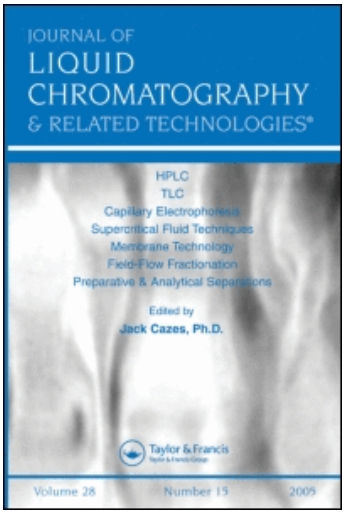

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Separation of Chlordiazepoxide and Selected Chlordiazepoxide Mixtures Using Capillary SFC

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SEPARATION OF CHLORDIAZEPOXIDE AND SELECTED CHLORDIAZEPOXIDE MIXTURES USING CAPILLARY SFC

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ABSTRACT

The analysis of chlordiazepoxide and selected chlordiazepoxide mixtures using supercritical fluid chromatography (SFC) has been investigated. The separations were carried out on four different stationary phases (SB-methyl-100, SB-biphenyl-30, SB-smectic and SB-cyanopropyl-50) with carbon dioxide as the mobile phase and flame ionization detection. The relative retention data obtained using the SFC method is compared with HPLC methods.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a analytical tool of increasing interest at the present time. After capillary columns were introduced into the marketplace, there has been an upsurge in the use of these columns with unmodified carbon dioxide for SFC (1).

There are some literature reports on the use of SFC in the analysis of pharmaceuticals (2-5). Wong and Dellafera have demonstrated the

use of capillary SFC in the therapeutic monitoring of phenobarbital in serum using a polysiloxane stationary phase with a carbon dioxide mobile phase (2). Later et al have also reported the analysis of steroids, antibiotics and cannabinoids on polysiloxane capillary columns using carbon dioxide (3). Crowther and Henion demonstrated the SFC-mass spectrometric analysis of codeine, caffeine, cocaine, phenylbutazone and methocarbamol using packed silica and amino columns and methanol modified carbon dioxide (4). Barbiturates were analyzed by Smith and Sangi using either an octadecylsilane or a polymer based polystyrene-divinylbenzene column with methanol modified carbon dioxide (5). In our laboratory, we have applied SFC to the separation of non-steroidal anti-inflammatory agents and estrogens (6,7). The compounds were separated on a cyanopropyl capillary column using carbon dioxide and flame ionization detection (FID).

In a continuation of our studies, there was an interest in the SFC separation of chlordiazepoxide and selected chlordiazepoxide mixtures. The use of SFC for the analysis of chlordiazepoxide has been mentioned by Smith and Sangi (8). The initial chromatography of chlordiazepoxide using carbon dioxide and a packed polystyrene-divinylbenzene column was unsuccessful. However, the same authors showed that chlordiazepoxide could be chromatographed in 18 min using carbon dioxide containing 4.3% methanol. They further indicated that the use of a methanol modifier in the carbon dioxide posed three problems: (a),

it was very difficult to reproducibly mix methanol with carbon dioxide in exact proportions; (b), the ratio of methanol to carbon dioxide changed over time; and (c), the presence of methanol in the carbon dioxide precluded the use of FID.

In this paper, the separation of chlordiazepoxide and selected chlordiazepoxide mixtures using capillary SFC with carbon dioxide as mobile phase is presented. The drugs mixed with chlordiazepoxide in this study included clidinium bromide, amitriptyline hydrochloride and the estrogens, estrone, equilin, d-equilenin, α-estradiol and β-estradiol.

EXPERIMENTAL

Reagents and chemicals

HPLC grade methylene chloride and methanol were purchased from J.T. Baker (Phillipsburg, NJ). Supercritical fluid chromatography grade carbon dioxide was obtained from Scott Specialty Gases (Plumsteadville, PA). Chlordiazepoxide, clidinium bromide and amitriptyline hydrochloride were gifts from Roche Laboratories (Nutley, NJ). Estrone, equilin, α-estradiol, β-estradiol and d-equilenin were purchased from Sigma Chemical Company (St. Louis, MO). The structural formulae of the compounds are shown in Fig. 1

Instrumentation

Chromatography was performed on a Lee Scientific Model 600D Supercritical Fluid Chromatograph (Salt Lake City, UT) equipped with a

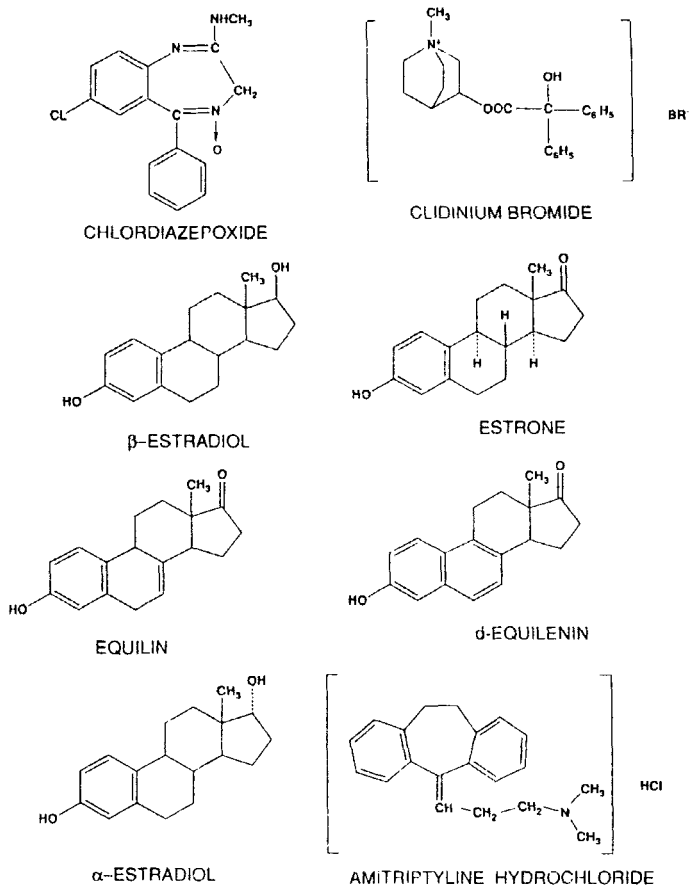

Figure 1 - Structural formulae of compounds studied

pump, oven and flame-ionization detector and controlled by a Dell computer (ACI 600D, software version 2.2). SFC was performed using four stationary phases: 5m x 100 μm i.d. SB-methyl-100 (200 μm o.d. and 0.25 μm film thickness); 10 m x 50 μm i.d. SB-biphenyl-30 and a 7 m x 50 μm i.d. SB-cyanopropyl-50 (both 195 μm o.d. and 0.25 μm

film thickness); and a 10m x 50m i.d. SB-smectic (195 μm o.d. and 0.15 μm film thickness). All four columns were purchased from Lee Scientific.

Preparation of Standard Solutions

Standard solutions of each analyte were prepared by accurately weighing 5 mg of each drug and dissolving in 5 ml of absolute methanol or methylene chloride to give a final concentration of approximately 1 mg/ml.

Chromatographic Parameters

Injection type: Time split set at 200 msec.

Detector: Flame ionization at 375°C.

Mobile Phase: Supercritical fluid chromatography grade carbon dioxide.

a. Chlordiazepoxide

Pump program - Multilinear pressure program: 8 min hold at an initial pressure of 100 atm; then 25 atm/min ramp to 400 atm.

Oven program - Isothermal at 130°C.

Column: 7 m x 50 μm SB-cyanopropyl-50

b. Chlordiazepoxide and Clidinium Bromide Mixture.

Pump program- Multilinear pressure program;

6 min hold at the initial pressure of 100

atm/min. 25 atm/min ramp to 250 atm. 4 atm/min ramp to 350 atm.

Oven program-isothermal at 100°C.

Column - 10 m x 50 μm SB-biphenyl-30.

c. Chlordiazepoxide and Amitriptyline Hydrochloride Mixture.

The program as described above for the chlordiazepoxide - clidinium bromide was used, except that the column was a 7 m x 50 μm SB-cyanopropyl-50.

d. Chlordiazepoxide and estrogens mixture.

Pump Program: Multilinear density program; 6 min hold at an initial density of 0.3000 g/ml, then 0.0064 g/ml/min ramp to 0.5000. Hold at 0.5000 g/ml for 90 min.

Oven Program - Hold at initial temperature of 50°C for 8.5 min, then 1°C/min ramp to 136°C.

Column: 10 m x 50 μm SB-cyanopropyl-50.

RESULTS AND DISCUSSION

Chlordiazepoxide, a 1,4 benzodiazepine derivative, is widely used therapeutically because of its muscle relaxant, taming, sedative, anti-anxiety and anticonvulsant properties (9-11). It is found in combination with clidinium bromide, amitriptyline hydrochloride and estrogens in pharmaceutical dosage forms. The goal of this study was to investigate the use of capillary SFC in the separation of chlordiazepoxide and selected chlordiazepoxide mixtures.

Initially, the SFC separation of chlordiazepoxide was studied. Four different capillary columns, SB-methyl-100, SB-biphenyl-30, SB-smectic and SB-cyanopropyl-50 were compared. The SB-methyl-100 column is crosslinked and is coated with 100% methylpolysiloxane. Various temperature and pressure gradients were investigated on the column, but the separation of chlordiazepoxide was not considered to be successful due to frontal tailing (tailing factor of 0.41).

The SB-biphenyl-30 column is coated with 30% biphenyl and 70% methylpolysiloxane and is crosslinked for SFC use. Several pressure gradients (25 atm/min to 3 atm/min) and oven temperatures (70 to 140°C) were investigated. The peak shape of chlordiazepoxide was better on this column compared to the SB-methyl-100 column (tailing factors of 0.63 vs 0.41), but it still was a fronted peak.

The SB-smectic is a liquid crystalline polysiloxane column. The selectivity mechanism of this column involves analyte size and shape, where analytes are separated on the basis of molecular geometry, with the length to breadth ratio of each compound determining the elution order (12). Different pressure gradients and temperatures were investigated, but chlordiazepoxide also gave a fronted peak on this column (tailing factor of 0.54).

The SB-cyanopropyl-50 column is coated with 50% cyanopropyl and 50% methylpolysiloxane and is the most polar of the commercially available SFC columns. Various density gradients and oven temperatures

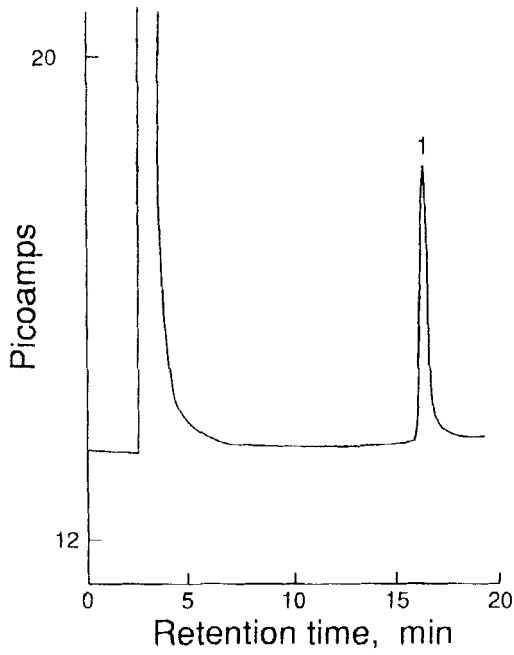

Figure 2 - Typical SFC chromatogram of chlordiazepoxide (1) on a SB-cyanopropyl-50 column.

were studied. It was found that the density gradient described for chlordiazepoxide (see Experimental Section) gave the best peak shape (tailing factor of 1.29) of any of the columns investigated. The retention time for chlordiazepoxide was approximately 14.5 min (see Fig. 2)

Next the separation of a chlordiazepoxide and clidinium bromide mixture was investigated on the four SFC columns. Different pressure gradients (2 atm/min to 50 atm/min) and oven temperatures (70 to 140°C) were studied. It was observed that the separation of both

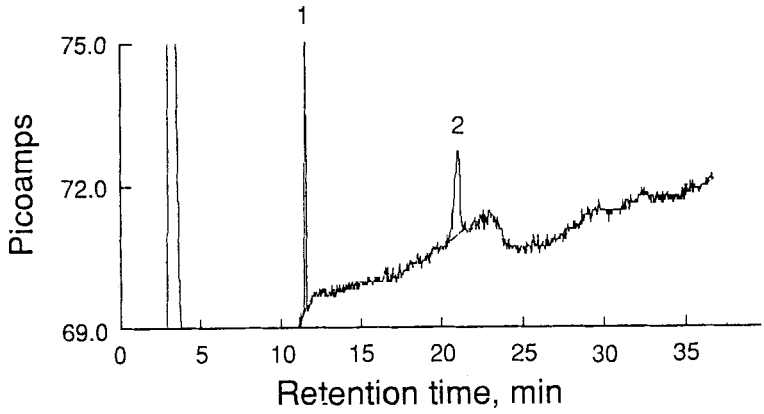

Figure 3 - Typical SFC separation of clidinium bromide (1) and chlordiazepoxide (2) on a SB-biphenyl-30 column.

compounds on the SB-methyl-100, SB-smectic and SB-cyanopropyl-50 stationary phases was not possible. However, the biphenyl column did provide a good separation of the two analytes when a pressure gradient (see chlordiazepoxide and clidinium bromide mixture, Experimental Section) was utilized. The retention times for clidinium bromide and chlordiazepoxide were approximately 11.5 and 21.0 min, respectively, as shown in Fig-3. The FID detector response had to be greatly magnified in order to show the chlordiazepoxide peak (1 mg/ml). The chromatogram was further complicated by the fact that both analytes eluted while a steep pressure gradient was being programmed.

A chlordiazepoxide and amitriptyline hydrochloride mixture was next to be investigated on the different stationary phases. Pressure

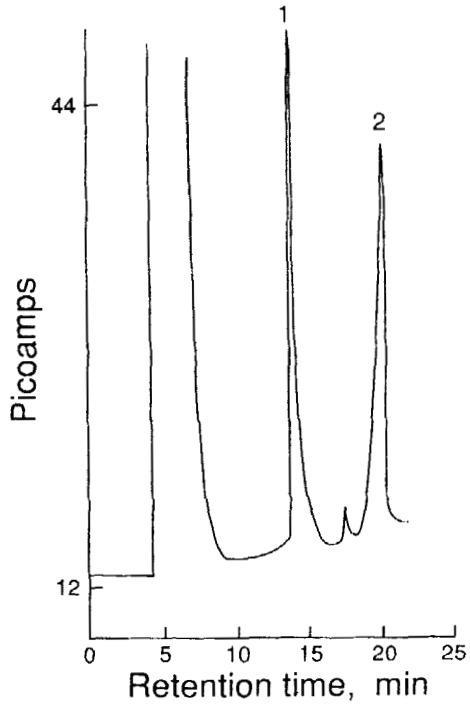

Figure 4 - Typical SFC separation of chlordiazepoxide (1) and amitriptyline hydrochloride (2) on a SB-cyanopropyl-50 column.

gradients of 1 atm/min to 25 atm/min and oven temperatures of 70 to 140° C were studied. It was observed that, under all of the conditions studied, chlordiazepoxide and amitriptyline hydrochloride coeluted on the SB-methyl, SB-biphenyl and SB-smectic columns. Using the pressure gradient described for the mixture in the Experimental Section, these compounds were separated on a SB-cyanopropyl-50 column in 21 min.

The retention times for chlordiazepoxide and amitriptyline were approximately 13.8 and 20.3 min, respectively (see Fig-4).

Finally, the separation of a chlordiazepoxide and estrogens mixture (estrone, equilin, d-equilenin, α-estradiol and β-estradiol) was investigated on the four different stationary phases. Several pressure gradients (2 to 50 atm/min) and oven temperatures (70 to 140°C) were studied on the SB-methyl-100 column. The analytes coeluted under all the conditions used. After using various pressure gradients on the SB-biphenyl-30 column, only d-equilenin and chlordiazepoxide could be separated using a pressure gradient (initial pressure at 100 atm, 20 atm/min ramp to 350 atm, hold at 350 atm for 15 min) and an oven temperature of 120°C. The retention times were approximately 24.0 and 26.8 min for chlordiazepoxide and d-equilenin, respectively. The remaining estrogens coeluted at 21.0 min.

On the smectic column, it was not possible to separate chlordiazepoxide and estrone even though separation of the other estrogens was achieved. A density gradient (initial density at 0.3500g/ml, 0.0060 g/ml/min ramp to 0.5700 g/ml) and an isothermal oven temperature of 130°C were used. The retention times were approximately 25.0, 25.7, 30.3, 30.6, 33.0 and 34.1 minutes for α-estradiol, β-estradiol, estrone, chlordiazepoxide, equilin and d-equilenin, respectively.

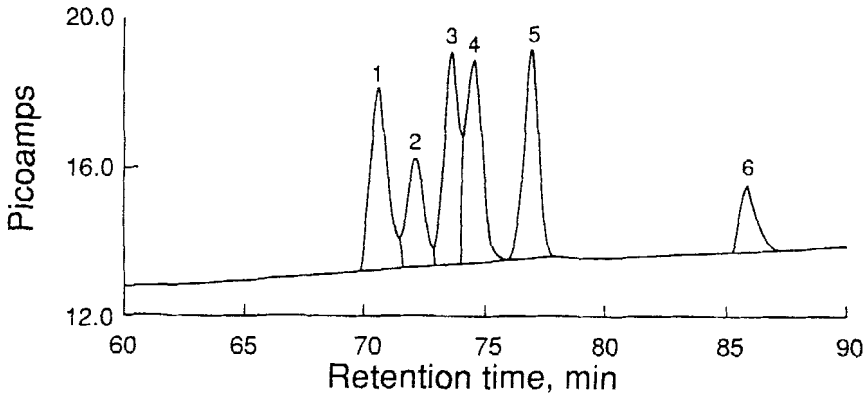

Figure 5 - Typical SFC separation of chlordiazepoxide (1), estrone (2), α-estradiol (3) β-estradiol (4), equilin (5), and d-equilenin (6), on a SB-cyanopropyl-50 column.

Several oven temperatures (40 to 140°C) and pressure or density gradients were investigated for the chlordiazepoxide-estrogens mixture on the SB-cyanopropyl-50 column. Separation of all the analytes was achieved using an oven temperature of 70°C with a density gradient (initial density of 0.5000 g/ml, 0.0064 gm/ml/min ramp to 0.7500 g/ml., hold at 0.7500 g/ml for 15 min). The chlordiazepoxide appeared as a shoulder on the front of the estrone peak. The retention times were approximately 28.3, 29.4, 31.1, 34.0, 35.2 and 37.2 min for chlordiazepoxide, estrone, equilin, α-estradiol, β-estradiol and d-equilenin, respectively.

It was then decided to program a temperature gradient along with the density gradient for the chlordiazepoxide-estrogens mixture. This

Table 1

Comparison of SFC and HPLC Relative Retention Data of Chlordiazepoxide Mixtures

Mixture		Relative Retention	
		SFC[a]	HPLC
1.	Clidinium Bromide	0.55	0.60[b]
	Chlordiazepoxide	1.00	1.00
2.	Chlordiazepoxide	0.68	0.71
	Amitriptyline hydrochloride	1.00	1.00
3.	Chlordiazepoxide	0.82	----[c]
	Estrone	0.83	0.85[d]
	α-Estradiol	0.86	1.00
	β-Estradiol	0.87	----[c]
	Equilin	0.90	0.75
	d-Equilenin	1.00	0.69

[a] See chromatographic parameters, Experimental Section.
[b] UPS XXII method, see reference 11.
[c] No data was reported.
[d] Mobile phase for estrogens was 44:55 pH 2.5 phosphate buffer containing 0.1% TEA/absolute methanol; octadecylsilane column (3.9 mm x 30 cm), flow rate, 1.2 ml/min; electrochemical detection at +600 mV versus Ag/AgCl, see reference 12.

dual programmed gradient mehtod (see Experimental Section) allowed the separation of all six compounds (Fig-5). The retention times for chlordiazepoxide, estrone, α-estradiol, β-estradiol, equilin and d-equilenin were approximately 70.5, 72.0, 73.5, 74.5, 76.9 and 85.9 min, respectively. Although α and β-estradiol were not baseline separated

under the experimental conditions used, the resolution of the two peaks is adequate for integration (R_s = 0.8).

The relative retention data of the selected chlordiazepoxide mixtures assayed by our SFC methods was compared with literature HPLC methods (see Table 1). It is interesting to note that the relative retention data obtained by SFC was comparable to that obtained using HPLC for all of the mixtures studied.

In summary, SFC has been shown to be amenable to the separation of chlordiazepoxide and chlordiazepoxide mixtures containing clidinium bromide, amitriptyline hydrochloride and estrogens. This study shows that SFC can be a complimentary technique to HPLC in the assay of these drugs.

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