HPLC of Trazodone in Serum after Microscale Protein Precipitation

Stanley Lam and Lucia Boselli
Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, U.S.A.

Trazodone, an anti-depressant medication, is found in serum in the 500-1000 ng/mL range in patients taking therapeutic doses. Because of this relatively high concentration, it has been possible to devise an HPLC assay system using the rapid, convenient microscale procedure described previously by Lam et al. (Clin. Chem. 26, 963 1980) to prepare the sample for chromatography. To 0.1 mL serum were added 0.1 mL acetonitrile and 10 μL of 10% zinc sulfate in water. The mixture was centrifuged and 50 μL of the clear supernatant was injected into a reversed-phase column which was eluted with 65% 0.05 M potassium phosphate-35% acetonitrile, with detection by ultraviolet absorbance at 210 nm. The trazodone elutes in 6 min, clearly resolved from endogenous interferences. The recovery of trazodone added to serum was better than 90%. Peak height was proportional to concentrations in the serum sample from 125 ng/mL to 3000 ng/mL.

INTRODUCTION

Trazodone, 2-{-[4-(3-chlorophenyl)-1-piperazine-yl]propyl}-1,2,4-triazolo[4,3-a]pyridin-3-(2H)-one, a new antidepressant, has been slowly introduced in the United States in recent years. Trazodone has a unique pharmacological property. It is like neither that of the tricyclic antidepressants nor that of the monoamine oxidase inhibitors (Brogden et al., 1981). Nevertheless, trazodone is as potent as the tricyclics. In the body, trazodone is well tolerated, and it seldom produces toxicities and anticholinergic activities, which are frequently associated with the more common antidepressants. Thus, trazodone is valuable addition to the panel of antidepressant drugs.

Measurement of trazodone levels in serum of patients is helpful in adjusting the therapeutic dosage to steady state levels and avoiding side effects. Methods for quantifying trazodone include GC (Caccia et al., 1981), GC-mass fragmentographic techniques (Belvedere et al., 1975) and HPLC (Ankier et al., 1981; Sukow et al., 1982; Wong et al., 1984). Most of these HPLC procedures require extraction of trazodone in serum samples into various organic solvents and are tedious, while others require mass spectrometers, which are not necessarily available in clinical laboratories. In this paper, we describe a simple and reliable procedure by which trazodone in the serum sample was extracted into the supernatant after removal of the proteins by micro precipitation using zinc sulfate in combination with acetonitrile (Lam et al., 1980; Lam and Karmen, 1982; Bock et al., 1984), and the supernatant introduced into a liquid chromatograph for trazodone quantitation.

EXPERIMENTAL

Equipment. The chromatograph consisted of a LDC Constametric model III pump (Riviera Beach, FL, USA), and a Kratos Spectroflow 757 absorbance detector (Kratos, Ramsey, NY, USA) set at 210 nm. The column was 0.42 X 15.0 cm packed with Nucleosil 5, C₈ by the downward slurry technique. The sample was introduced through a 7120 syringe-loading Rheodyne injector. The detector signals were output to the Model 4416 data station by Nelson Analytical (Cupertino, CA, USA) and linear strip-chart recorder.

Sample preparation. 10 μL of 10% w/v zinc sulfate solution and 100 μL of patient sample or serum standard were added to a 10 X 75 mm disposable culture tube. The content of the tube turned cloudy upon mixing. Then, 100 μL of acetonitrile was added with mixing. The culture tube was centrifuged at 3000 rev/min for 1 min and 50 μL of the supernatant was injected into the chromatograph. The drug was eluted with a mobile phase containing 35% acetonitrile in 50 mM potassium phosphate (KH₂PO₄) at 1.0 mL/min.

RESULTS AND DISCUSSION

After the oral administration of a single dose of trazodone, the plasma drug level peaks at about 2-4 h. Steady state drug level is reached after 4 days of administering 25 mg of trazodone 3 times a day (Allori et al., 1978). The therapeutic range for trazodone has not been established. However, in our laboratory, trazodone is usually found in serum in the 500-1000 ng/mL range in patients taking the therapeutic doses. The HPLC procedures reported thus far for the measurement of trazodone all require extraction of the drug in serum samples into various organic solvents. Subsequently, the drug was recovered by back-extracting into an acidic solution or by resolubilizing the residue in a small volume of mobile phase after removal of the extractant by evaporation, before injecting into the liquid chromatograph. The procedures, necessitating evaporation of large volumes of organic solvents, are tedious and impractical because of variable recoveries that depend on the extraction solvent and the pH of the aqueous phase. An internal standard is usually employed to compensate for recovery, in order to improve assay precision. A micro protein precipitation
procedure, deproteinizing the serum before injecting the sample into the chromatograph for measuring trazodone, is a more efficient and simpler approach. We have previously devised methods using methanol or acetonitrile in combination with zinc sulfate in a final concentration of 0.05% for deproteinizing serum (Lam et al., 1980; Lam and Karmen, 1982; Bock et al., 1984). Presumably, these small amounts of zinc form complexes with serum proteins. The zinc complexes are dehydrated upon the addition of acetonitrile or methanol, and precipitate out of solution. Incomplete protein precipitation resulted, however, when zinc sulfate, methanol or acetonitrile alone was used as the protein precipitant.

The chromatograms of a serum blank, a serum standard containing 250 ng/mL of trazodone, and a patient specimen containing 963 ng/mL trazodone are shown (Fig. 1). Excellent resolution of the drug from the endogeneous interferences was accomplished in six minutes. Recovery of trazodone added to serum was better than 90%. Trazodone was quantified by calibration with six external standards at concentrations of 250, 500, 700, 1000, 2000 and 3000 ng/mL. The standard curves exhibit good linearity with correlation coefficients 0.9996 (Fig. 2). As little as 20 ng/mL trazodone was detected with a signal to noise ratio better than five.

Since the procedure did not involve solvent extraction and evaporation steps, an internal standard was not used for compensating recovery losses. Excellent precision of measurement equal to those of our earlier works was achieved. The coefficient of variance (CV) of five repeated analyses of 2000 ng/mL trazodone was 1.4% and of 1000 ng/mL was 4.6%.

In summary, a micro sample clean up procedure using the combination of zinc sulfate-acetonitrile provides an effective way to precipitate serum proteins prior to the chromatographic analysis of trazodone. Sample clean-up and batch processing of 12 samples takes less than 5 min. The supernatant thus prepared is very clean; column life extends far beyond 6 months. Because of the excellent recovery and reproducibility of the microprotein precipitation technique, internal standards (which in many instances are not readily available to clinical laboratories, and far too often suffer from interferences from sample matrix) may be omitted. This procedure also affords rapid sample turn around and fast throughput.
REFERENCES


Received 7 October 1986