BIOMEDICAL CHROMATOGRAPHY, VOL. 9, 254–256 (1995)

Injection-Generated Solvent and pH Gradients for Sample Enrichment on Injection of Large Volumes in Microcolumn Liquid Chromatography

B. Streel, A. Ceccato, P. Chiap, Ph. Hubert and J. Crommen

Laboratoire d'Analyse des Médicaments, Institut de Pharmacie, Université de Liège, Rue Fusch, 5, B-4000 Liège, Belgium

INTRODUCTION

Some important advantages of the use of microcolumns in liquid chromatography are an increase in mass sensitivity, a lower consumption of mobile and stationary phases and the possibilities of reaching higher efficiency and of direct coupling to detectors such as mass spectrometers. The gain in mass sensitivity is mainly related to the lower dispersion associated with the reduction of the internal diameter of the column. The injection of large volumes of sample without causing a distortion of the analyte zone involves the formation of a spontaneous gradient by the sample itself

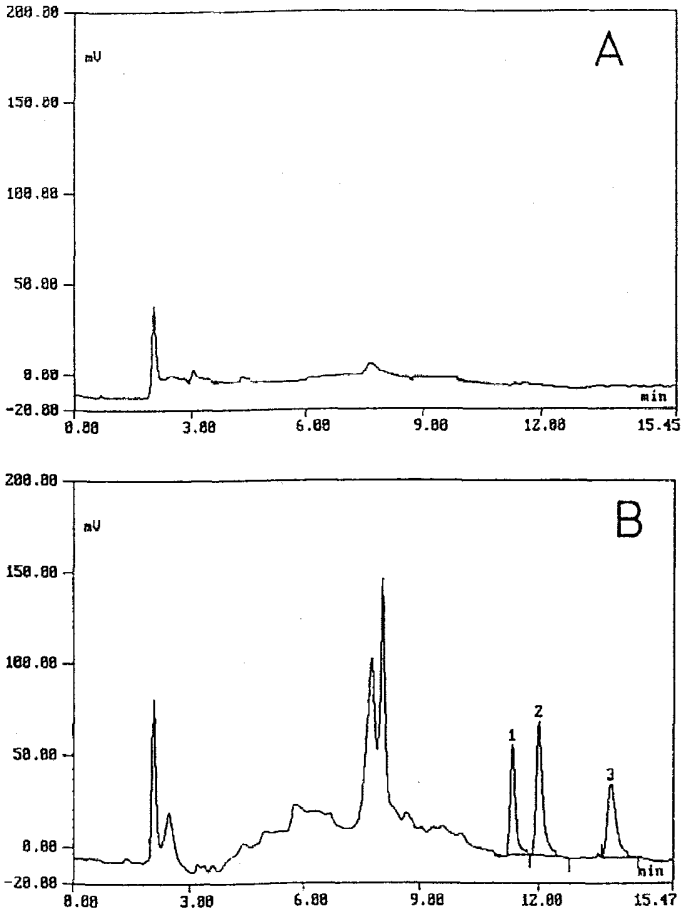

Figure 1. Effect of injection-generated solvent gradient. (A) Sample solution containing 45% of acetonitrile, (B) sample solution containing 0% acetonitrile. Column, 150 x 0.32 mm i.d., stationary phase, Hypersil C-18 (3 μm), mobile phase, acetate–citrate buffer:acetonitrile (55:45, v:v), adjusted to pH 4.0, sample loop, 20 μL; sample solution, 0.025 μg/mL of each phenothiazin (1, levomepromazine; 2, chlorpromazine; 3, thioridazine).

CCC 0269–3879/95/060254–03
© 1995 by John Wiley & Sons, Ltd.

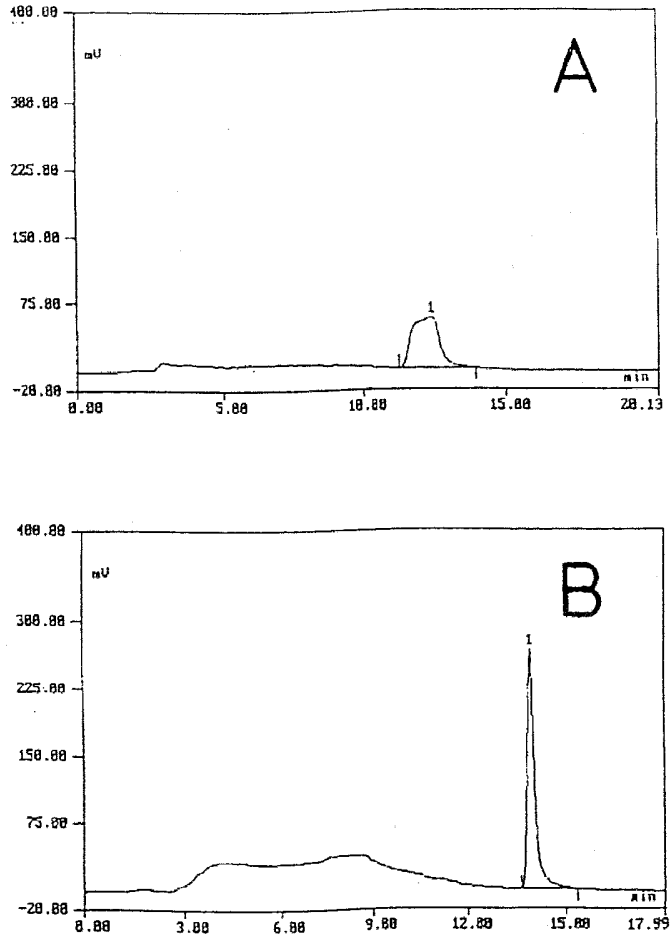

Figure 2. Effect of injection-generated pH gradient. (A) Sample solution adjusted to pH 6.0; (B) sample solution adjusted to pH 2.5. Column, 150 × 0.32 mm i.d.; stationary phase, Hypersil C-18. (3 μm); mobile phase, acetate–citrate buffer:acetonitrile (55:45, v:v), adjusted to pH 6.0; sample volume, 20 μL; sample solution, 0.25 μg/mL of indomethacin.

which gives rise to a compression of the analyte zone at the top of the column.

In this study, two means of obtaining peak compression at the top of the column were investigated: the generation of solvent gradients and of pH gradients, respectively, on sample injection.

In the case of solvent gradients, the influence of the organic modifier concentration in the sample solution and of the injection volume has been studied using three different chromatographic columns: a classical column (4.6 mm i.d.) and two microcolumns (0.80 and 0.32 mm i.d.). The HPLC mobile phase was a mixture of acetate–citrate buffer (pH 4.0) and acetonitrile (55:45 v/v), and levomepromazine, chlorpromazine and thioridazine were used as model compounds. Injection volumes were varied from 60 nL to 2 mL. The effect of the solvent gradient, for a given column, was found to increase with the injection volume and the difference in the organic modifier concentration between the injected sample solution and the HPLC mobile phase. This tendency was particularly pronounced when the diameter of the column decreased, as can be seen from chromatograms presented in Fig. 1. In both cases, 20 μL of sample were injected on the microcolumn of 0.32 mm i.d. The chromatogram of Fig. 1A was obtained with a sample dissolved in the HPLC mobile phase, whilst in Fig. 1B the sample injected did not contain acetonitrile. Moreover, the retention times of the analytes increased considerably when the internal diameter of the column was decreased.

In the study of pH gradients, a microcolumn (0.32 mm i.d.), a sample loop of 20 μL and a HPLC mobile phase (pH 6.0) were used. The pH of the sample solution was changed from 2.5 to 6.5, causing a variation of the dissociation of the acidic model compound, indomethacin. As expected, the analyte peak height increased when the pH of the sample solution was decreased, the analyte being then less dissociated and therefore more strongly retained on the C18 stationary phase. Figure 2 shows chromatograms obtained by injection of sample solutions of pH 6.0(A) and 2.5(B), respectively.

Finally, limits of detection and quantification obtained with the classical column (4.6 mm i.d.) and the two microcolumns (0.80 and 0.32 mm i.d.) were determined and compared on injection of a same sample volume (20 μL).

The limits of detection and quantification obtained

with the microcolumn (0.32 mm i.d.) at 254 nm were ca. 0.4 and 1.2 ng/mL, respectively. They were about 10 fold lower than those observed with the microcolumn (0.80 mm i.d.) and about 100 fold lower than those found with the classical column (4.6 mm i.d.).