Analysis of Doxylamine in Plasma by High-Performance Liquid Chromatography

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Abstract □ A rapid and sensitive high-performance liquid chromatographic (HPLC) assay for the quantitative determination of doxylamine in plasma is described. The drug levels of doxylamine in plasma were monitored after the oral administration of a single 25-mg tablet of doxylamine succinate to each of 20 male volunteers. The compound was extracted from the plasma samples, concentrated under a nitrogen stream, and analyzed by HPLC using normal-phase chromatography with detection at 254 nm. The detection limit is ~5 ng/ml.

Keyphrases □ Doxylamine—in human plasma, determination by high-performance liquid chromatography □ High-performance liquid chromatography—quantitation of doxylamine in human plasma □ Antihistamines—doxylamine succinate, determination of doxylamine in human plasma by high-performance liquid chromatography

Doxylamine, one of the oldest drugs exhibiting antihistaminic properties, also possesses impressive hypnotic properties. Although the characteristic activity of the antihistamines is their ability to antagonize the effects of histamine on various peripheral structures (1), these compounds are known to have a wide spectrum of pharmacological and therapeutic effects. Since many of these drugs cause sedation, they are frequently used as hypnotic agents or sedatives, in spite of their mixed excitatory and depressant actions within the CNS (2). According to Sjöqvist and Lasagna, 25–50 mg of doxylamine is more effective as a sedative than 100 mg of secobarbital (3), and it is often used as a hypnotic antihistamine at bedtime (4).

A previous method, tested by the Food and Drug Administration in 1972, describes a doxylamine assay in urine using GC (5). This paper describes a simple, sensitive, high-performance liquid chromatographic (HPLC) method for the measurement of doxylamine in plasma using normal-phase chromatography.

EXPERIMENTAL

Materials—Reagent grade sodium hydroxide, ammonium hydroxide, and ammonium chloride; HPLC grade acetonitrile, chloroform, methanol, and dichloromethane; and USP reference standard dextroamphetamine sulfate and doxylamine succinate were used.

Standard Preparation—A set of standard samples was prepared by adding 80, 50, 30, 20, and 10 μl of a 10-ng/μl solution of doxylamine succinate in water to 3.0-ml plasma blanks with a microliter syringe. The

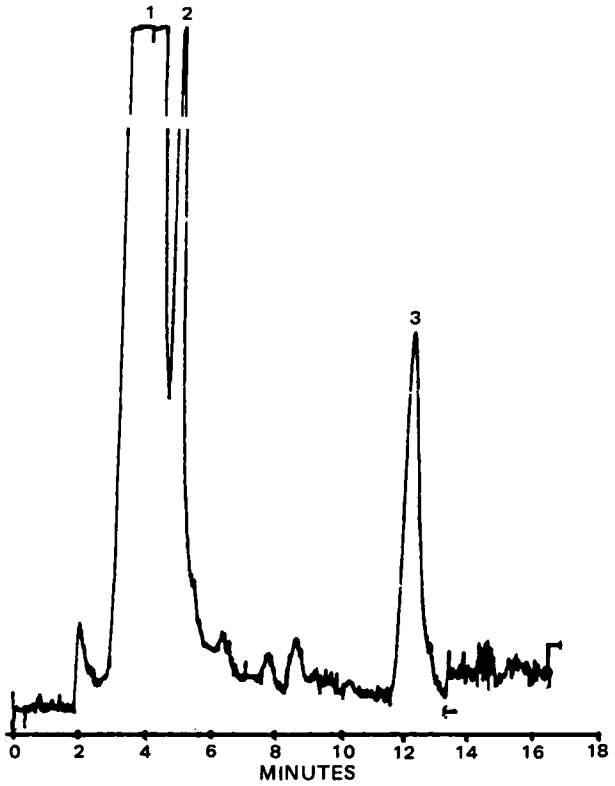

Figure 1—*Chromatogram of a plasma blank. Key: (1) and (2) unidentified components of plasma; (3) amphetamine peak.*

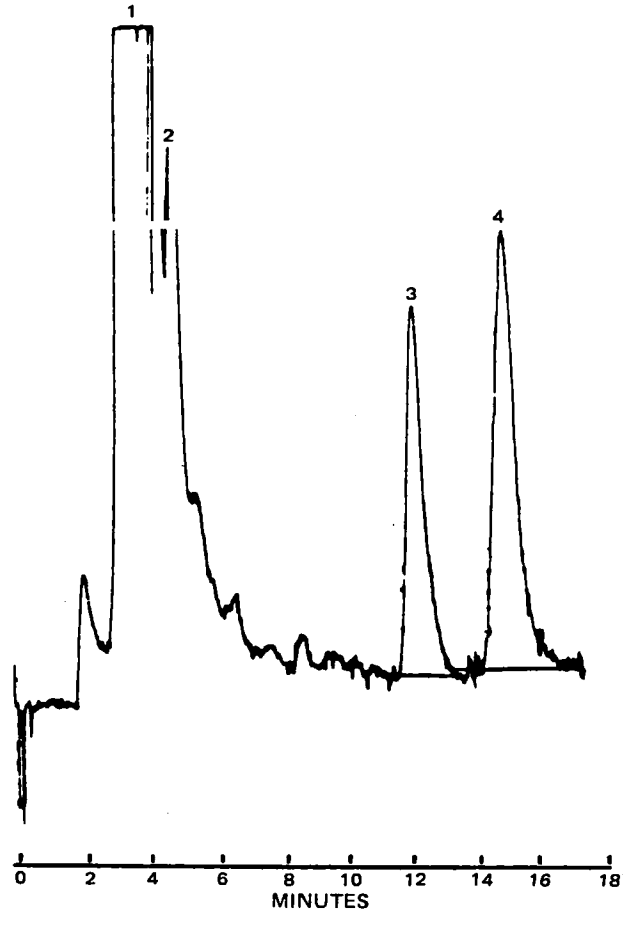

Figure 2—*Chromatogram of a plasma sample taken from a human volunteer 2 hr after oral administration of 25 mg of doxylamine succinate in tablet form. Key: (1) and (2) unidentified components of plasma; (3) amphetamine; (4) doxylamine.*

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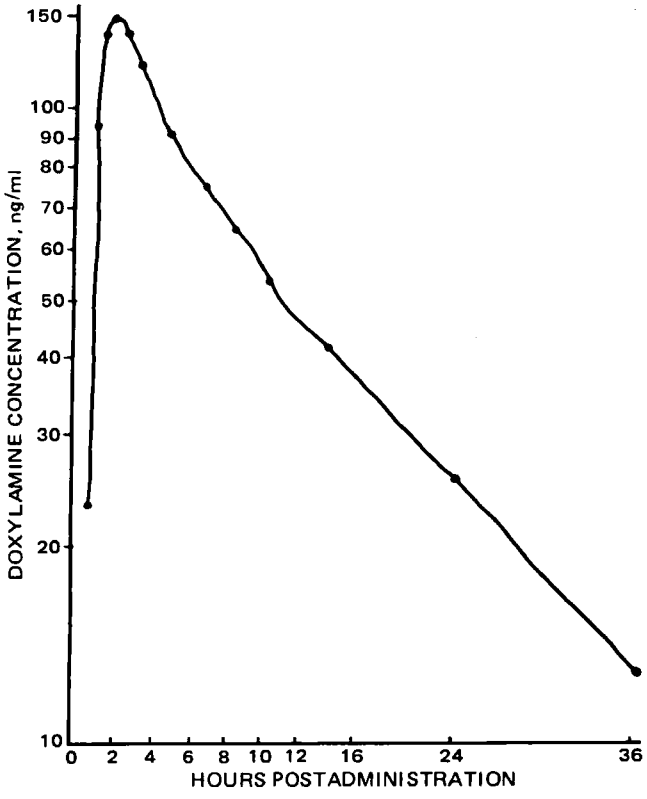

Figure 3—*Plasma levels of doxylamine in a human volunteer following the oral administration of 25 mg of doxylamine succinate in tablet form.*

internal standard was prepared by adding 10 ml of 0.3 *N* NaOH and 20 ml of dichloromethane to 20 mg of dextroamphetamine sulfate in a 125-ml separatory funnel and shaking manually for 5 min. After the layers separated, the organic layer was collected in a 50-ml volumetric flask. The extraction was repeated with 20 ml of dichloromethane, and the organic phase was combined with the first extract. This was diluted to volume with dichloromethane to obtain a 300-μg/ml concentrated solution of dextroamphetamine in dichloromethane. A 5-ml aliquot of this solution was transferred to a 50-ml volumetric flask and diluted to volume with the eluting solvent. The concentration of the resulting solution was 30 μg/ml.

Subjects—After an overnight fast, 20 healthy male volunteers (20–50 years old, 59–68 kg) were administered a single 25-mg dose of doxylamine succinate in tablet form. Blood samples of sufficient volume to obtain 10 ml of plasma were drawn prior to administration and at regular intervals postadministration. All samples were collected in heparinized evacuated containers[1] and centrifuged at 2500 rpm. The separated plasma was stored at −20° until analysis.

Plasma Extraction—Plasma (3.0 ml) was pipetted into a 20-ml screw-cap[2] test tube, and 10 ml of 0.3 *N* NaOH and 5 ml of dichloromethane were added. The tubes were placed on a rocking shaker[3] at a slow speed for 15 min and then centrifuged. Any emulsion formed was broken up by lightly tapping the tubes and the samples were centrifuged again. The organic layer was transferred to a 12-ml glass-stoppered conical-tip tube using a Pasteur pipet. The extraction of the aqueous layer was repeated with an additional 5 ml of dichloromethane. This was centrifuged, the organic layer was combined with the first extract in the conical-tip tube, and the tubes were placed in a water bath with the temperature maintained at 40°. The combined extracts were evaporated under a nitrogen stream, and 200 μl of the dilute dextroamphetamine solution was added with a 100-μl syringe. The tube was capped with the glass stopper, vortexed[4], and centrifuged.

Chromatographic Analysis—The HPLC system[5] consisted of a solvent delivery pump, a fixed-wavelength UV detector at 254 nm, and a μPorasil column (10 μm, 3.9 mm × 30 cm). The eluting solvent was 8 parts chloroform, 1 part acetonitrile, and 1 part of a mixture of methanol–ammonium hydroxide–ammonium chloride (57:2:1); the flow rate was 1.5 ml/min. The UV absorption was monitored using an electronic integrator[6] with an attenuation of 2^4–2^5, as necessary.

Eighty microliters of the plasma extract was introduced into the column using an automatic injector[7]. Retention times for doxylamine and amphetamine were 14.6 and 12.1 min, respectively. The peak height ratio of doxylamine to the internal standard in the sample plasma was measured and compared with the peak height ratio in the standards to achieve quantitation.

Calibration Curve—The linearity of the calibration curve was determined by adding 100–800 ng of doxylamine succinate[8] to 3-ml plasma blanks. The coefficients of variation were determined using replicate plasma standards spiked with doxylamine succinate.

Table I—Reproducibility of Doxylamine Determination in Human Plasma [a]

Doxylamine Succinate Concentration, ng/ml	Doxylamine–Amphetamine Peak Height Ratio, ng/ml	*CV*, %
268	1.600	2.5
167	0.982	3.8
100	0.598	2.5
67	0.406	3.2
34	0.203	6.2

[a] n = 20 for all concentrations.

RESULTS AND DISCUSSION

The method was evaluated in terms of extraction efficiency, interference, linearity, and precision. Preliminary experiments demonstrated the need for adding amphetamine at the end of the extraction steps. When known amounts of amphetamine were added to the dichloromethane solution in the beginning of the extraction procedure, its recovery was negligible. To ensure the availability of a fixed amount of amphetamine in the sample solution, 200 μl of amphetamine was added to each tube after the dichloromethane was evaporated.

The peaks observed for doxylamine and amphetamine were well separated from any naturally occurring plasma constituents or any metabolites of the drug. With the blank plasma, when the standard doxylamine was eliminated from the assay, no peaks absorbing at 254 nm that had the same retention time as doxylamine were observed (Fig. 1). Figure 2 shows plasma extracted from a typical subject 2 hr after the administration of 25 mg of doxylamine succinate. From 0.5–36 hr postadministration, the concentration of doxylamine in plasma was generally in the range of 10 to 150 ng/ml (Fig. 3). The peaks encountered for doxylamine and the internal standard were symmetrical and well defined.

The mean values of the peak height ratio of doxylamine to amphetamine obtained from 20 calibration curves are given in Table I. The method exhibits excellent linearity: the correlation coefficient of the doxylamine to amphetamine peak height ratio *versus* doxylamine succinate added to plasma was 0.99 over the range of values obtained (Table I). The absolute recovery when 570 ng of doxylamine succinate was added to 3 ml of plasma was 84.8% (based on three determinations). The assay is suitable for the study of doxylamine succinate pharmacokinetics, representing a reproducible and accurate method for the detection of doxylamine in plasma to 5 ng/ml.

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(5) "Dextromethorphan, *O*-Desmethyldextromethorphan, Doxylamine Assay in Urine," Food and Drug Administration, Washington, D.C., November 16, 1972. (Analytical Method No. 1652E.)

[1] Vacutainer; Becton, Dickinson & Co., Oxnard, Calif.
[2] Teflon-lined screw cap, No. 9826; Pyrex.
[3] Buchler Instruments, Fort Lee, N.J.
[4] Vortex-Genie, Model K-550-G, Scientific Industries, Bohemia, N.Y.
[5] Solvent delivery pump Model M6000, UV detector Model 440, Waters Associates, Milford Mass.
[6] Automation System No. 3385A, Hewlett-Packard, Palo Alto, Calif.
[7] WISP Model 710B, Waters Associates, Milford, Mass.
[8] The plasma levels of doxylamine were calculated as doxylamine succinate.