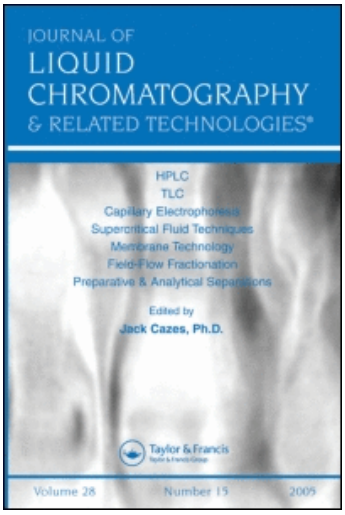

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Separation of Chlorobenzazepine Isomers by Preparative Liquid Chromatography

Karl F. Erhard[a]
[a] Department of Medicinal Chemistry, Smith Kline & French Laboratories, Pennsylvania, Swedeland

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SEPARATION OF CHLOROBENZAZEPINE ISOMERS BY PREPARATIVE LIQUID CHROMATOGRAPHY

Karl F. Erhard
Department of Medicinal Chemistry
Smith Kline & French Laboratories
709 Swedeland Road
Swedeland, Pennsylvania 19479

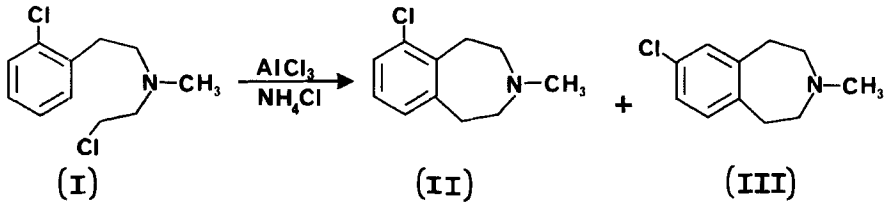
ABSTRACT

The multi-gram separation of 6-chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine (SK&F 86466) from its 7-chloro isomer was accomplished by preparative HPLC, using radially compressed silica gel columns. The effects of flow rate, injection mode and recycle on the preparative throughput of SK&F 86466 were investigated. A concentration of 1% diethylamine in the mobile phase for the preparative separation was necessary to maintain acceptable resolution and peak symmetry of the isomer mixture.

INTRODUCTION

6-chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine (II; SK&F 86466) is a potent, alpha$_2$ adrenergic receptor antagonist, which has undergone extensive biological evaluation in our laboratories (1). For extended pathology/toxicology studies, the preparation of large quantities of highly purified

SK&F 86466 became necessary. The original synthesis of SK&F 86466 involved an intramolecular Friedel-Crafts alkylation of the chloroamine precursor (I), using a melt of aluminum chloride and ammonium chloride at 175°C (2). Under certain conditions, this reaction produced a substantial amount of the undesired 7-chloro isomer (III). A preparative HPLC system was developed to purify large quantities of SK&F 86466, from a mixture containing approximately 40% of the 7-chloro isomer.



(I) (II) (III)

SKF 86466

MATERIALS

Reagents

Chloroform, hexane, isopropanol, methanol, methylene chloride and toluene were reagent grade solvents from J.T. Baker Chemical Co. Diethylamine (99%) was purchased from Aldrich Chemical Co. (Solvent compositions of the mobile phases are expressed as percent by volume).

Chromatographic Media

Thin layer chromatography (TLC) plates (silica gel GF; 250 u) were obtained from Analtech, Inc.

Analytical HPLC was performed on an Altex Ultrasphere silica gel column (4.6 mm i.d. x 25 cm; 5 u).

Preparative HPLC columns were obtained from Waters Associates. Each Preppak cartridge contained approximately 325 g of 37-75 u irregularly shaped silica gel, with dimensions of 55 mm i.d. x 30 cm. The approximate void volume for one cartridge was 500 mL.

Instrumentation

The analytical HPLC consisted of an Altex Model 110A solvent pump, Rheodyne Model 7125 injector with 20 ul loop, an LDC Spectromonitor III variable wavelength uv detector, and a Shimadzu Model C-R1A integrator.

Preparative HPLC separations were carried out on the Waters Associates Prep LC System 500, using refractive index detection.

Sample Mixture

All preparative HPLC separations were carried out using one lot of isomer mixture (200 g; 57:43 - II:III). Both purified isomers obtained by preparative HPLC were characterized by IR, NMR, and GC/MS.

METHODS

TLC plates were deactivated by placing them in a chamber of NH_4OH vapors for 15 minutes prior to use. (Similar results were also obtained by deactivation with diethylamine.)

The analytical HPLC silica gel column was deactivated by eluting with 5 column volumes (~15mL) of 25:75:2.0 - isopropanol: hexane:diethylamine, followed by equilibration with 25:75:0.1 - isopropanol:hexane:diethylamine.

The preparative HPLC columns were deactivated by the following procedure:

1) Radially compress 2 Preppak silica gel cartridges to 35 atm.
2) Elute with and discard the first 2.0 liters of 25:75:2.0 - isopropanol:hexane:diethylamine.
3) Elute with and discard 1.0 liter of the desired mobile phase.
4) Equilibrate with 10 liters of mobile phase.

Initial deactivation and equilibration were done at a flow rate of 500 mL/min, with a solvent pressure of 7-8 atm. The R.I. detector baseline was stable after 3-5 liters of mobile phase had been eluted through the 2 Preppak cartridges.

RESULTS

The separation of the 6- and 7-chlorobenzazepine isomers was optimized by using TLC (3). Deactivated silica gel plates were necessary to minimize tailing of the benzazepine free base isomers. Solvents were chosen from various solvent groups, in order to maximize selectivity differences between the two isomers (4). As indicated in Table 1, the optimum solvent system was found to be 22:78 - isopropanol:hexane, which gave a

TABLE 1
Effect of Different TLC Systems on Isomer Separation

Solvent System	R_f II	R_f III	k'II	k'III	α
5 CH_3OH 95 $CHCl_3$	0.41	0.38	1.4	1.6	1.1
5 CH_3OH 95 CH_2Cl_2	0.30	0.25	2.3	3.0	1.3
10 $(CH_3)_2CHOH$ 90 $C_6H_5CH_3$	0.39	0.29	1.6	2.4	1.5
22 $(CH_3)_2CHOH$ 78 C_6H_{14}	0.51	0.35	1.0	1.9	1.9

$k' = \frac{1 - R_f}{R_f}$ = Capacity Factor

$\alpha = \frac{k' \text{ III}}{k' \text{ II}}$ = Selectivity Factor

selectivity factor, α of 1.9. This system was chosen for the preparative separations.

In order to assay the approximate isomer content of the sample mixture, an analytical HPLC method was used. As shown in Figure 1, good separation and peak symmetry of the mixture were obtained, using 0.1% diethylamine in the mobile phase. A quantitative assay based on peak area at 265 nm was developed by using the uv extinction coefficients of each purified isomer obtained from preparative HPLC.

The first preparative HPLC separation is shown in Figure 2. 5.0 g of isomer mixture was chromatographed to give 2.1 g (70% recovery) of the desired SK&F 86466 (II), using a mobile phase

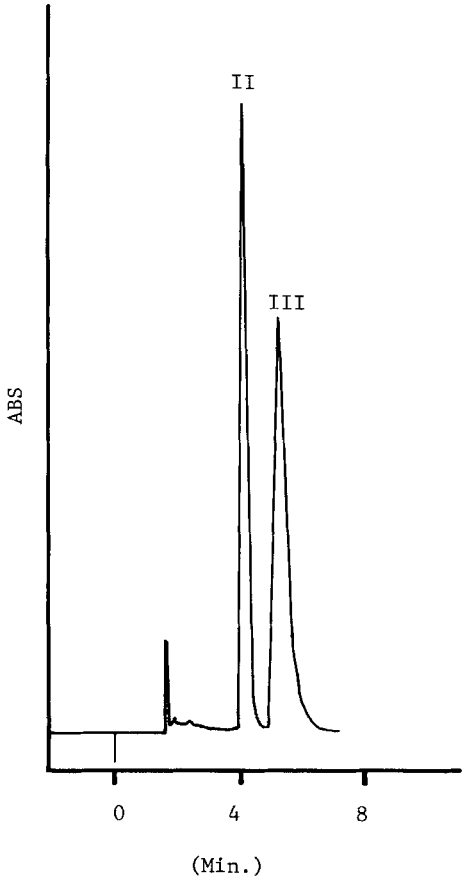


FIGURE 1. Analytical HPLC of Isomer Mixture.

Conditions: Ultrasphere Si; (5U); 4.6 mm i.d. x 25 cm.
25:75:0.1 - Isopropanol:Hexane:Diethylamine
2.0 mL/min; UV Detection @ 265 nm
Isomer Mixture = 57% II (SK&F 86466)
43% III (7-Chloro Isomer)
R_t (II) = 3.9 min.; R_t (III) = 5.0 min.

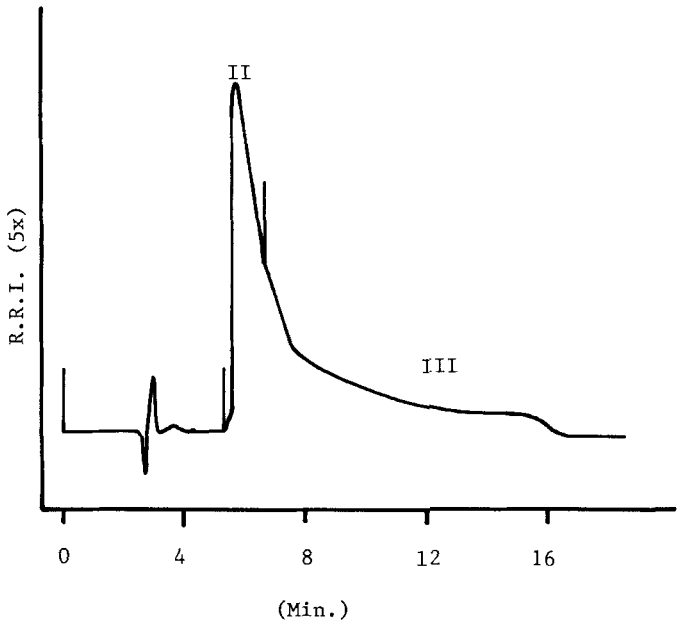


FIGURE 2. Preparative HPLC of Isomer Mixture.

Standard Conditions: Waters Prep LC 500
2 Preppak Silica Columns
25:75:0.1 - Isopropanol:Hexane:Diethylamine
500 mL/min.
5.0 g Isomer Mixture in 25 mL of Mobile Phase

composition of 25:75:0.1 - isopropanol: hexane:diethylamine. The purity of chromatographed SK&F 86466 was shown to be $\geq$ 99% by analytical HPLC (Figure 3). Although this system is capable of producing gram quantities of purified SK&F 86466, there is substantial tailing of the 7-chloro isomer, which results in excessive solvent consumption.

The effect of increased diethylamine concentration on the preparative separation is shown in Figure 4. The use of 1.0%

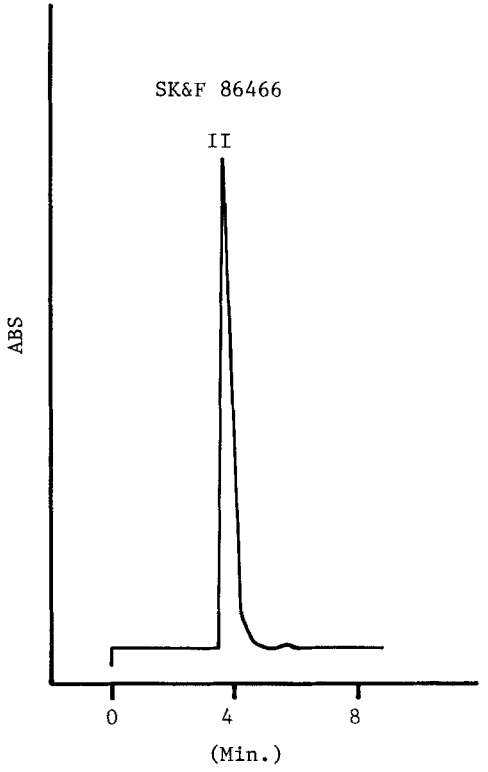


FIGURE 3. Analytical HPLC of Chromatographed SK&F 86466.

diethylamine in the mobile phase effectively eliminates the tailing of the 7-chloro isomer. Solvent consumption per run is reduced from 8.2L to 4.3L. In addition, separation time is reduced from 16 to 9 minutes per run.

The effects of flow rate and injection mode on the preparative separation were examined, and the results summarized in Table 2. There is only a slight loss in purity of recovered SK&F 86466, when the separation is run at maximum flow rate (500

TABLE 2
Effects of Flow Rate and Injection Mode on Preparative Separation

Flowrate	Injection Mode	Purity of Chromatographed SK&F 86466 (5)
150 mL/min.	Syringe	99%
500 mL/min.	Syringe	97%
150 mL/min.	Pump	99%

Standard Conditions, Using 22:78:1.0 - Isopropanol:Hexane:Diethylamine; 10 g Isomer Mixture.

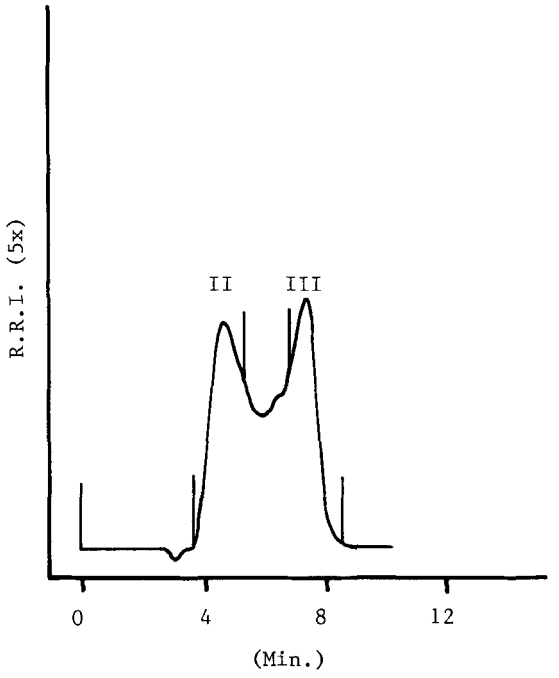


FIGURE 4. Effect of Increased Diethylamine Concentration on Isomer Separation.

Standard Conditions: Using 25:75:1.0 - Isopropanol:Hexane: Diethylamine

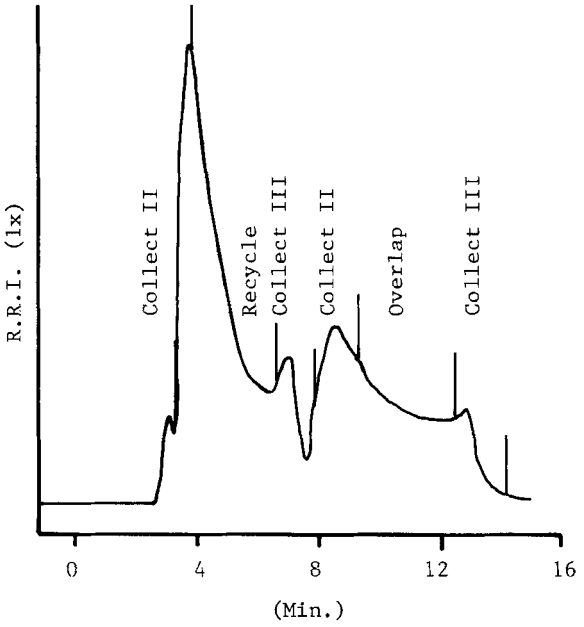


FIGURE 5. Effect of Recycle on Preparative HPLC.

Standard Conditions: Using 22:78:1.0 - Isopropanol:Hexane: Diethylamine
25 g Isomer Mixture

mL/min). Also, there is negligible loss in purity of SK&F 86466, when the sample mixture is injected through the solvent pump.

In order to increase the preparative HPLC throughput, the use of recycle was examined. As indicated in Figure 5, 25 g of isomer mixture was chromatographed effectively using one recycle. A comparison of the standard and recycle modes is shown in Table 3. By using one recycle, the throughput of isomer mixture can be increased 43%, while solvent consumption is decreased 20%. No

TABLE 3
Effect of Recycle on Preparative Separation

Wgt. Isomer Mixture	# Recycles	Recovery/ Purity SK&F 86466	Solvent Consumption (25 g Mixture)	Mixture Throughput
10 g	0	65%/97%	7.5 Liters	75 g/hr.
25 g	1	68%/98%	6.0 Liters	107 g/hr.

TABLE 4
Comparison of Chromatographic Methods for Isomer Separation

Method	Mobile Phase	k' II	k' III	α
TLC	22:78 Isopropanol:Hexane	1.0	1.9	1.9
Prep HPLC (10 g mixture)	22:78:1.0 - Isopropanol:Hexane: Diethylamine	1.0	2.3	2.3
Prep HPLC (5 g mixture)	25:75:1.0 - Isopropanol:Hexane: Diethylamine	1.3	2.7	2.1
Analytical HPLC	25:75:0.1 - Isopropanol:Hexane: Diethylamine	1.6	2.3	1.4

loss in recovery or purity of SK&F 86466 was observed using the recycle mode.

A comparison of capacity factors for TLC, analytical HPLC, and preparative HPLC is shown in Table 4. The k' values are in general agreement, indicating that a basic correlation exists among these chromatographic methods. Variances in the k' and α values can be attributed to the different types of silica gel used, the extent of deactivation of each silica gel, and the different mass load in each chromatographic system.

DISCUSSION

The main goal of preparative HPLC is the maximum throughput of a mixture, with acceptable resolution and recovery of pure components. In analytical HPLC, the resolution of a binary mixture depends on column efficiency, retention, and selectivity.

$$R = 1/4 \, (\sqrt{N})(k'/k'+1)(\alpha-1)$$

According to this equation, the selectivity factor, α has the largest effect on resolution, and it is thus desirable to maximize α before scale up to preparative HPLC.

Optimization of α is generally part of analytical methods development, using an HPLC system to determine k' and α values for various mobile phases and adsorbents. Recent developments in HPLC instrumentation have allowed unattended methods development using either high speed HPLC (6) or computer assisted simplex optimization (7). In conjunction with analytical HPLC, TLC can often be used as a fast scouting method to determine the maximum α value. As shown in Table 1, an optimum α value of 1.9 was determined from TLC R_f data by varying solvent system components.

Organic bases, such as triethylamine, have been used to prevent tailing of basic compounds on silica gel. A mixture of threo-erythro aminoalcohols was separated by preparative HPLC, using 20:80:0.1 - acetonitrile:toluene: diethylamine as the mobile phase (8). However, in that system, significant tailing of the isomer mixture was observed, and recycle chromatography was not feasible. In the current study, it has been demonstrated that, in addition to deactivation of the silica gel columns with

2.0% diethylamine, it is critical to use a higher percentage of diethylamine (1.0%) in the preparative mobile phase than is normally used in analytical HPLC (0.1%). The tailing observed in the preparative HPLC using 0.1% diethylamine could be caused by the inability of this concentration of amine to compete effectively with the higher concentrations of solutes for certain acidic silanol sites.

An investigation of flow rate and injection mode variables indicates that the preparative separation can be effected at high flow rates and by injection through the solvent pump. In addition, the use of recycle chromatography allows increased throughput of isomer mixture with a corresponding decrease in solvent consumption. With manual, repetitive injections, a total of 200 g of isomer mixture was separated on two Preppak silica cartridges, with no observable loss in resolution or retention of the isomer mixture.

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