High-Pressure Liquid Chromatographic Determination of Plasma Dobutamine Concentrations

D. W. McKENNON and R. E. KATES *x

Received February 17, 1978, from the *College of Pharmacy, Ohio State University, Columbus, OH 43210.* Accepted for publication March 31, 1978. *Present address: Cardiology Division, Stanford University Medical Center, Stanford, CA 94305.

Abstract □ A sensitive and specific high-pressure liquid chromatographic method was developed for measuring dobutamine in human plasma samples. Nylidrin is employed as an internal standard. Following extraction and separation on a C_{18} reversed-phase column, the drug is detected by a fluorescence detector with an excitation wavelength of 195 nm and a 330-nm emission cutoff filter. The retention times of dobutamine and nylidrin are 5.2 and 19.2 min, respectively. The minimum level of sensitivity is 10 ng/ml. Reproducibility was ±5% over a 25–300-ng/ml range. Several drugs were screened for possible interference, but none interfered with the dobutamine analysis.

Keyphrases □ Dobutamine—high-pressure liquid chromatographic analysis in plasma □ High-pressure liquid chromatography—analysis, dobutamine in plasma □ Cardiotonic agents—dobutamine, high-pressure liquid chromatographic analysis in plasma

Dobutamine (I), a synthetic catecholamine, was developed in an effort to find an ideal inotropic drug (1). Studies in patients with severe heart failure showed that dobutamine increases ventricular contractility and cardiac output without substantially increasing heart rate or systemic blood pressure (2). Dobutamine is structurally similar to dopamine (II) but does not appear to share the positive chronotropic or pressor effects seen with dopamine or other inotropic agents.

To investigate the pharmacokinetics and plasma level–effect relationships for this drug, it was necessary first to develop a rapid, specific, and sensitive analytical method for measuring dobutamine in human plasma samples. Since dobutamine is a naturally fluorescing compound, a high-pressure liquid chromatographic (HPLC) procedure utilizing a fluorescence detector was developed.

EXPERIMENTAL

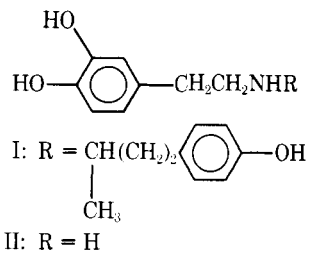
Chemicals and Reagents—Dobutamine[1] and nylidrin[2] were obtained as the hydrochloride salts, and methoxydobutamine[1] was obtained as the free base. Glass-distilled[3] ethyl acetate, methanol, and acetonitrile were used as obtained without further purification. A solution of 5 *M* K_2HPO_4 and 0.1 *M* phosphate buffer (pH 2) were prepared using double-distilled deionized water.

HPLC—A 4.6-mm × 25-cm, 5-μm particle-size, C_{18} reversed-phase column[4] was used. The eluting solvent was 22% acetonitrile–78% 0.1 *M* phosphate buffer (pH 2.0). The solvent was routinely degassed prior to use by applying a vacuum. A dual-piston reciprocating pump[5] was used to pump the solvent isocratically through the column at 2.0 ml/min, which developed a precolumn pressure of about 2500 psi (175.75 kg/cm^2).

A valve loop injector[6] was used with a 50-μl loop. On the variable wavelength fluorescence detector[7], the range was set at 0.5 and the time constant was set at 3. The excitation wavelength was 195 nm, and an emission cutoff filter of 330 nm was employed.

Procedure—To 1 ml of plasma were added 1000 ng of nylidrin, 0.1 ml of 5 *M* K_2HPO_4, and 4 ml of ethyl acetate. The mixture was then shaken for 5 min and centrifuged to separate the organic and aqueous layers. The organic layer then was transferred to a clean tube. A second 4-ml aliquot of ethyl acetate was added to the plasma, which was then shaken and centrifuged. The second ethyl acetate extract was added to the first and evaporated to dryness on a nitrogen evaporator[8] at 70°.

To the residue was added 0.5 ml of methanol. Following adequate mixing, the methanol was transferred to a clean tube, leaving behind an oily residue, which was discarded. The methanol then was evaporated under nitrogen, and the residue was dissolved in 200 μl of the eluting solvent. After adequate mixing, a 50-μl sample was injected onto the column.



RESULTS AND DISCUSSION

The only previously published assay for dobutamine is an enzymatic procedure utilizing ^{3}H-methyl-*S*-adenosylmethionine (3). In this procedure, dobutamine is converted to the labeled methyl derivative by reaction in the presence of catechol *O*-methyltransferase. General procedures for measuring catecholamines in biological fluids include HPLC separations of dansyl derivatives (4), GLC separation after derivatization with trimethylsilane–imidazole (5), and extensive extraction procedures involving adsorption and selective desorption from aluminum oxide (6). The method reported here is relatively simple and rapid and does not require derivatization.

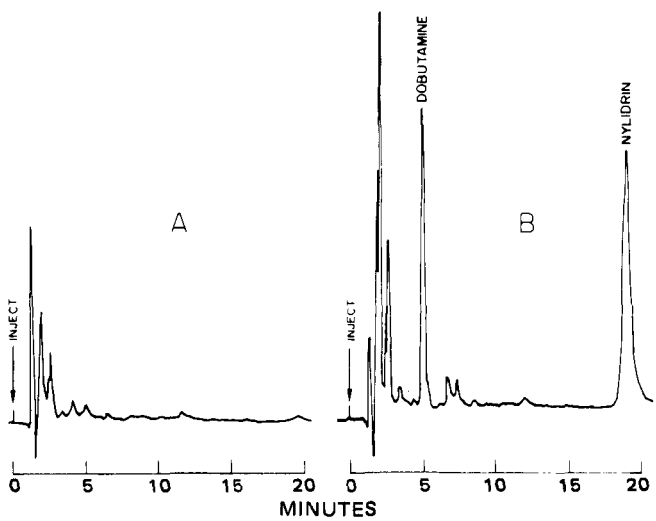


Figure 1—*Chromatograms of a blank plasma sample (A) and a plasma sample to which had been added 150 ng of dobutamine/ml and 1000 ng of nylidrin/ml (B).*

[1] Eli Lilly & Co., Indianapolis, Ind.
[2] Zenith Laboratories, Northvale, N.J.
[3] Burdick and Jackson Laboratories, Muskegon, Mich.
[4] Laboratory Data Control, Riviera Beach, Fla.
[5] Constametric II G, Laboratory Data Control, Riviera Beach, Fla.
[6] Model 7120, Rheodyne, Berkeley, Calif.
[7] Schoeffel Instrument Corp., Westwood, N.J.
[8] N-Evap, Organomation Associates, Shrewsbury, Mass.

0022-3549/78/1200-1756$01.00/0

Dobutamine extraction from plasma was evaluated with several buffers at various pH levels. The optimal condition was found with 0.1 ml of 5 *M* K_2HPO_4. The resulting pH of the plasma was 8.9–9.1. The use of a pH 9 borate buffer or 1.0 *M* $NaHCO_3$ did not yield as adequate an extraction. The extraction efficiency, as determined by adding known amounts of dobutamine to plasma and comparing the peak heights with nonextracted samples, was 76.7 ± 4.3%. Since an internal standard was employed when analyzing plasma samples, variability in the extraction efficiency did not create any difficulties.

A fairly polar solvent was needed for extraction. Nonpolar solvents such as benzene were not effective in extracting dobutamine from plasma. Following extraction of the plasma with ethyl acetate and subsequent evaporation of the organic layer, the residue was partially dissolved in methanol. While dobutamine and nylidrin are readily soluble in methanol, an oily component did not dissolve in the alcohol. It is important to separate out this oily component prior to injecting the sample, because it collects on the precolumn filter and necessitates frequent filter replacement.

The nonpolar C_{18} column was selected for use after comparison studies with a moderately polar μ-CN column. The more polar column resulted in longer retention times for dobutamine, its metabolite, and the internal standard. For both columns, the use of an organic–aqueous mixture was necessary to elute the desired catechols. Higher concentrations of the organic phase, acetonitrile, lowered the retention times of the catechols while higher pH values in the aqueous media increased the retention times. Both columns gave the same elution order: dobutamine, 3-methoxydobutamine, and nylidrin.

Chromatograms of an extracted blank plasma sample (A) and an extract of a plasma sample to which was added 150 ng of dobutamine/ml (B) are shown in Fig. 1. The retention times of dobutamine and nylidrin were 5.2 and 19.2 min, respectively. As can be seen in the chromatogram of the blank plasma, no endogenous catecholamines present in plasma eluted with retention times that would interfere with the analysis of dobutamine.

Pure samples of epinephrine and L-norepinephrine were injected directly onto the column. They eluted together very rapidly with a retention time of about 2 min. When injected onto the column, dopamine also eluted with a retention time of about 2 min. The primary nonconjugated metabolite of dobutamine is the 3-methoxy derivative (3). This metabolite had a retention time of 7.9 min. It is apparently very rapidly conjugated to the glucuronide since only very low concentrations have been detected in plasma.

Several drugs commonly administered to cardiology patients were tested for possible interference with the assay. Included were allopurinol, colchicine, warfarin, chlorothiazide, digoxin, lidocaine, procainamide, furosemide, spironolactone, and quinidine. With the exception of quinidine, none of these drugs or their metabolites present in patient plasma interfered with the assay. Quinidine eluted with a retention time similar to that of dobutamine, and the fluorescence was extremely intense. It was necessary to extract quinidine from the plasma prior to extracting dobutamine. Since quinidine can be selectively extracted into benzene without removing dobutamine, an initial extraction with benzene can be performed before the ethyl acetate extraction.

Standard curves are routinely prepared from spiked plasma samples containing 25, 100, and 300 ng of dobutamine/ml. Each concentration is evaluated in triplicate. Peak height ratios (dobutamine to nylidrin) are plotted as a function of concentration of dobutamine added. The mean slope obtained from averaging six standard curves was 0.0061 ± 0.0002. Intercepts were not significantly different from the origin. Standard curves were linear up to 500 ng of dobutamine/ml.

The lower limit of sensitivity of the assay is around 10 ng/ml. However, this limit can be extended downward by using a larger injection loop and a smaller final dilution volume. To date, however, no patient samples with a dobutamine concentration of less than 10 ng/ml have been analyzed.

The assay reproducibility and precision were evaluated over a range of 25–300 ng of dobutamine/ml. Seven replicates of each concentration (25, 50, 150, and 300 ng/ml) were analyzed; the concentrations of dobutamine measured were 24.7 ± 0.87, 52.2 ± 3.01, 148.9 ± 4.05, and 303.1 ± 7.10 ng/ml ± *SD*, respectively. The average standard deviation was only 3.6% of the mean, and the measured mean concentrations only differed from the added concentrations by an average of 1.8%.

Metabolism of dobutamine in the plasma must be prevented once the sample has been drawn from a patient. Catechol *O*-methyltransferase, the enzyme primarily responsible for the metabolism of dobutamine, is present in plasma. By immediately placing samples in ice, this metabolic process can be arrested.

Whole blood, as well as plasma, may be analyzed by the described procedure. It was found that, following addition of dobutamine to whole blood, whole blood and plasma concentrations are equal. This result indicates that dobutamine readily partitions into red blood cells and reaches a rapid equilibrium.

This procedure has been used routinely to analyze patient samples containing dobutamine. Patients are generally administered the drug by constant infusion at rates of 2.5–15.0 μg/kg/min. Plasma dobutamine levels of 50–350 ng/ml have been observed in patient samples.

REFERENCES

(1) R. R. Tuttle and J. Mills, *Circ. Res.*, **36,** 185 (1975).

(2) C. V. Leier, J. Webel, and C. A. Bush, *Circulation,* **56,** 468 (1977).

(3) P. J. Murphy, T. L. Williams, and D. L. Kau, *J. Pharmacol. Exp. Ther.*, **199,** 423 (1976).

(4) G. Schwedt and H. H. Bussemas, *Chromatographia,* **9,** 17 (1976).

(5) Y. Maruyama and A. E. Takemori, *Anal. Biochem.*, **49,** 240 (1972).

(6) A. H. Anton and D. F. Sayre, *J. Pharmacol. Exp. Ther.*, **138,** 360 (1962).

ACKNOWLEDGMENTS

Supported in part by Health Professions Special Projects Grant 05D000714-010 of the Department of Health, Education, and Welfare.

The authors thank Ms. Suzanne Hessler for assistance in preparation of the manuscript.