	4.45

[*] peak splitting.
[a] Injection volume.

Table 2
Effect of diluent on peak width. Peak width measured at 10% peak height ($PW_{0.10}$), mean of two injections.

Diluent	Analyte A, $PW_{0.10}$ (min)		Analyte B, $PW_{0.10}$ (min)		Analyte C, $PW_{0.10}$ (min)	
	10 μL[a]	20 μL[a]	10 μL[a]	20 μL[a]	10 μL[a]	20 μL[a]
10% MeCN	0.099	0.108	0.085	0.090	0.091	0.094
100% MeCN	0.756	1.661	0.197	0.500	0.109	0.177
EtOAc	0.113	0.140	0.057	0.050	0.356	1.021
i-PrOAc	0.109	0.124	0.094	0.110	0.088	0.102
MIBK	0.106	0.115	0.094	0.106	0.100	0.120

[a] Injection volume.