Peak Compression Effects in Packed-Column Supercritical Fluid Chromatography

J. Thompson Strode III, Olle Gyllenhaal, Arne Torstensson, Anders Karlsson, and **Lars Karlsson***
Pharmaceutical Research and Development, Astra Hässle AB, S-431 83 Mölndal, Sweden

Abstract

Peak compression phenomena are studied and applied to the analysis of lipid emulsion samples of clevidipine. Alcohols and water are found to generate system peaks on a silica column with 2-propanol-modified carbon dioxide. The retention times of the system peaks are found to vary as a function of type (water or alcohol) and chromatographic conditions (pressure, temperature, and modifier). By selecting an appropriate system peak generator and chromatographic conditions, the peak compression effect is created for the analysis of an emulsion sample of clevidipine solution containing water, methanol, and acetonitrile. The presence of buffer or lipids in the sample does not affect the peak compression phenomena.

Introduction

Supercritical fluid chromatography (SFC) is currently being recognized as a suitable alternative to normal-phase liquid chromatography (LC) in the analysis of pharmaceutical substances and formulations (1,2). The technique generates lower amounts of organic solvent waste and offers faster column equilibration and analysis times. These features, combined with good chromatographic selectivity, provide an excellent complement to reversed-phase liquid chromatography. Packed-column SFC has been used in the analysis of antipsychotics (3), antidepressants (4), calcium channel-blocking agents (5–6), a proton pump inhibitor (7), and chiral compounds (8). A recent paper reviewed SFC applications within the pharmaceutical field (2). The detection of low amounts of degradation products, on the other hand, is sparsely reported. In our laboratories, comparative studies have shown that the signal-to-noise ratio is often lower in SFC than in comparable runs by LC (9).

In general, the analyte detectability may be increased by increasing the on-column peak concentration of the analyte. This may be accomplished by compressing the peak via peak compression phenomena, studied earlier in reversed-phase LC systems (10–12). The principle of SFC peak compression was observed when samples containing various amounts of water were injected (13,14) on a silica column. In these systems, the mobile phase was a mixture of alcohol (e.g., 2-propanol) and carbon dioxide. Initially, an equilibrium was established between the alcohol modifier and the silica support. When the sample-containing water was injected, the water molecules displaced alcohol from the stationary phase, thus creating a plug containing an excess of alcohol. When the conditions were such that this plug migrated with a speed similar to that of the analyte plug, interaction between the excess alcohol and the analyte occurred. The displaced alcohol competed efficiently with the analyte for active sites on the column. This, in turn, caused the analyte to move quickly (relative to the migration rate in the bulk mobile phase) within the plug of displaced alcohol until it reached the end of the plug. Hence, the analyte plug was compressed, and high apparent plate numbers were obtained. The principle is illustrated in Figure 1.

As shown in Figure 1A, the system peak, which contained an excess of modifier, eluted well before the analyte peak. The analyte peak was not compressed much because the modifier excess plug moved faster than the analyte through the column. The first positive peak is the displaced alcohol reaching the detector, whereas the negative peak represents the alcohol deficiency where water eluted. Maximum peak compression was obtained when the analyte moved in front of the displaced modifier plug, as illustrated in Figure 1B. Figure 1C shows conditions in which the analyte peak was well in front of the system peak. The analyte moved faster through the column than the modifier plug and was slightly compressed.

The peak compression effects in packed-column SFC were first reported in direct injection of emulsion samples (13). In a later study, Carlsson et al. found that the peak compression effect was influenced by temperature, pressure, and modifier concentration using a chemometric approach (14). The aim of the present paper is to describe how the peak compression phenomena

* Author to whom correspondence should be addressed.

are generated and controlled for different analytes. Peak compression phenomena are also applied to the analysis of clevidipine from an emulsion sample.

Experimental

Instrumentation

A Hewlett-Packard G1205A SFC instrument (Hewlett-Packard, Wilmington, DE) equipped with dual reciprocating pumps, a variable-wavelength detector, and an autosampler was used throughout this work. Flow rate, fluid composition, and column outlet pressure were independently controlled by the system software. Samples were injected using a 5-µL injection loop on a Hypersil column (200 × 4.6-mm i.d.) (Hewlett-Packard). Detection was performed at wavelengths of 254 and 210 nm.

Conditions

The temperature of the oven housing the column was set to 31°C. The column outlet pressure was constant at 200 bars, whereas the flow rate was 2.0 mL/min. Modifier concentration was varied in the range of 10 to 40% (v/v). A limited number of experiments with altered temperature and pressure and a constant modifier concentration and flow rate were also investigated. In the optimization experiments, temperature, pressure, and modifier concentration were varied in the ranges of 30–50°C, 150–250 bars, and 17–23%, respectively. A statistical experimental design approach was used to optimize the system based on previous work (14).

Chemicals

Carbon dioxide (3.5 grade, AGA, Lidingö, Sweden), delivered in a tank with a diptube, was used as the main component of the mobile phase.

The solvents (all of pro-analysis quality) used in the study were acetonitrile, 1-butanol, 2-methyl-1-propanol, 1-propanol, 2-propanol, methylene chloride, methanol, and ethanol. Ethanol was obtained from Kemetyl AB (Stockholm, Sweden), and the rest were obtained from from Merck AG (Darmstadt, Germany). Clevidipine (Figure 2) was obtained from the Department of Medicinal Chemistry (Astra Hässle AB).

Methods

The sample in Figure 1 (clevidipine, 0.2 mg/mL) was dissolved in a 30:70 mixture of 1-propanol and water. For the initial screening experiments, two types of sample solutions were prepared. In one sample solution, the analyte was dissolved in pure 2-propanol at a concentration of 0.1 mg/mL. In the other sample solution, the analyte was dissolved in a 30:70 (v/v) mixture of 2-propanol and system peak generator (i.e., water, methanol, and acetonitrile). The analyte solutions were injected separately, and the chromatographic data were calculated as the mean of duplicate injections.

Experimental designs (15) were created using Modde 3.0 software (Umetri AB, Umeå, Sweden). Multivariate analysis was performed by the partial least squares method (16). Multivariate

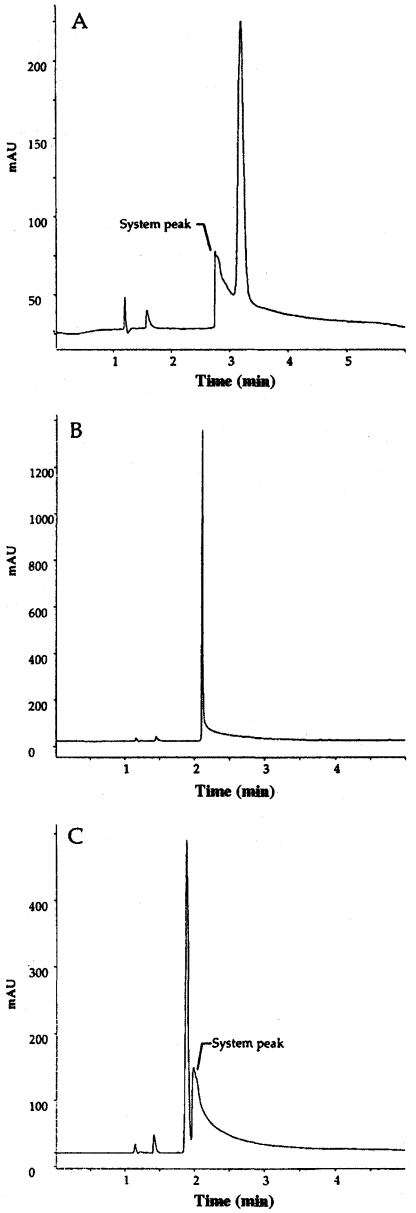

Figure 1. Injection of samples (0.2 mg/mL clevidipine in 30:70 [v/v] 1-propanol and water) at conditions with varying amounts of modifier. Pressure, 200 bar; temperature, 31°C; injection volume, 5 µL; detection, 210 nm; modifier, 2-methyl-1-propanol at (A) 15% (v/v), (B) 24% (v/v), and (C) 28% (v/v).

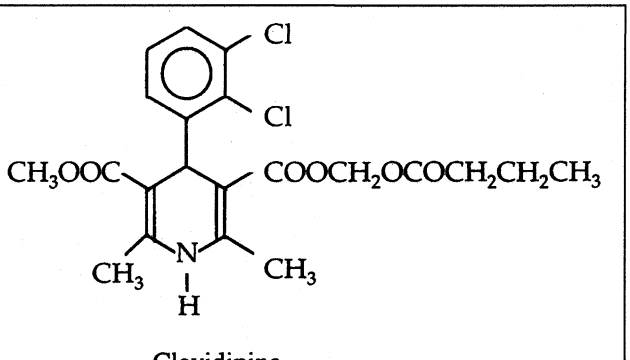

Figure 2. Structure of clevidipine.

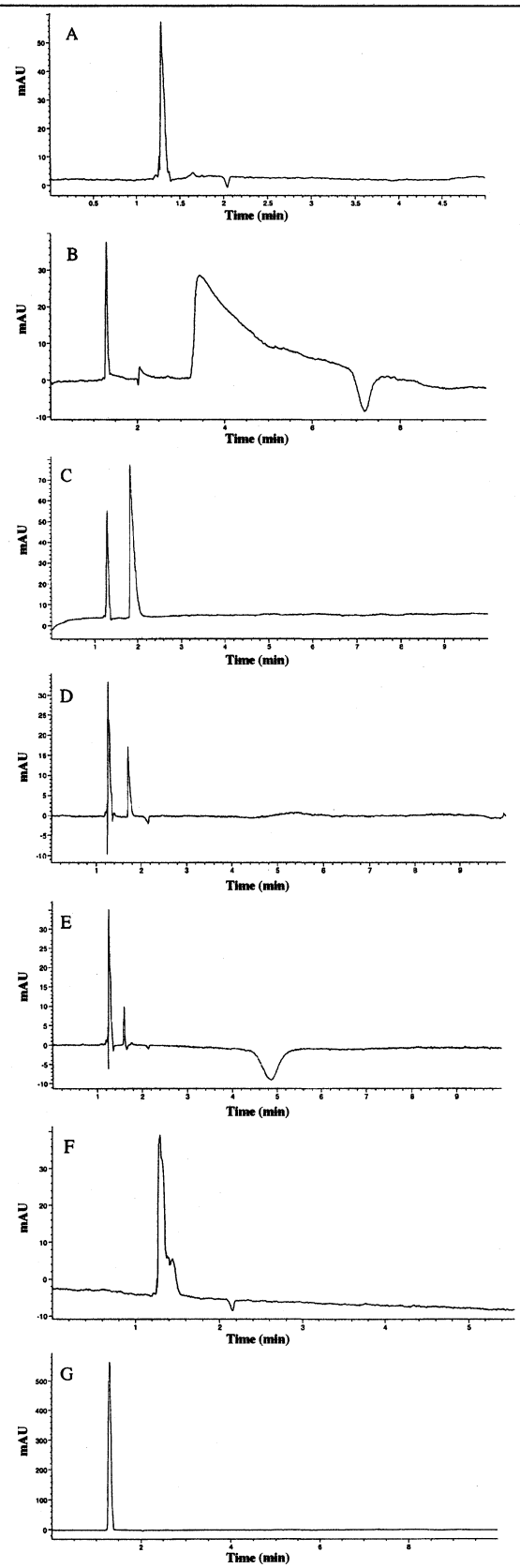

Figure 3. Injection of peak-compression-generating solutions. Conditions: pressure, 200 bar; temperature, 31°C; injection volume, 5 µL; detection, 210 nm; modifier, 20% 2-propanol-modified CO_2. (A) 100% 2-propanol; (B) 30% water–70% 2-propanol; (C) 30% methanol–70% 2-propanol; (D) 30% ethanol–70% 2-propanol; (E) 30% butanol–70% 2-propanol; (F) 30% acetonitrile–70% 2-propanol; (G) 30% methylene chloride–70% 2-propanol.

models were validated by cross-validation (17). This software was also used for modeling the chromatographic data.

The data retrieved from the chromatograms were analyte peak width and retention time. The plate numbers were calculated with a macro program created in HP SFC 2D ChemStation (Hewlett-Packard, Waldbronn, Germany). The peak width, retention time, and calculated plate number were linked to Microsoft Excel 5.0 (Microsoft Corporation, Seattle, WA) through a Dynamic Data Exchange (DDE) with another ChemStation macro program created in-house. The plate numbers were calculated according to the following equation:

$$N = 16(t_r/t_w)^2 \qquad \text{Eq 1}$$

where t_r is the retention time of the peak and t_w is the peak width.

Results and Discussion

Initial screening experiments

Previous papers have reported on the presence of the peak compression effect in packed-column SFC (13,14). The system peaks in both these reports were created by the presence of water. As described above, the water displaces the adsorbed alcohol modifier, thus generating the peaks. In this paper, other solvents were tested to see if system peaks could be generated by injecting them onto the SFC system. Throughout the screening study, 2-propanol was used as the modifier. Water, methanol, ethanol, *n*-butanol, acetonitrile, and methylene chloride were mixed with 2-propanol (30:70). These samples were injected onto the SFC system at 200 bar, 30°C, 2 mL/min, and 20% 2-propanol-modified CO_2. To ensure that no system peaks were generated by 2-propanol, 2-propanol was also injected onto the SFC system under the same conditions. System peaks were observed. Methanol, ethanol, *n*-butanol, and water created system peaks (Figure 3).

No retained system peak was observed when methylene chloride was used. Therefore, the displacement of 2-propanol modifier occurs by a specific interaction that does not occur with methylene chloride or acetonitrile. According to the theory of adsorption chromatography, alcohols and water all interact with silica through localized interactions. This interaction is most likely hydrogen-bonding between the hydroxyl group and the active silica sites (i.e., silanol groups). Because acetonitrile and methylene chloride did not generate system peaks, they were no longer studied as system peak generators.

The condition of the column was found to be important when water was used. The first injection of water on an activated silica column generated only a positive system peak (Figure 4A). This indicated that the water did not elute from the column. In subsequent injections of water and 2-propanol, both positive and negative system peaks were observed. Therefore, it can be concluded that the silica column is not a homogeneous surface. Part of the silica surface is more active than the rest of the surface. During the first injection of the water–2-propanol solution, the more active silica sites became deactivated by adsorbing the

water. During the subsequent injections, the new water only interacted with the regular silica surface (Figures 4B and 4C). Therefore, the column should be conditioned prior to use with water by injecting water samples before an analysis is performed.

The system peaks generated by the alcohols and water did not have the same retention times. Water was found to be most retained on the silica. The alcohols, on the other hand, had lower retention factors; methanol was the most retained, and butanol was the least retained. By reducing the modifier concentration, the retention times of the system peaks for the alcohols and water were increased. Therefore, retention times of the positive and negative system peaks can be controlled by altering the chromatographic conditions. Moreover, the system peaks can also be controlled by the sample solvent used.

Liquid–solid extractions with aqueous solutions are typically used to dissolve drugs and degradates from pharmaceutical formulations. One reason is to match the sample solvent to the mobile phase of the reversed-phase LC method. Because the aqueous phase can consist of some combination of water and acetonitrile, a mixture of 30% water and 70% acetonitrile was injected onto the SFC system under the conditions described previously (Figure 5). Peaks similar to those produced in the water–2-propanol system were seen. This indicated that the water was generating the system peak independently of other solvents.

Once the formation of the system peaks was understood, the peak compression of clevidipine was undertaken. This was accomplished by selecting an appropriate solvent system and optimizing the chromatographic conditions so that the positive system peak would coelute with clevidipine. Clevidipine dissolved in 2-propanol was injected on the SFC system at 200 bar, 30°C, 2 mL/min, and 20% 2-propanol-modified CO_2. Water was selected as the system peak generator because the positive peak in the water–2-propanol system had the closest retention time to clevidipine. Next, the analysis of clevidipine dissolved in 30% water–70% 2-propanol was optimized so that the clevidipine peak coeluted with the positive system, resulting in the peak compression

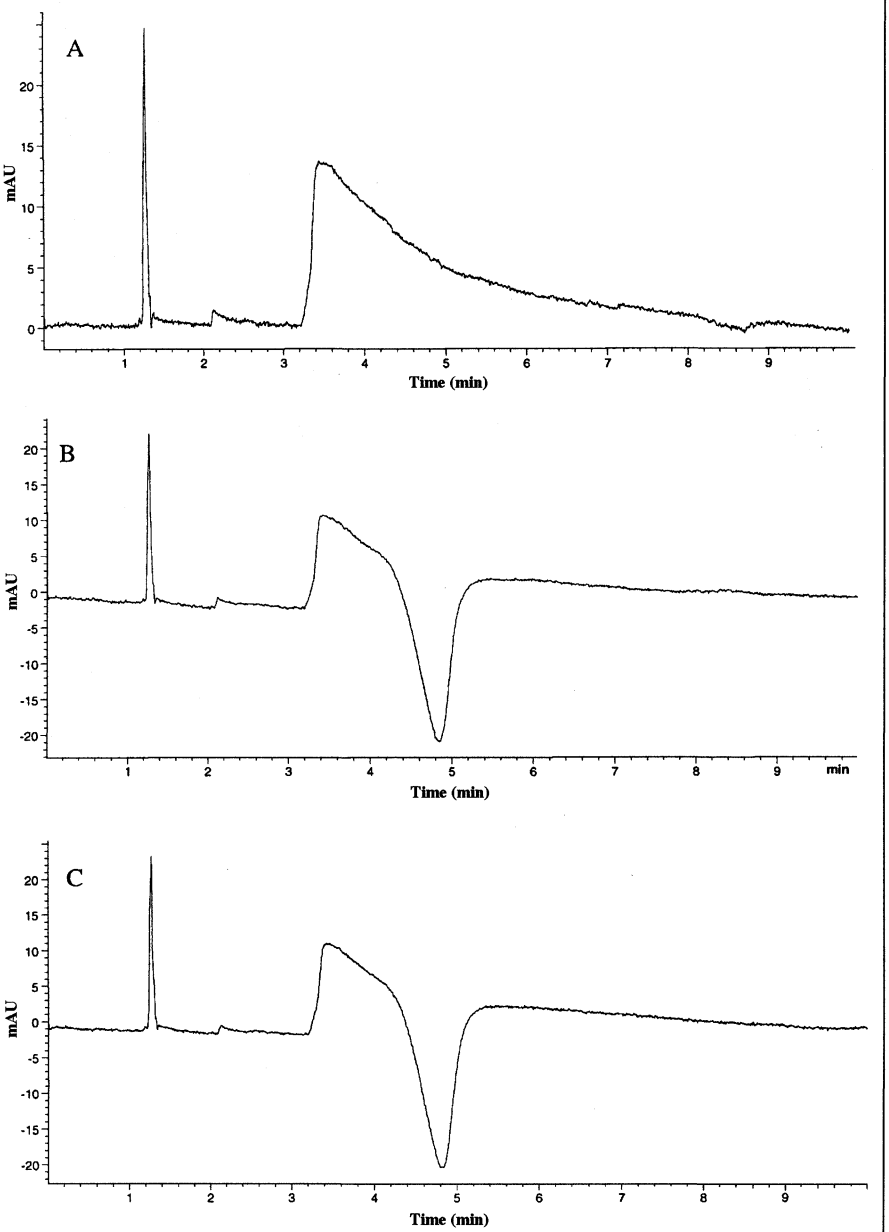

Figure 4. Replicate injections of 30% water–70% 2-propanol. Conditions are listed in Figure 3.

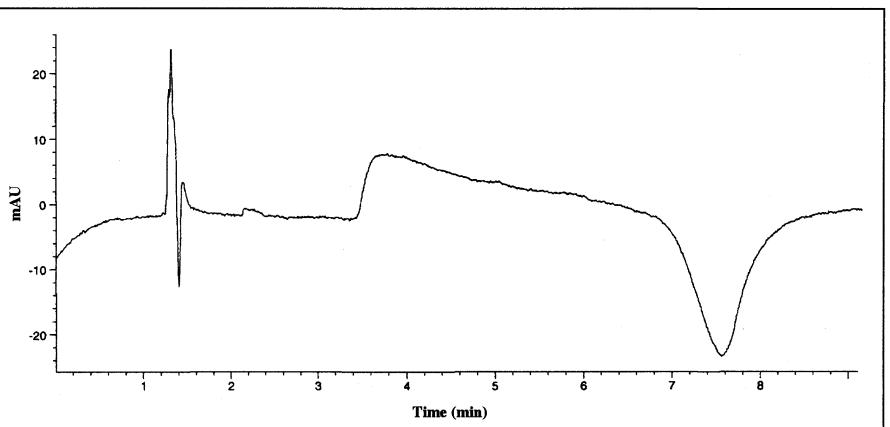

Figure 5. Injection of 30% water–70% acetonitrile. Conditions are listed in Figure 3.

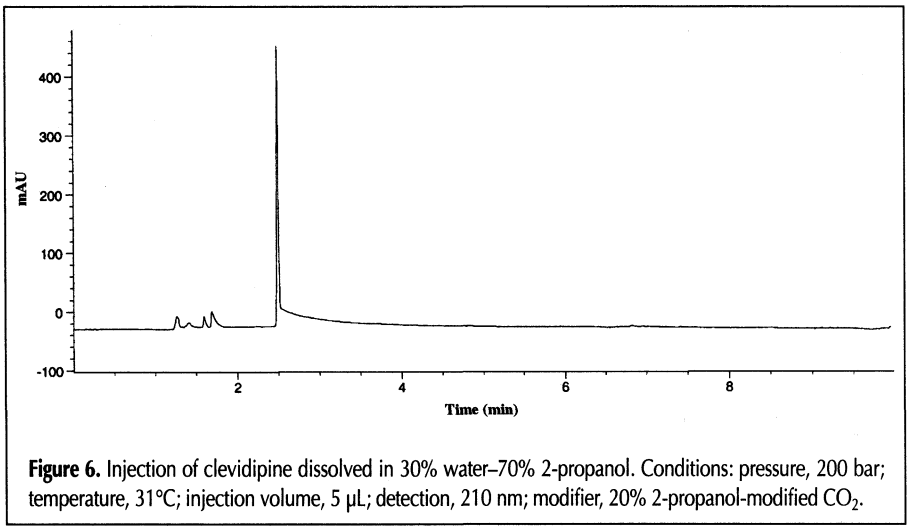

Figure 6. Injection of clevidipine dissolved in 30% water–70% 2-propanol. Conditions: pressure, 200 bar; temperature, 31°C; injection volume, 5 µL; detection, 210 nm; modifier, 20% 2-propanol-modified CO_2.

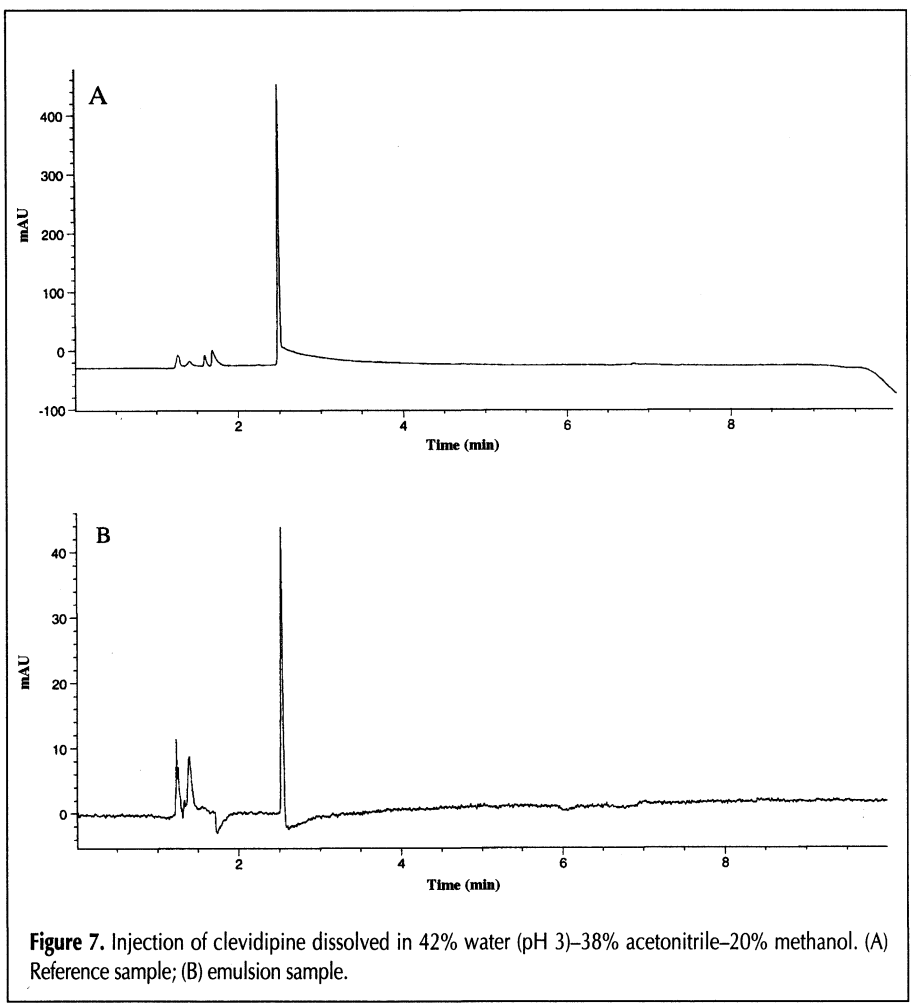

Figure 7. Injection of clevidipine dissolved in 42% water (pH 3)–38% acetonitrile–20% methanol. (A) Reference sample; (B) emulsion sample.

effect. Based on a previous report (14), the peak compression effect is controlled by the temperature, pressure, and modifier concentration of the chromatographic system. The peak compression of clevidipine was optimized using a chemometric approach that was reported previously by Carlsson et al. In this study, the peak compression of clevidipine was observed to occur at 160 bar, 30°C, 2 mL/min, and 20% 2-propanol-modified carbon dioxide (Figure 6). This compared well with the work of Carlsson et al. (14).

Peak compression of emulsion samples

The sample preparation of emulsion samples containing clevidipine used a buffered aqueous organic solution. The aqueous solution (42%) was buffered to pH 3 with a phosphate buffer; the organic solution comprised 38% acetonitrile and 20% methanol. A reference solution (0.1 mg/mL) of clevidipine was prepared in the aqueous organic solution. This standard solution was then injected onto the SFC under the conditions used in Figure 6. As shown in Figure 7A, clevidipine was compressed into a narrow band. This meant that the buffer, acetonitrile, and methanol did not affect the generation of system peaks by the water. Furthermore, the aqueous organic injection solvent is suitable not only for SFC but also for peak compression.

An emulsion sample containing clevidipine was prepared so that 0.01 mg/mL of clevidipine was dissolved in the aqueous organic solution. This sample solution was then injected onto the SFC system (Figure 7B). The clevidipine peak was compressed under the same conditions of the standard. The retention time and peak area precision were 2 and 3% relative standard deviation (RSD) (five replicates), respectively, at optimized peak compression conditions. Furthermore, this meant that the lipid fraction of the emulsion did not interfere with the peak compression phenomena. This allows the researcher to optimize a separation with a standard solution before injecting the actual sample, which may be in short supply.

Conclusion

Alcohols and water were found to generate system peaks on a silica column with 2-propanol-modified carbon dioxide. The retention times of the system peaks were found to vary as a function of type (water or alcohol) and chromatographic conditions (pressure, temperature, and modifier). By selecting an appropriate system peak generator and chromatographic conditions, the peak compression effect was created for an emulsion sample of clevidipine solution containing water, methanol, and acetonitrile. The presence of buffer or lipids was found not to affect the peak compression phenomena. By selecting other system peak generators and altering the chromatographic conditions, the degradation products of the parent compound could be compressed.

Acknowledgments

The authors wish to thank Daniel Carlsson for writing several macro programs for the calculation of peak parameters.

References

1. T.A. Berger. *Packed Column SFC, RCS Chromatography Monographs.* Roger M. Smith, Ed. Royal Society of Chemistry, Cambridge, UK, 1996.
2. A. Salvador, M.A. Jaime, G. Becerra, and M. De La Guardia. Supercritical fluid chromatography in drug analysis: A literature survey. *Fres. J. Anal. Chem.* **356:** 109–22 (1996).
3. T.A. Berger and W.H. Wilson. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics. *J. Pharm. Sci.* **83:** 281–86 (1994).
4. T.A. Berger and W.H. Wilson. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants. *J. Pharm. Sci.* **83:** 287–90 (1994).
5. J.T. Strode III, L.T. Taylor, A.L. Howard, D.P. Ip, and M.A. Brooks. Analysis of felodipine by packed column supercritical fluid chromatography with electron capture and ultraviolet absorbance detection. *J. Pharm. Biomed. Anal.* **12:** 1003–14 (1994).
6. O. Gyllenhaal and J. Vessman. Poster 68, 6th International Symposium on Analytical SFC and SFE, September 6–8, 1995, Uppsala, Sweden.
7. O. Gyllenhaal and J. Vessman. Packed-column supercritical fluid chromatography of omeprazole and related compounds. Selection of column support with triethylamine- and methanol-modified carbon dioxide as the mobile phase. *J. Chromatogr.* **628:** 275–81 (1993).
8. K. Anton, J. Eppinger, L. Frederiksen, E. Francotte, T.A. Berger, and W.H. Wilson. Chiral separations by packed column super- and subcritical fluid chromatography. *J. Chromatogr. A* **666:** 395–401 (1994).
9. O. Gyllenhaal, L. Tekenbergs-Hjelte, and J. Vessman. Poster 66, 6th International Symposium on Analytical SFC and SFE, September 6–8, 1995, Uppsala, Sweden.
10. L.B. Nilsson and D. Westerlund. Peak compression effects in ion-pair reversed-phase liquid chromatography of substituted benzamides. *Anal. Chem.* **57:** 1835–40 (1985).
11. T. Fornstedt, A. Sokolowski, and D. Westerlund. Studies on system peaks inion-pair adsorption chromatography. II. Effects on analyte peak compression and deformation. *J. Liq. Chromatogr.* **11:** 2645–84 (1988).
12. T. Fornstedt, A. Sokolowski, and D. Westerlund. Studies on system peaks inion-pair adsorption chromatography. III. Regulation of system peak gradient retention for obtaining analyte peak compression. *J. Chromatogr.* **506:** 61–74 (1990).
13. L. Karlsson, O. Gyllenhaal, A. Karlsson, and J. Gottfries. Packed-column supercritical fluid chromatography of a new dihydrypyridine drug based on direct injection of emulsion samples. *J. Chromatogr. A* **749:** 193–99 (1996).
14. D. Carlsson, J.T. Strode III, O. Gyllenhaal, A. Karlsson, and L. Karlsson. Investigation of peak compression effectsin packed column supercritical fluid chromatography using chemometrics. *Chromatographia* **44:** 289–94 (1997).
15. G.E.P. Box, W.G. Hunter, and J.S. Hunter, et al. *Statistics for Experimenters.* John Wiley & Sons, New York, NY, 1978, p. 306.
16. S. Wold, C. Albano, W.J. Dunn III, U. Edlund, K. Esbensen, P. Geladi, S. Hellberg, E. Johansson, W. Lindberg, and M. Sjöström. in *Chemometrics - Mathematics and Statistics in Chemistry.* B.R. Kowalski, Ed. Reidel, Dordrecht, The Netherlands, 1984, p. 17.
17. S. Wold. Cross-validatory estimation of the number of components in factor and principal components models. *Technometrics* **20:** 397–405 (1978).

Manuscript accepted December 11, 1997.