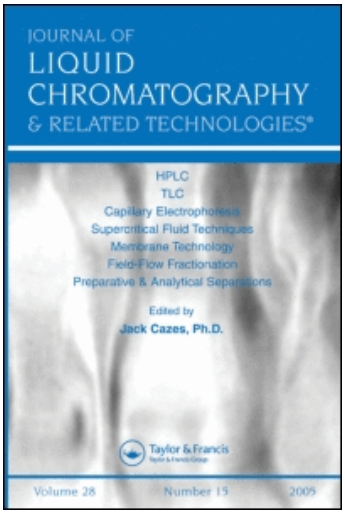

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Purification of Zopiclone by Preparative High Performance Liquid Chromatography

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PURIFICATION OF ZOPICLONE BY PREPARATIVE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The zopiclone was obtained in a 99.5% pure form, and in good yield by preparative scale high performance liquid chromatography. Purification was from a complex reaction mixture. A reversed phase preparative scale HPLC column is used. This work demonstrates how kilogram quantities of pure material can be conveniently obtained using preparative HPLC, even when the components in a mixture are difficult to resolve.

INTRODUCTION

Zopiclone is a cyclopyrrolone which is reported to have similar sedative, anxiolytic, muscle relaxant, and anticonvulsant properties to those of the benzodiazepines. It is used as a hypnotic in the short-term management of insomnia.[1-5] The correct chemical name of zopiclone is 6-(5-chloro-2-pyridyl)-6,7-dihydro-7-oxo-5H-pyrrolo [3,4-b] pyrazin-5-yl 4-metylpiperazine-1-carboxylate. Chemical structure of zopiclone is shown in Figure 1.

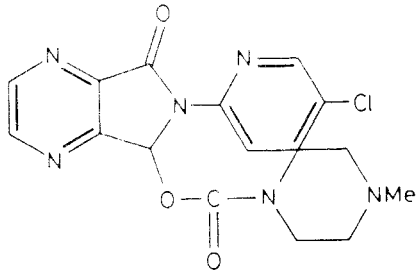

Figure 1. Chemical structure of zopiclone.

The quality requirements for pharmaceutical compounds continue to become even more stringent. As a result, the choice of purification processes and the optimization of purification conditions have became increasingly important. Purification is a key process for the commercialization of chemical compounds. This is especially the case of pharmaceutical compounds, which require high quality.[6]

Chromatographic processes are important industrial unit operations used for purification of a large range of pharmaceutical products. The development of a large scale chromatographic process starts with small scale studies which may be developed through pilot-plant equipment.[7-9]

In this paper some of the factors involved in the scale up of a chromatographic process to industrial scale are studied. Initially it is necessary to consider the overall objectives of the separation and purification process, particularly the purity and yields requirements. The purity is defined by the amount of contaminants which can be accepted, and the yield determines how much product is available for sale from the process.[10-11]

This paper reports the results of preparative HPLC to isolate gram quantities of analytically pure zopiclone (used as standard for chemical analysis) and kilogram quantities of zopiclone for commercial use.

MATERIALS AND METHODS

Apparatus

The instrumentation used for analytical chromatography was from Waters (Milford, MA, USA) consisting of a Waters 600E gradient module, a Waters 484

tunnable absorbance detector and a Waters 745B recorder. The Waters 484 detector is equipped with a 10 mm flow cell path length. For preparative chromatography, the high performance liquid chromatographic apparatus consisted of a Waters Delta Prep 4000 equipped with a Rheodyne sample injection valve (Model 7010), an automatic sample loader (Waters 170), a Waters 484 tunnable absorbance detector, a Waters 745B recorder and a Waters fraction collector.

The 3 mm path length of the 484 detector's preparative flow cell is ideal when high concentrations of UV absorbing material are present. Large volumes of the sample solution could be loaded onto the column by the automatic sample loader.

Materials

Zopiclone was supplied by Astur Pharma (Asturias, Spain) and it was obtained by chemical synthesis. Solvents for analytical chromatographic separations (acetonitrile and dichloromethane) were LiChrosol grade (Merck, Darmstadt, Germany) and for preparative separations were acetonitrile Prepsolv grade (Merck, Darmstadt, Germany) and dichloromethane (Panreac, Montcada i Reixac, Spain). Mobile phases were filtered through a 0.2 μm filter and degassed prior to use.

Columns

For analytical separations a Nucleosil C_{18} 100 Å (4.6 x 250 mm, 10 μm) column and a Kromasil C_{18} 100 Å (4.6 x 250 mm, 10 μm) were used. Preparative separations were conducted on a Nucleosil C_{18} 100 Å (22 x 250 mm, 10 μm) column and on a Prochrom Dynamic Axial Compresion (DAC) column (Prochrom, Champigneulles, France). The DAC column offers a convenient means of packing and maintaining high performance preparative HPLC. This column consists of a piston which moves within a stainless steel column body. The piston is mounted on a hydraulick jack, fitted with an air operated hydraulic pump. The compression pressure is maintained on the column bed throughout its lifetime to take up voids that may be formed if the bed rearranges during column usage. A pressure up to 100 bar may be applied to the packing material.[12-14]

The Prochrom preparative column was packed as follows: A suspension of the desired amount of packing material [Kromasil C_{18} (10 μm)] in acetone-isopropanol (50:50) was maintained in an ultrasonic bath for 5 min, poured into the column, axially compresed until a selected packing pressure was reached (80 bar), and finally conditioned by passing several times the intersticial volume of

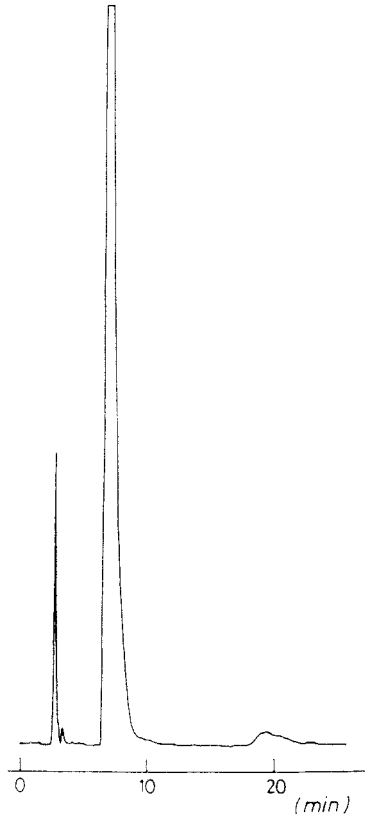

Figure 2. Analytical chromatography of zopiclone on a Nucleosil C_{18} 100 Å column (10 µm), 4.6 x 250 mm, in acetonitrile-dichloromethane (80:20) at a flow rate of 1 mL/min. Detection was at 254 nm. Pressure: 3.5 MPa.

eluent. The same column can be used successfully for several injections. Usually, at the end of a day of multiple runs, the column was washed with 100% acetonitrile. In order to recover the packing material, the piston was allowed to push out the compressed bonded phase.

RESULTS AND DISCUSSION

Analytical separation of the zopiclone was achieved using a reverse phase column Nucleosil C_{18} 100 Å. 40 µg sample of crude zopiclone was injected into the column and it was chromatographed at a flow rate of 1 mL/min.

The analyses were carried out with acetonitrile-dichloromethane (80:20) as mobile phase at ambient temperature. Good separation between zopiclone and the impurities was obtained. The column effluent was monitored at 254 nm. Figure 2 shows the analytical chromatogram. The analysis showed a zopiclone purity of 92%.

Having demonstrated the utility of a reverse phase packing, we proceeded to scale up our preparative reversed phase procedure, achieving higher sample loading while maintaining good resolution. By keeping the concentration of feed solution at a constant value (200 mg/mL of crude zopiclone dissolved in dichloromethane) and varying the total injection sample, we examined the loading effect.[15]

Separation of 20 mg crude zopiclone was carried out under the same conditions on the same Nucleosil C_{18} 100 Å column (4.6 x 250 mm). The resulting chromatogram is shown in Figure 3. This chromatogram indicates that the reasonable reversed phase partition is taking place although a decrease in peak resolution is observed.

The optimized mobile phase of the analytical system was transferred without any modification to preparative HPLC. The column diameter was enlarged from 4.6 mm (analytical column) to 22 mm and the flow rate was increased from 1 to 25 mL/min. The isocratic preparative separation was carried out with 500 mg of crude zopiclone. Concentration overload has been chosen because this is the most economical way of carrying out a preparative separation. Practically the same baseline resolution was achieved as in the analytical column. A minimal loss of resolution of the zopiclone peaks from the other components of the sample at high loading demonstrates that the 100 Å pore size of the packing material has a high available surface area.

A few grams of zopiclone in high purity (100%) were then isolated by preparative HPLC from crude zopiclone. The collected fractions were analyzed with analytical HPLC. The purpose of this isolation was to obtain a standard for quantitative analysis.

The purification on an industrial scale has been carried out using the dynamic axial compression (DAC) column of Prochrom and Kromasil C_{18} (10 μm) as packing material. DAC eliminates void formation and channeling and a homogeneous bed structure is obtained minimizing sample dispersion in the column. Kromasil is mechanically stable up to 10000 psi, making it possible to slurry-pack at very high pressures, giving extremely stable beds. It has a high specific surface area and high degree of carbon coverage, giving a high loadability for preparative work.

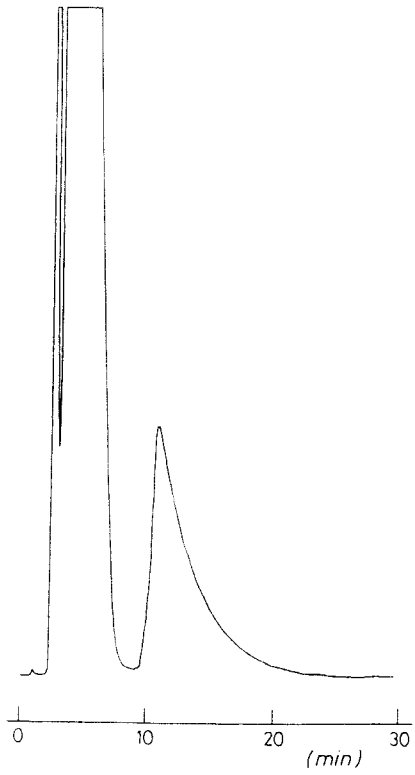

Figure 3. Overload separation of zopiclone on a Nucleosil C_{18} 100 Å column (10 µm), 4.6 x 250 mm, in acetonitrile-dichloromethane (80:20) at a flow rate of 1 mL/min. Detection was at 254 nm. Pressure: 3.5 MPa.

In an industrial process, the solvent often represents the main problem of liquid chromatography. Two types of solvent processing have to be distinguished: the recovery of the products in the eluent and the eluent recycle. Solvent recycling is very often needed in order to minimize purification cost. The contribution of the packing material is often very minor, as is that of the equipment.[11,16,17]

In the present study, the purification of zopiclone in large scale has been developed using one single solvent as eluent. Several solvents as acetonitrile, dichloromethane, tetrahydrofuran, methanol and ethyl acetate have been tested with an analytical column of Kromasil C_{18} 100 Å. Figure 4 shows the chromatogram obtained when acetonitrile is used as mobile phase. A reasonable resolution between zopiclone and its impurities is obtained.

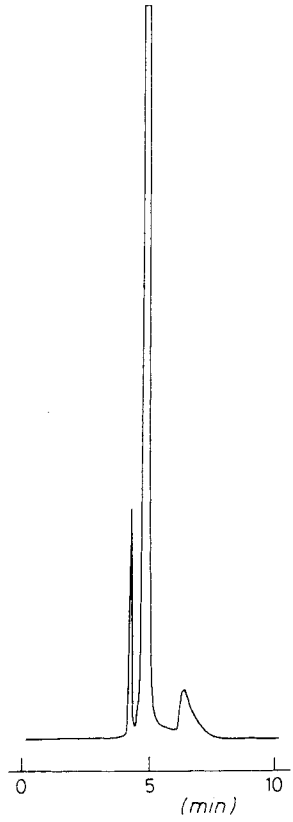

Figure 4. Analytical chromatography of zopiclone on a Kromasil C_{18} 100 Å (10 μm) column, 4.6 x 250 mm, in acetonitrile (100%) at a flow rate of 0.51 mL/min. Pressure: 1.9 MPa.

Various sample amounts from 40 μg up to 2.5 mg of crude zopiclone were injected onto the analytical column. Mass overload occurs at a load of 2.5 mg. The performance of the analytical column prompted scale up to the preparative column (50 x 250 mm). Separation of 300 mg crude zopiclone was carried out under the same conditions on the preparative column at an acetonitrile flow rate of 60 mL/min. An eluent flow rate of 60 mL/min for this preparative column was required to maintain a linear flow velocity comparable to that obtained on the analytical column at a flow rate of 0.51 mL/min. Figure 5 shows the chromatogram obtained. Many injections were made during several hours with a cycle period of 6 min.

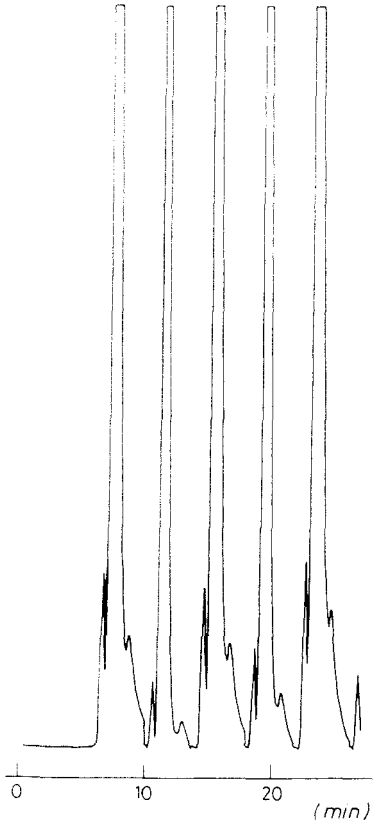

Figure 5. Preparative chromatogram of zopiclone showing several injections on a Kromasil C_{18} 100 Å (10 μm) column, 50 x 250 mm, in acetonitrile (100%) at a flow rate of 60 mL/min. Sample size: 300 mg. Pressure: 1.8 MPa.

Although the peaks corresponding to the zopiclone do not appear to be well resolved in the preparative chromatogram, it is possible to obtain high-purity zopiclone by collecting the center portions of the peaks only. Analytical chromatography was used to check the purity of zopiclone, which was found to be greater than 99.5 when a 300 mg sample load was used. Also, the purity of zopiclone was checked potentiometrically according to British Pharmacopoeia.[18] Recovery of the zopiclone from the preparative run was calculated to be approximately 92 % of the original crude zopiclone sample. By multiple sample injections large amounts can be processed per day. The solvent was removed at 40 °C in a rotary evaporator.

Zopiclone can be isolated and purified on both an analytical and a preparative scale from a crude feedstock using phase reverse columns. The high efficiency of axially compressed columns allows a peak resolution comparable to that obtainable under analytical conditions. Thus, the isolation of reaction products of high purity and, in almost quantitative yield, may be easily attained. The results obtained in these cases were satisfactory and indicated the general usefulness of the method.

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