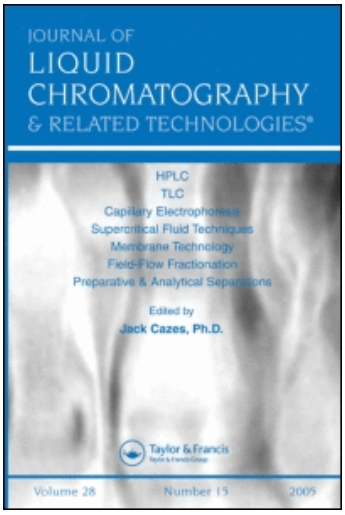

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Analysis of Diazepam and Chlordiazepoxide and Their Related Compounds Using Supercritical Fluid Chromatography

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ANALYSIS OF DIAZEPAM AND CHLORDIAZEPOXIDE AND THEIR RELATED COMPOUNDS USING SUPERCRITICAL FLUID CHROMATOGRAPHY

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ABSTRACT

The analysis of diazepam, chlordiazepoxide and their by- and degradation products using supercritical fluid chromatography (SFC) was investigated. The separations were carried out using a capillary SB-cyanopropyl-50 column with carbon dioxide as mobile phase and flame ionization detection. Typical analysis time was in the range of 20-23 min. Accuracy and precision of the SFC method were both in the 1-4% range. The SFC method was then applied to dosage forms containing diazepam and chlordiazepoxide. The data obtained using the SFC methods was compared with HPLC methods.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a complementary technique to high performance liquid chromatography (HPLC) and gas chromatography (GC). The advantages of SFC include the possibility of

analysis of thermally labile compounds and the use of both HPLC and GC type detectors including UV-Vis and flame-ionization (1). Commercial instruments for capillary and packed column SFC are available.

There have been reports of the use of SFC in the analysis of pharmaceuticals (2-7). Wong and Dellafera (2) demonstrated the use of capillary SFC in therapeutic drug monitoring of phenobarbital in serum using a polymethylsiloxane stationary phase and a carbon dioxide mobile phase. Later et al (3) have reported the analysis of steroids, antibiotics and cannabinoids on polymethylsiloxane capillary columns using a carbon dioxide mobile phase. Crowther and Henion (4) demonstrated the SFC-mass spectrometric analysis of codeine, caffeine, cocaine, phenylbutazone and methocarbamol by using packed amino and silica columns and a modified direct liquid-introduction interface. The mobile phase was carbon dioxide modified with methanol. Smith and Sangi (5) have reported the SFC analysis of barbiturates using a packed column containing a polystyrene-divinylbenzene or octadecylsilane stationary phase with methanol modified carbon dioxide as mobile phase. Perkin et al (6) have reported the analysis of veterinary antibiotics (levamisol, furazolidone, chloramphenicol and lincomycin) on a packed column containing an aminopropyl stationary phase also utilizing carbon dioxide with modifier.

There is no report in the scientific literature on the separation of diazepam and chlordiazepoxide from their by- and degradation products

using SFC. The USP XXII/NF XVII TLC limit tests for the related compounds of diazepam and chlordiazepoxide is based on visual detection of the spots by short wavelength UV light (7). The USP/NF monograph also specifies a separate assay for the drug. The use of SFC in the analysis of a diazepam and chlordiazepoxide mixture is limited to a report by Smith and Sangi in which they have analysed these two drugs using methanol modified carbon dioxide and a packed polystyrene-divinylbenzene column (8). The two compounds could not be eluted from the column using only carbon dioxide.

In this paper, the use of SFC in the separation of diazepam and its related products, nordiazepam, 3-amino-6-chloro-1-methyl-4-phenyl-carbostyril (ACMPC) and 2-methylamino-5-chlorobenzophenone (MCAB) is presented. The separation of chlordiazepoxide and its related products, namely 7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one-4-oxide (demoxepam) and 2-amino-5-chlorobenzophenone (ACB) is also presented. The structural formulae of the compounds studied are shown in Fig 1. The separations were achieved using a SB-cyanopropyl-50 capillary column and a FID detector with a carbon dioxide pressure gradient. The typical analysis time for each drug mixture was 20-23 min.

EXPERIMENTAL

Reagents and chemicals: HPLC grade absolute methanol and methylene chloride were purchased from J.T. Baker (Phillipsburg, NJ). Puradisc

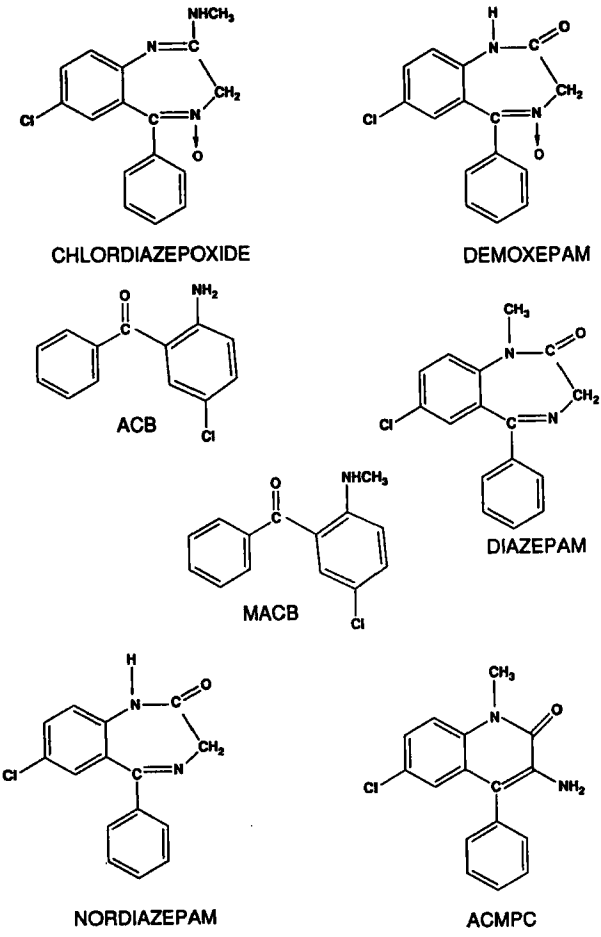

Figure 1 - Structural Formulae of Compounds Studied.

25TF, 0.45 μm filters were obtained from Whatman (Maidstone, U.K.). Supercritical grade carbon dioxide was obtained from Scott Speciality gases (Plumsteadville, PA).

Diazepam, nordiazepam, ACMPC, MCAB, chlordiazepoxide, demoxepam and ACB were gifts from Hoffmann-La Roche (Nutley, NJ).

Commercial dosage forms of Valium[R] and Librium[R] were obtained from a local pharmacy.

Instrumentation: Chromatography was performed on a Lee Scientific Model 600D supercritical fluid chromatograph (Salt Lake City, UT) equipped with a pump, oven and flame-ionization detector and controlled by a Dell computer (ACI 600D, software version 2.2). SFC was performed on a 7m SB-cyanopropyl-50 column (195 μm od and 0.25 μm film thickness) purchased from Lee Scientific.

Preparation of standard solutions: Standard solutions of each compound was 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 conditions:

Column: 7m x 50 μm SB-cyanopropyl-50 (195 μm o.d. and 0.25 μm film thickness).

Injection type: Time split set at 200 msec; rotor size of 200nl.

Detector: Flame ionization at 375°C.

Mobile phase: Supercritical fluid grade carbon dioxide

Analysis time: 20-23 min.

Diazepam and related compounds:

Pump program: Multilinear pressure program: 1 min hold at an initial pressure of 120 atm., then 20 atm/min ramp to 190 atm., followed by a 1.0 atm/min ramp to 205 atm, then a 30 atm/min

ramp to 300 atm., followed by a hold of 3 minutes.

Oven Program: Hold at initial temperature of 150°C for 6 min. Ramp at 25°C/min to 200°C, hold at 200°C for 17 min.

Chlordiazepoxide and related compounds:

Pump program: Multilinear pressure program: 9 min hold at initial pressure of 200 atm., then 50 atm/min ramp to 300, followed by 20 atm/min ramp to 400 atm., then hold for 5 min at 400 atm.

Oven program: Isothermal at 120°C.

RESULTS AND DISCUSSION

The goal of this study was to investigate the applicability of SFC in the separation of diazepam, chlordiazepoxide and their by- and degradation products. The SB-cyanopropyl-50 column was initially chosen to investigate the separation of these mixtures based on our previous success with the column in the separation of non-steroidal anti-inflammatory agents and estrogens (8,9).

The first mixture to be studied was diazepam and its related compounds, nordiazepam, ACMPC and MCAB. Several different pressure gradients (3-50 atm/min) and oven temperatures (50-160°C) were investigated for the separation. It was observed that lowering the temperature did not aid in separation. The best separation at an isothermal oven temperature was obtained at 160°C using a pressure gradient (Hold at 100 atm for 9 min, ramp at 5 atm/min to 200, followed by a 2 atm/min ramp to 250 atm). Under these conditions, nordiazepam

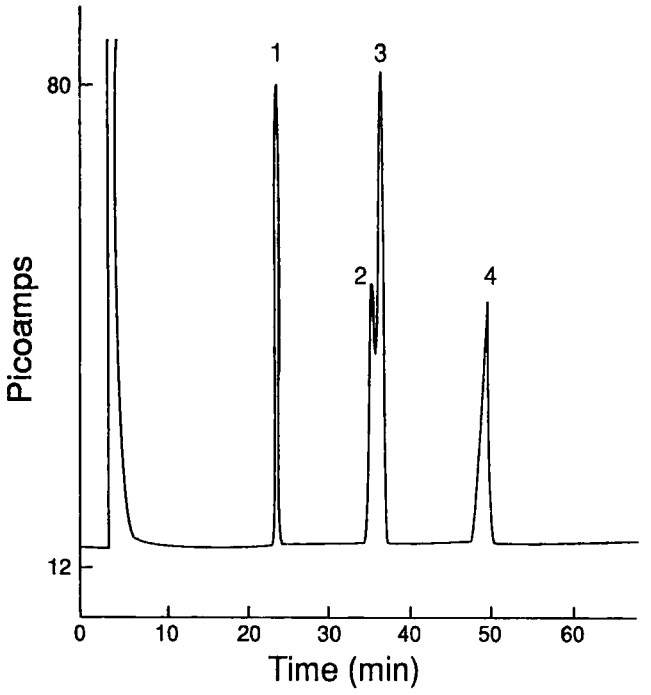

Figure 2 - Typical SFC Separation of MCAB(1), ACMPC(2), diazepam(3), and nordiazepam(4) on a SB-cyanopropyl-50 column at 160°C oven temperature and a pressure gradient (see Experimental Section). Concentration of each compound was 1 mg/ml in methylene chloride.

and MCAB were well separated, but ACMPC appeared as a shoulder on the front of the diazepam peak (Fig 2). When the oven temperature was increased to 200°C, diazepam and ACMPC appeared as twin peaks, but were not baseline separated. It was noted, however, that MCAB was starting to degrade at this temperature. It was then decided to perform a temperature gradient in addition to the pressure gradient. The

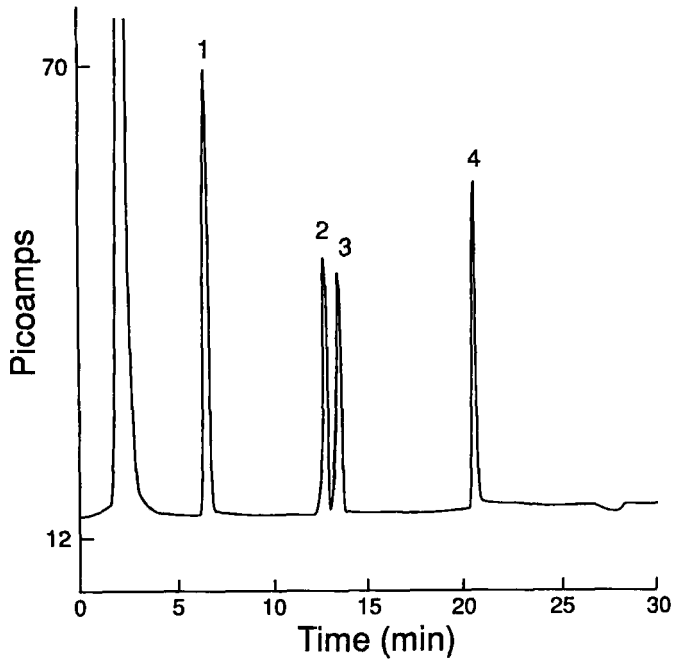

Figure 3 - Typical SFC Separation of MCAB(1), diazepam(2), ACMPC(3) and nordiazepam(4) on a SB-cyanopropyl-50 column using temperature and pressure gradients described in Experimental Section. Concentration of each compound was 1 /mg/ml in methylene chloride.

procedure described in the experimental Section for diazepam and related compounds was finally developed with baseline separation of all 4 compounds being achieved in 23 min (Fig 3). The retention times were 6.5, 12.8, 13.5 and 20.6 min for MCAB, diazepam, ACMPC and nordiazepam, respectively. It was interesting to note that the elution order of diazepam and ACMPC were reversed with the change in oven

TABLE 1

Analytical Figures of Merit for Diazepam, Chlordiazepoxide and the By- and Degradation Products on a 7 m SB-Cyanopropyl-50 Column.

Compound	Retention time, min	Tailing factor[a]	Limit of detection (μg/ml)[b]
MACB	6.5	1.06	150
Diazepam	12.8	1.13	225
ACMPC	13.5	1.15	225
Nordiazepam	20.6	1.05	175
ACB	8.4	1.27	200
Chlordiaze-poxide	13.2	1.09	150
Demoxepam	18.6	1.20	200

[a] Calculated at 10% peak height
[b] Limit of detection based on a signal to noise ratio of 3.

temperature. The detection limits of the method are in the range of 150-225 μg/ml. Table 1 lists the analytical figures of merit for these compounds.

Next, the separation of chlordiazepoxide and its related products, demoxepam and ACB, was studied on the cyanopropyl-50 column. After investigating various pressure gradients (3-50 atm/min) and oven temperatures (50-140°C), the method as described in the Experimental Section gave excellent separation of all the compounds (Fig 4). The retention times were 8.4, 13.2 and 18.6 min for ACB, chlordiazepoxide

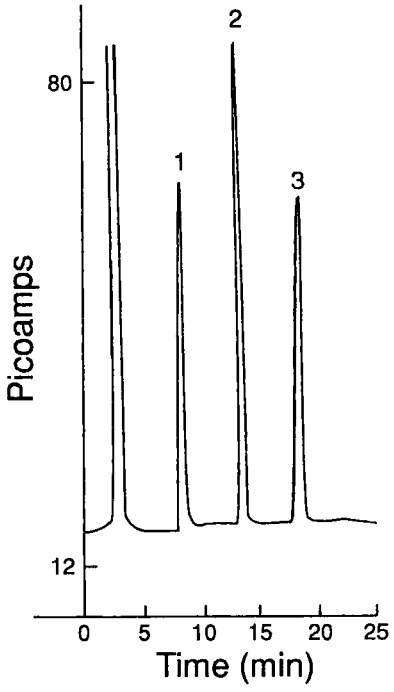

Figure 4 - Typical SFC Separation of ACB(1), chlordiazepoxide(2) and demoxepam(3) on a SB-cyanopropyl-50 column at 120°C oven temperature and a pressure gradient (see Experimental Section). Concentration of each compound was 1 mg/ml in methylene chloride.

and demoxepam, respectively. The analytical figures of merit for each compound are listed in Table 1.

Current USP XXII specifications allow a maximum of 0.3% w/w nordiazepam, 0.1% w/w ACMPC and 0.01% w/w MACB in diazepam drug substance. Current specifications for chlordiazepoxide drug substance allow a maximum of 0.1% w/w demoxepam and 0.01% ACB.

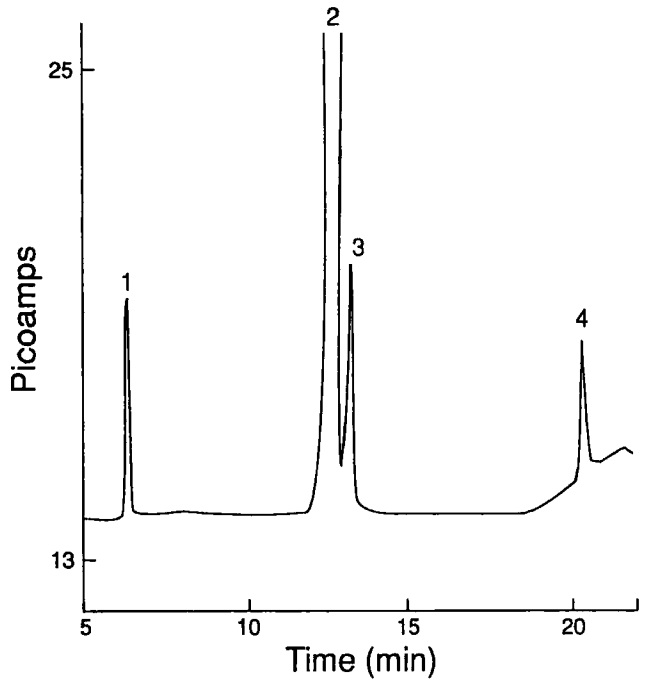

Figure 5 - Typical SFC Chromatogram of MCAB(1), diazepam(2), ACMPC(3) and nordiazepam(4) on a SB-cyanopropyl-50 column using temperature and pressure gradients. Concentration of peaks 1,3 and 4 were 2% w/w of the peak 2 level.

In the monograph for the capsule dosage form, demoxepam and ACB levels are higher at 3.0 and 0.1% w/w, respectively. Due to presently *obtainable* detection limits for the by- and degradation products of each drug based on existing SFC injection hardware, the levels of the by- and degradation products for both diazepam and chlordiazepoxide can only be analysed down to the 1% w/w level. Figs 5 and 6 show the

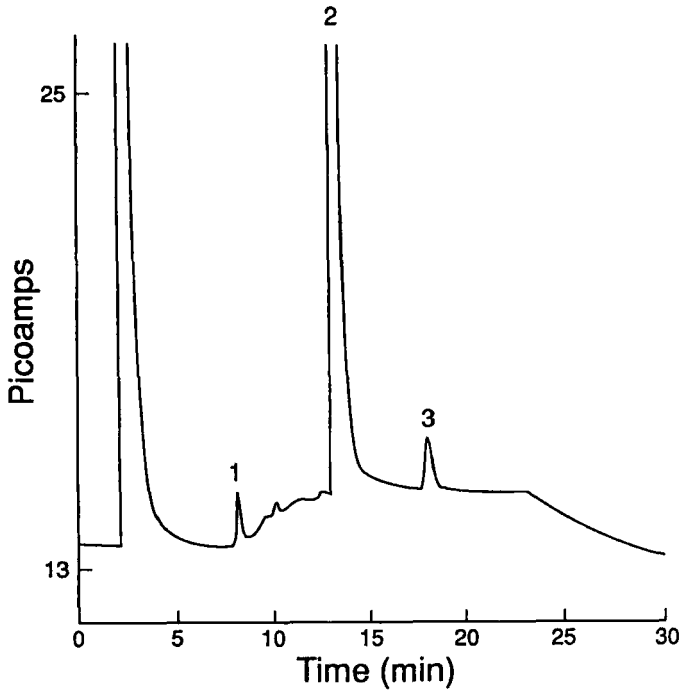

Figure 6 - Typical SFC Chromatogram of ACB(1), chlordiazepoxide(2) and demoxepam(3) on a SB-cyanopropyl-50 column using a 120°C oven temperture and a pressure gradient. Concentration of peaks 1 and 3 were 2% w/w of the peak 2 level.

separation of the drugs and their by- and degradation products at the 2% w/w level. Future improvement in SFC injection hardware will most certainly facilitate the trace analysis of these by- and degradation compounds. In order to show applicability of the SFC method to the drug substance, pharmaceutical dosage forms containing each drug were analysed. The accuracy and precision of the SFC method were

TABLE 2
Accuracy and Precision of Spiked Drug Samples

Compound	Concn Added mg/ml	Concn found[a] mg/ml	%Error	%RSD
Diazepam[b]	1.50	1.46 ± 0.029	2.67	1.99
	2.50	2.48 ± 0.045	0.80	1.81
Chlordia-zepoxide[c]	1.50	1.44 ± 0.058	4.00	4.03
	2.50	2.51 ± 0.059	0.40	2.35

[a] Mean ± standard deviation based on n=3.
[b] Regression coeff. = 0.9994 (n=3).
[c] Regression coeff. = 0.9982 (n=3).

TABLE 3
Comparison of Diazepam and Chlordiazepoxide Dosage Form Analysis Using SFC and HPLC.

Compound	Labelled Amount, mg	Amound found by SFC, mg	Amount found by HPLC, mg
Diazepam[a]	10	10.29 ± 0.42[b] RSD, 4.08%	10.16 ± 0.12 RSD, 1.18%
Chlordiaze-poxide[c]	10	10.13 ± 0.32 RSD, 3.16%	10.08 ± 0.04 RSD, 0.40%

[a] Valium tablet, 10 mg, Roche lot unknown.
[b] Mean ± Standard Deviation based on n=3.
[c] Librium capsule, 10 mg, Roche lot 6159-02.

evaluated using spiked samples of diazepam and chlordiazepoxide. Typical accuracy and precision shown in Table 2 were in the 1-4% range. Recoveries of 101-103% of the labelled amount were obtained for these analytes in their respective dosage forms. The SFC results

were also compared with HPLC methods outlined in the USP XXII monographs for these compounds. The data shown in Table 3 indicates that both methods give comparable results for each drug from a dosage form.

SFC has been shown to be a successful analytical tool in the separation of diazepam and chlordiazepoxide and their related compounds. The method can be utilized for the routine determination of the intact drug substance, but future improvements in SFC hardware will be necessary before the detection and analysis of their by- and degradation product at trace levels can be facilitated.

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