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Note

Determination of papaverine in plasma and urine by high-performance liquid chromatography

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Papaverine (6,7-dimethoxy-1-veratrylisoquinoline) is commonly used as a peripheral vasodilator. A number of analytical methods for the determination of papaverine in biological fluids such as plasma and urine have been described in the literature. The turbidimetric method [1], which involves phosphomolybdic acid, lacks in specificty for papaverine, whereas the differential spectrophotometric method [2] exhibits a specificity for papaverine, but unfortunately the limit of detection for papaverine is about 0.5 μg/ml. The gas chromatographic methods [3—5] for the determination of papaverine have the sensitivity necessary to detect low concentrations in plasma, however, these methods generally require large sample volumes and involve various extraction and purification steps. This leads to a considerable increase in the analysis time.

This paper reports a rapid and simple high-performance liquid chromatographic (HPLC) assay for papaverine in plasma and urine. The assay is quite specific for papaverine and requires a short sample preparation procedure prior to the chromatographic analysis.

EXPERIMENTAL

Reagents

Chloroform, isopropanol, methanol and sodium borate decahydrate were all reagent grade and purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Laudanosine, the internal standard, was obtained from Aldrich (Milwaukee, WI, U.S.A.). Papaverine was obtained from S.B. Penick & Co. (Lyndhurst, NJ, U.S.A.).

Apparatus

A Milton Roy Mini Pump (Milton Roy, Laboratory Data Control Division,

Riviera Beach, FL, U.S.A.) was used to deliver the mobile phase to a high-pressure loop injector (Model No. 7120 injector, Rheodyne, Berkeley, CA, U.S.A.), fitted with a 20-μl loop and a C_8 reversed-phase column (25 cm × 4.6 mm I.D., 10 μm particle size, Brownlee Labs., Santa Clara, CA, U.S.A.). The chromatography was carried out at ambient temperature. A variable-wavelength UV—Vis detector (Vari-chrom, Varian Instrument, Palo Alto, CA, U.S.A.) was used to monitor the column effluent at 239 nm. The output from the detector was connnected to a 1-mV potentiometric recorder (Linear Instrument, Irvine, CA, U.S.A.). The HPLC mobile phase was methanol—0.015 *M* sodium borate, pH 8.5 (58:42). This was pumped through the HPLC system at a rate of 2.7 ml/min and the resulting pressure was approximately $1.517 \cdot 10^7$ Pa.

Procedure

The plasma or urine sample (1.0 ml) was pipetted into a 16 × 125 mm culture tube and mixed with 0.3 ml of laudanosine solution (0.2 mg/l), followed by the addition of 10 ml of a mixture of chloroform—isopranol (95:5). After sealing the tube with a PTFE-lined screw cap, it was vortexed for 2 min and centrifuged for 30 min. The supernatant aqueous layer was aspirated and 8 ml of the organic phase were transferred to a clean tube. The organic phase was evaporated with an air stream at room temperature and the evaporated residue was reconstituted with 100 μl of methanol. A 20-μl aliquot of this solution was injected. The standard curves were developed by spiking blank plasma or urine samples with known amounts of papaverine to give concentrations from 0.0— 10.0 μg/ml.

Calculation

A standard curve was developed for each series of analyses by plotting the ratio of the height of papaverine peak to the height of laudanosine peak versus the concentration of papaverine. The concentrations of the unknown samples were subsequently determined from the standard curve.

RESULTS AND DISCUSSION

The chromatographic conditions for the analysis of papaverine in plasma and urine were selected after appropriate preliminary investigations with a number of different mobile phases. It was observed that the elution of papaverine from the HPLC column was considerably influenced by the pH and methanol concentration of the mobile phase.

The chromatograms resulting from the analysis of a blank plasma sample and a plasma sample spiked with a known amount of papaverine are shown in Fig. 1. Fig. 2 presents the chromatograms from blank and spiked urine samples. Under the chromatographic conditions selected for this assay, papaverine eluted from the HPLC column with a retention time of 5.0 min while laudanosine, the internal standard, eluted at 9.5 min. No endogenous components with a retention time similar to either papaverine or laudanosine were observed during the analyses of the plasma or urine samples. The specificity of the assay was tested by spiking the plasma and urine samples with theophylline, caffeine, theobromine, tetracycline, oxytetracycline, gentamycin,

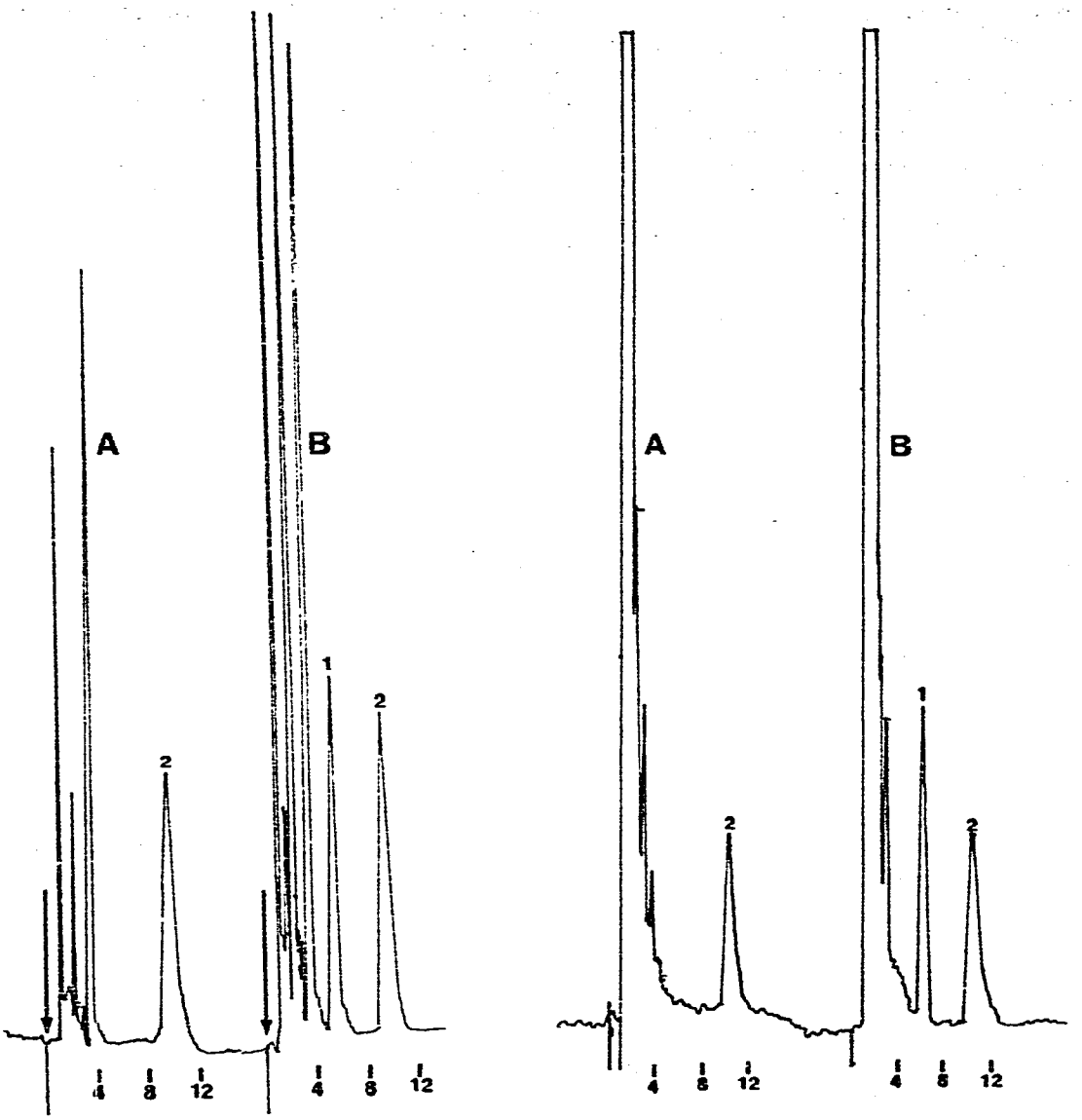

Fig. 1. Chromatograms of blank plasma (A) and plasma spiked with 0.5 μg/ml papaverine (B). Peaks: papaverine (1) and laudanosine (2).

Fig. 2. Chromatograms of blank urine (A) and urine spiked with 0.5 μg/ml papaverine (B). Peaks: papaverine (1) and laudanosine (2).

chlorothiazide and hydrochlorothiazide. These samples were carried through the entire assay procedure and none of these drugs were found to interfere with the analysis of papaverine.

The standard curve for papaverine from the plasma and urine samples was linear over the concentration range studied (Table I). The linearity of the standard curve was studied over a concentration range of 0.05- 1.0 μg/ml of

TABLE I

STANDARD CURVE DATA FOR PAPAVERINE

$n = 5$.

Sample	Conc. of papaverine (μg/ml)	Ratio*	Equation
Plasma	0.00	0.00	$Y = 2.058X + 0.01$
	0.05	0.1858	$r = 0.997$
	0.10	0.3927	
	0.50	1.2058	
	1.00	2.1120	
Urine	0.00	0.00	$Y = 1.567X + 0.02$
	0.05	0.0973	$r = 0.999$
	0.10	0.1989	
	0.50	0.8253	
	1.00	1.5758	

*Ratio of papaverine peak height to laudanosine peak height.

papaverine, both in plasma and urine, and was found to be linear with correlation coefficients for linear regression of 0.997 and 0.999, respectively. The maximum sensitivity for papaverine detection was 25 ng/ml of plasma and urine, since at this concentration the signal-to-noise ratio was about 4 or 5 to 1.

The efficiency of the extraction step was checked by extracting known concentrations of papaverine in replicates of five, from plasma and urine samples. The recovery of papaverine from plasma samples ranged from 78.6–87.2% with a mean recovery of 84.3%, whereas the recovery from urine samples ranged from 78.57—104.46% with a mean recovery of 88.2%.

The precision and reproducibility of the assay procedure was determined by analyzing papaverine at three concentration levels (0.1, 1.0, 10.0 μg/ml), in triplicate. In plasma, at these levels, the coefficients of variation were found to be 6.16, 1.93 and 7.5% while the coefficients of variation at the same levels from the urine samples were 3.39, 1.13 and 0.8% (Table II).

TABLE II

REPRODUCIBILITY DATA FOR PAPAVERINE

Sample	Conc. of papaverine (μg/ml)	Ratio* (mean ± S.D.)	C.V. (%)
Plasma	0.10	0.4250 ± 0.0262	6.16
	1.00	1.9593 ± 0.0378	1.93
	10.0	45.583 ± 3.42	7.5
Urine	0.10	0.5152 ± 0.017	3.39
	1.00	4.0747 ± 0.0463	1.13
	10.0	39.3900 ± 0.345	0.8

*Ratio of papaverine peak height to laudanosine peak height.

The method described is the first HPLC-method which is rapid, simple and with sufficient sensitivity for the quantitative determination of papaverine in plasma and urine. The lower limit of detection of papaverine in plasma and urine is 25 ng/ml with excellent reproducibility. The method is specific for papaverine and no interference from other drugs was observed.

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