Organic Process Research & Development **1997**, *1*, 68−71

HPLC Purification of Pergolide Using Silica Gel

Joseph H. Kennedy

Chemical Process Research and Development, Lilly Research Laboratories, Division of Eli Lilly and Company, Indianapolis, Indiana 46285

Abstract:

Pergolide is a synthetic ergot alkaloid approved for the treatment of Parkinson's disease. Process-related impurities from the synthesis are difficult to remove chemically without significant yield loss. An alternative purification procedure was necessary. Pergolide is soluble in nonaqueous solvents such as chloroform, methylene chloride, and dimethylformamide. Solubility is improved when the halocarbon is mixed with an alcohol such as methanol. These characteristics are desirable for silica gel chromatography. This paper describes the evaluation of solubility as a function of halocarbon and the development of a silica gel system to separate the process-related impurities from pergolide. The criteria for choosing a commercially available silica gel and results from purification using axial compression column technology are also discussed.

Introduction

Chromatographic properties of silica gel are dependent on specific pore volume, average pore diameter of the silica, and concentration of silanol groups per unit surface area.[1] The highest surface area is obtained when irregular silica with 60 Å pore size is used. Particle size distribution will also affect loading parameters and, more importantly, column back pressure. A particle size of 15−30 μm was found to be optimum for back-pressure restrictions on the production pumping system.

Large-scale HPLC is most efficiently operated under "column overload" conditions. Column overloading gives rise to asymmetrical peak shapes, displacement affects, and selectivity reversals. Knowing the type of isotherm behavior that compounds exhibit is important when optimizing a preparative separation since this affects the loading and purification rate. When a compound exhibits Langmuir isotherm behavior, retention decreases as loading is increased. A second type of behavior is the S-shaped isotherm. S-shaped isotherms are characterized by an increase in retention time for the peak maximum up to a certain concentration. At this point, a maximum concentration for the column is reached and retention time decreases, causing a concentration "shock wave" where the peak ends abruptly at the long retention time.[2] Preliminary experiments indicated S-shape isotherm behavior for pergolide on an IMPAQ silica gel column with acetic acid, methanol, and dichloroethane eluent.[3] A chromatogram of pergolide illustrating this behavior is presented in Figure 1. Although the shape of the isotherm cannot be completely controlled, modification

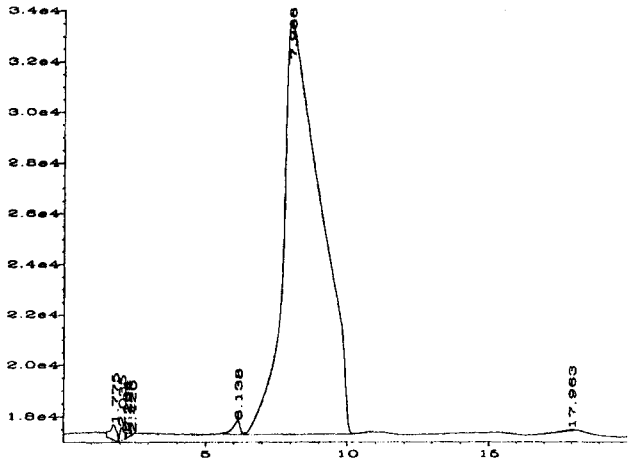

Figure 1. **S-shaped isotherm behavior on IMPAQ silica gel. The column was 4.6 × 250 mm with an eluent consisting of 80% dichloroethane and 20% methanol containing 10% acetic acid at a flow rate of 1.0 mL/min. The column loading was 1.35 mg.**

of the chromatographic eluent and the silica gel surface can be used to achieve acceptable separation of components. This paper describes how the chromatographic eluent was modified to achieve acceptable behavior on various silica surfaces and successfully used to purify pergolide in production scale equipment.

Experimental Section

Preparative chromatography experiments were carried out using a Gilson Medical Electronics, Inc., system (Middleton, WI, USA) consisting of Model 303 pumps with 10 and 25 mL pump heads, Holochrome UV/vis detector with a 2 mm flow cell, and a Model 201C fraction collector. Experiments were monitored at a wavelength of 290 nm. Scale-up experiments and evaluation of different batches of silica gel were carried out on 10 × 250 mm prepacked silica gel columns. Column parameters used in the scale-up experiments of the separation and calculated loading capacities of pergolide are presented in Table 1. The initial loading experiments were carried out by loading 60−100 mg of pergolide on a 4.6 × 250 mm column.

The preparative HPLC system in the pilot plant was an integrated dynamic axial compression (DAC) system from Prochrom, Inc. (Indianapolis, IN, USA), consisting of a 15 cm diameter column with a 1000 mm stroke length, Model LC150VE.1000.70, Lewa three-head diaphragm metering pump Model EK3H, and Custom Sensors and Technology UV detector Model 4700. A wavelength of 254 nm was used for the production lots. An automation system utilizing a Compaq 286 PC was supplied by Prochrom.

(1) Engelhardt, H.; Muller, H. *J. Chromatogr.* **1981**, *281*, 395.
(2) Svoboda, V. *J. Chromatogr.* **1990**, *518*, 77.
(3) G. Cox, Prochrom Inc., unpublished results.

 S1083-6160(96)00003-5 CCC: $14.00

Table 1. **Column parameters**

column size, mm	column vol	packing[a]	flow rate, mL/min	loading
4.6 × 250	4.15 mL	2.1 g	0.8	100 mg
10 × 250	19.6 mL	9.8 g	3.8	472 mg
50 × 250	491 mL	245 g	100	11.8 g
150 × 250	4.42 L	2.21 kg	900	106.2 g

[a] Analytical (4.6 mm) and semipreparative (10 mm) columns were slurry packed with $CHCl_3$ and used as received from the different vendors. Preparative columns (50 and 150 mm) were packed by DAC at a piston pressure of 150 bar. The silica gel was slurried in $CHCl_3$ prior to packing.

Table 2. **Bulk sources of silica gel**

type	source	av diam, μm	pore size, Å
IMPAQ RG1020	PQ Corporation	20	100
IMPAQ RG0620	PQ Corporation	20	60
SORBSIL C60	Jones Chromatography	20	60
AI-023-10/25	YMC Corporation	16	120

Silica gel from PQ Corporation (Conshohocken, PA, USA), Jones Chromatography USA, Inc. (Littleton, CO, USA), and YMC, Inc. (Wilmington, NC, USA), were evaluated. Each manufacturer supplied a 10 × 250 mm prepacked column containing a batch of silica for evaluation. On the basis of this evaluation, bulk material was purchased. Properties of the different silica gels evaluated in these experiments are summarized in Table 2.

Results and Discussion

The criteria for column purification were that related impurities in the recovered pergolide must be less than 0.1%; column loading should be 20 g/L of column volume/cycle; and recovery of pergolide should be greater than 90%. A process based on RP-HPLC has been successfully employed using axial compression column technology for purification of biosynthetic human insulin.[4] The structures of pergolide and related compounds that were generated in processing are illustrated in Figure 2. Initial efforts to develop a column purification of pergolide entailed a reverse phase method using acetonitrile and pH 7 buffer on a C8 type column (Zorbax C8, MAC MOD, Chadds Fort, PA, USA). These results indicated that purification would be possible but column loading would be low (<5 gm/L) due to the solubility characteristics of pergolide. Solubility as a function of pH was evaluated. At low pH, pergolide was soluble in acetonitrile/buffer in the range of 40−60 mg/mL. However, at low pH, pergolide is not retained on typical reverse phase columns. At higher pH values, solubility was reduced and column loading decreased significantly due to volume restrictions of the column.

Other media for column purification of pergolide were investigated, and HP-20 SS (Mitsubushi Chemical Industries, LTD) was evaluated as an alternative to C8 packing. *S*-Methylpergolide was absorbed on HP-20 SS and separated from pergolide. However, 6-methylpergolide did not separate from pergolide when HP-20 SS was used, and thus total purification on this resin was not feasible. Concurrent studies on silica gel indicated that the 6-methylpergolide could be separated from pergolide and that a combination of the two chromatographic techniques could be used for purification.[5]

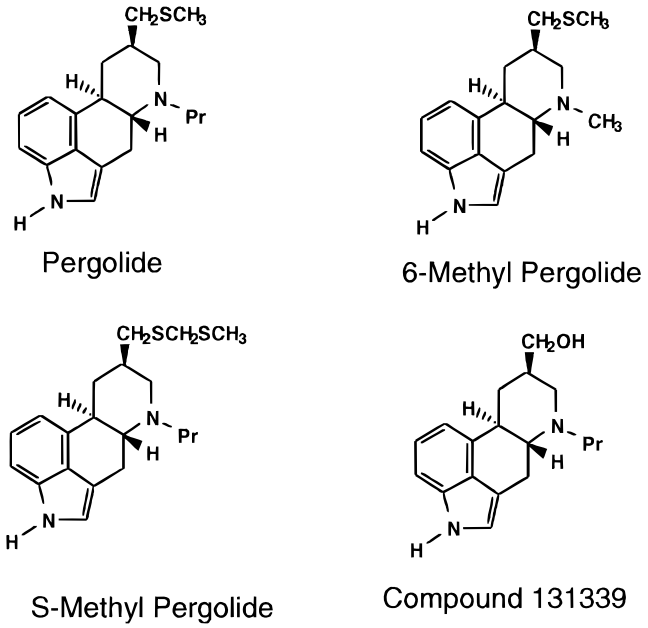

Figure 2. **Structures of pergolide and related compounds.**

Chromatography Development. Separation conditions for pergolide and related impurities were initially developed on a Spectra Physics analytical HPLC system using 4.6 × 250 mm prepacked columns containing different batches of silica gel. Analytical separation of pergolide and related compounds shown in Figure 2 was achieved on a YMC silica gel column using a basic system consisting of ethylene dichloride, isopropyl alcohol, and triethylamine. The solubility of pergolide under these conditions (<15 mg/mL) was not sufficient for scale-up. Similar results were obtained with other systems utilizing bases such as ammonium hydroxide. Solubility was better when acids such as acetic or trifluoroacetic were used in conjunction with an alcohol and halocarbon. Pergolide exhibited nonlinear, S-shaped isotherm behavior on YMC silica with ethylene dichloride or methylene chloride and isopropyl alcohol based eluents. The isotherm behavior of pergolide was improved when methanol and methylene chloride containing trifluoroacetic acid were used as eluent rather than acetic acid. However, this system was not optimal in terms of solubility, and pergolide did not exhibit the desired Langmuir isotherm behavior. Chromatography was improved when trifluoroacetic acid was replaced with methanesulfonic acid. Increasing the acidity of the eluent improved the behavior of pergolide on the silica gel surface. Methanesulfonic acid was used to make the mesylate salt in a separate crystallization step; thus, using it in the chromatographic purification was a way to avoid possible different salt forms of pergolide which might result from other acids in the process. A chromatogram using methanesulfonic acid for separation of pergolide and impurities is presented in Figure 3. Pergolide solubility for this

(4) Kroeff, E. P.; Owens, R. A.; Cambell, E. L.; Johnson, R. D.; Marks, H. I. *J. Chromatogr*. **1989**, *461*, 45.

(5) Kennedy, J. H. Midwest Pharmaceutical Process Chemistry Consortium '93, Poster 11, Indianapolis, IN, Oct 11, 1993.

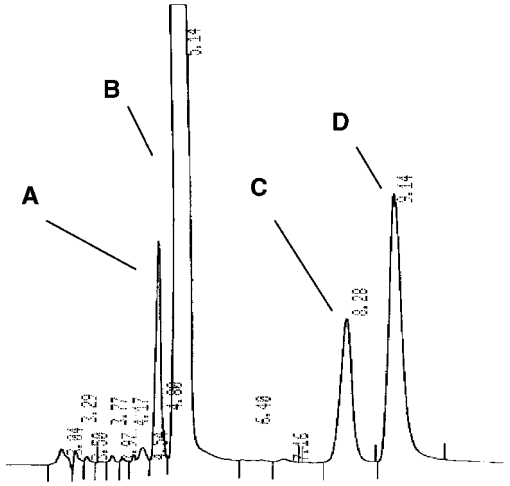

Figure 3. **Separation of pergolide and related compounds: (A) *S*-methylpergolide; (B) pergolide; (C) 6-methylpergolide; (D) 131339. The column was a 4.6 × 250 mm YMC silica column. The eluent consisted of 5% methanol and 95% methylene chloride with 0.2% methanesulfonic acid modifier. The column flow rate was 1.0 mL/min.**

system was approximately 25 mg/mL. Substituting chloroform for methylene chloride increased solubility to 40 mg/mL and improved the separation factor (α) for pergolide and 6-methylpergolide.

The effect of other solvents on solubility and loading was evaluated by substituting different halocarbons in the eluent in place of chloroform. Various solvent combinations were evaluated for the separation of pergolide and related compounds. The ratio of methanol to halocarbon was controlled to provide equivalent elutropic strength.[6,7] After pergolide solubility was measured, the α value for separation of pergolide and 6-methylpergolide was obtained using a SORBSIL C60 column. Loading was not calculated for solubility less than 10 mg/mL since this would not be a practical eluent to use. This data indicated that only chloroform or methylene chloride was suitable for a production process. Screening experiments with a 4.6 × 250 mm column indicated that loading of approximately 66 g/cycle should be possible on a 15 × 25 cm column using SORBSIL C60 silica gel. A summary of solubility and loading data is shown in Table 3.

Silica Gel Evaluation. An eluent consisting of 5% methanol and 95% chloroform with 0.2% methanesulfonic acid was used to determine loading parameters on the prepacked 10 × 250 mm silica columns from the different vendors. A stock solution of pergolide at a concentration of 30 mg/mL was prepared. Varying amounts of pergolide were pumped onto the column using a Gilson pump. The initial volume was 10 mL on column. Eluent was pumped through the column using the second Gilson pump at a rate of 5 mL/min. After the pergolide peak was collected, the flow rate was increased to 12 mL/min to elute the impurities. System back flushing was evaluated in the production unit to clean the column, but this option was not available on the lab unit. Loading was a function of column volume, solubility, and volume of eluent required to elute the

(6) Snyder, L. R. *J. Chromatogr*. **1974**, *92*, 223.
(7) Snyder, L. R. *J. Chromatogr. Sci.* **1979**, *16*, 223.

Table 3. **Pergolide solubility/loading as function of halocarbon solvent**

solvent[a]	solubility (mg/mL)	loading (g/cycle)	α[b]
chloroform	40	66	2.50
methylene chloride	25	23	2.00
1,2-dichloroethane	20	5.5	1.47
tetrachloroethylene	13	3.5	1.44
1,1,2-trichloroethane	10	2.7	1.33
1,1,2-trichloroethylene	10	2.7	1.35
1,1,1-trichloroethane	10	2.7	1.26
1,1,1-trichloroethane[c]	2	0.5	2.33
toluene	7.3		
acetonitrile	3.6		
tetrahydrofuran	3.6		
ethyl acetate	<1.0		

[a] Mixture of methanol/solvent with same elutropic strength. [b] α calculated from separation of pergolide/6-methylpergolide. [c] Elutropic strength changed to maximize α.

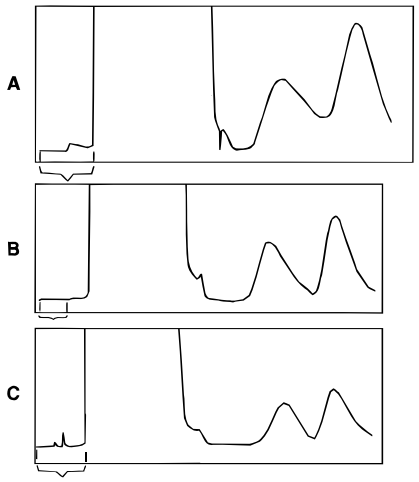

Figure 4. **Loading experiments on lot 257K SORBSIL C60: (A) 999 mg; (B) 520 mg; (C) 325 mg.**

pergolide. The 10 × 250 mm column had a volume of approximately 20 mL. On the 10 × 250 mm column, pergolide was eluted with 33 mL of eluent. At a 30 mg/mL concentration of pergolide, loading was limited to 999 mg. Chromatograms from the lab trials of SORBSIL C60 with column loading of 325, 520, and 999 mg are illustrated in Figure 4. The impurities were resolved at a loading of 999 mg. This indicates that more pergolide could possibly be loaded on this silica and the desired purity of the recovered pergolide still be achieved.

Similar loading profiles were obtained on IMPAQ silica gel. Chromatograms from IMPAQ experiments with column loading of 345 mg are presented in Figure 5. The IMPAQ silica used in these studies was from two different lots, one being 60 Å (RG0620) and the other being 100 Å (RG1020). Both lots were 20 μm particle size. Both IMPAQ and SORBSIL C60 gels were found to be acceptable, with the 60 Å materials having higher loading capacity based on the increase in α between pergolide and 6-methylpergolide.

Pilot Plant Scale-Up. All scale-up experiments in the pilot plant were done with SORBSIL C60 silica. The column length was increased to 50 cm in the pilot plant in order to increase the column volume and thus increase the loading

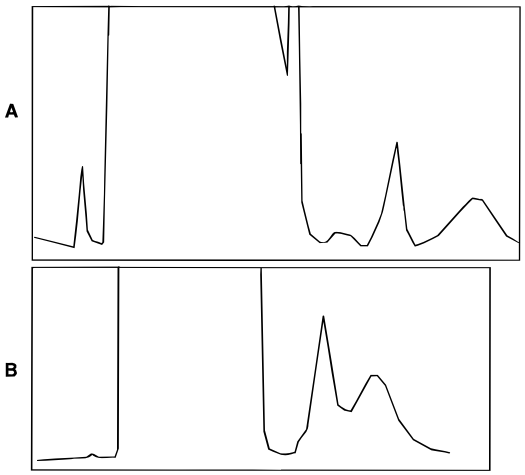

Figure 5. **Loading versus pore size experiments on IMPAQ silica gel: (A) 60 Å type RG0620; (B) 100 Å type RG1020. The loading was 345 mg on each column.**

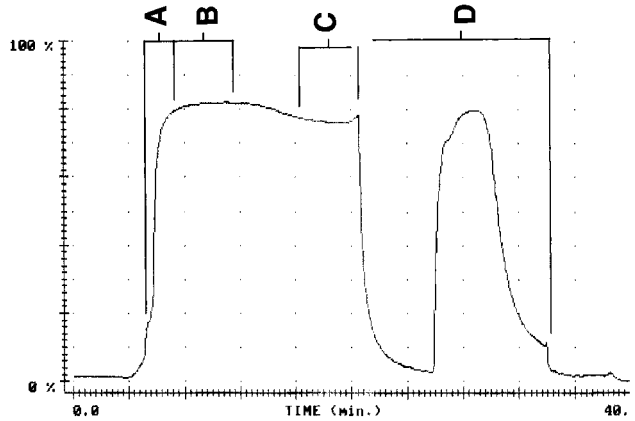

Figure 6. **Chromatogram from 100 g load on 15 × 50 cm dynamic axial compression column: (A) fraction 1; (B) fraction 2; (C) fraction 3; (D) wash. The eluent consisted of 5% methanol and 95% chloroform containing 0.2% methanesulfonic acid. The flow rate was 1.5 L/min at a pressure of 650 psi. The wash eluent consisted of 20% methanol in chloroform.**

capacity of the column. Flow rates were found to be limited to 1.5 L/min with a back pressure of approximately 650 psi on the 15 × 50 cm column. After elution of pergolide, the methanol concentration was increased from 5% to 20% in the eluent to clean the column for subsequent runs. This procedure was preferred due to pressure problems changing to back flush at the 1.5 L/min flow rate. A chromatogram from a 100 g loading on the column is illustrated in Figure 6. Individual fractions were monitored on a 5 μm APEX silica column, and analytical chromatograms are presented in Figure 7.

Decreasing detector sensitivity on the production unit was necessary to detect the break between pergolide/6-methylpergolide and accurately begin the wash cycle. The detector path length was reduced from 4 to 2 mm to decrease the sensitivity. By the decrease in sensitivity, detection of the break between pergolide and 6-methylpergolide was possible at higher loading. Two hundred fifty grams of pergolide was loaded on the 15 cm column with satisfactory separation of impurities and recovery of product.

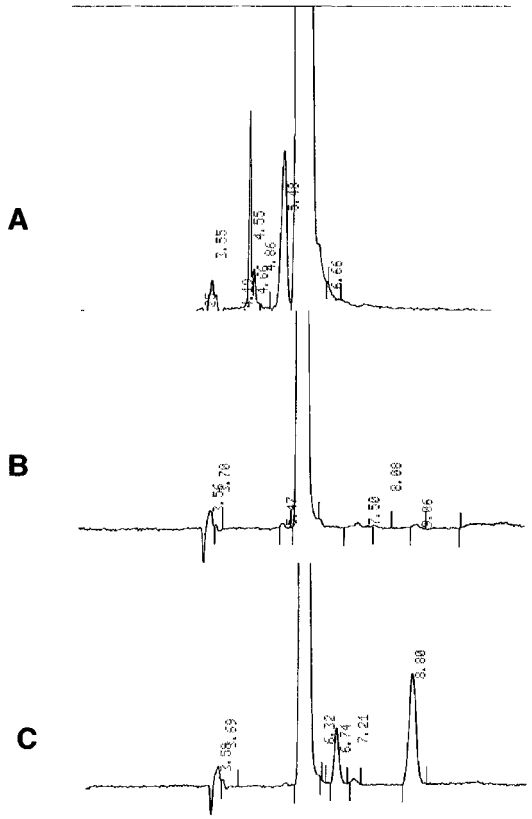

Figure 7. **Analytical chromatograms from 100 g loading: (A) fraction 1; (B) fraction 2; (C) fraction 3. The analytical conditions on 5 μm APEX silica were 10% methanol and 90% methylene chloride containing 0.2% methanesulfonic acid with a flow rate of 1.0 mL/min. The column fractions were monitored at 280 nm, and the column was heated to 40 °C.**

This procedure has been successfully run in the pilot plant using a 250 g loading per cycle on the 15 × 50 cm column. The cycle times were approximately 1 h with a 98% recovery of pergolide from the silica gel column.

Conclusions

A purification procedure for pergolide using commercially available silica gel has been developed and scaled up in a pilot plant. The combination of the silica gel procedure and HP-20 SS column to remove *S*-methylpergolide made total purification of pergolide possible. The initial goals of less than 0.1% of process-related impurities in final material; a 20 g/L column loading; and a 90% yield were accomplished.

Acknowledgment

The author wishes to thank the many Lilly associates who made significant contributions to this work, in particular, the members of Process Research and Development including Mr. S. Biggs, Mr. T. Lobdell, Mr. J. W. Misner, and Mr. M. Mann as well as Dr. G. Cox from Prochrom Inc. for suggestions about sources of silica gel and packing the initial columns for evaluation.

Received for review October 16, 1995.[⊗]

OP960003P

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1996.