Simple and Rapid High-Pressure Liquid Chromatographic Determination of Papaverine in Plasma

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Abstract □ A rapid and sensitive high-pressure liquid chromatographic (HPLC) assay is described for the quantification of papaverine in plasma. A paired-ion, reversed-phase system was employed with detection at 254 nm. This procedure yielded reproducible results with a sensitivity of 2 ng/ml. Total extraction and quantification time was <1 hr. The suitability of this procedure for plasma sample analysis from a bioavailability study was demonstrated.

Keyphrases □ Papaverine—analysis, high-pressure liquid chromatography, human plasma, bioavailability □ Relaxants (smooth muscle)—papaverine, high-pressure liquid chromatographic analysis, human plasma, bioavailability □ High-pressure liquid chromatography—analysis, papaverine in human plasma, bioavailability

Papaverine, a smooth muscle relaxant, is used primarily as a peripheral vasodilator. *In vitro* studies implicated cyclic AMP as a mediator of the papaverine pharmacological effect (1). Its therapeutic effectiveness is limited by its rapid metabolism. The resultant necessity for frequent dosages has generated interest in the development of a dosage formulation to sustain therapeutic blood levels over longer dosage intervals (2–5).

Relatively few methods are available for the measurement of blood papaverine levels following oral administration. GLC methods require lengthy cleanup procedures and large sample volumes, which are cumbersome for the numerous samples generated by a bioequivalency study. In addition, the detection limits of these methods (generally in the 10-ng/ml range) are such that half-life determination of some formulations has been quite difficult (3, 6, 7). A recent GLC–mass spectroscopy procedure offers improved sensitivity (5 ng/ml) but requires lengthy extraction and expensive instrumentation (8).

Recently, the use of paired-ion, reversed-phase high-pressure liquid chromatography (HPLC) for quantitative drug analysis was demonstrated (9–11). This paper reports a simple, rapid, and highly sensitive HPLC method for papaverine determination in plasma.

EXPERIMENTAL

Chemicals and Reagents—1-Heptanesulfonic acid sodium[1] and papaverine hydrochloride[2] were used as received; all other chemicals and reagents were USP, NF, or ACS grade. Methanol and ether were distilled in glass prior to use.

HPLC System—A high-pressure liquid chromatograph[3] equipped with a valve injector[4], a guard column[5], a microparticulate reversed-phase HPLC column[6], a fixed-wavelength UV detector[7], and a potentiometric recorder[8] was used for separation and quantification. The system was operated at ambient temperature with a flow rate of 2.0 ml/min, and the detector was set at 254 nm.

The mobile phase consisted of 55% (v/v) methanol, 1% (v/v) acetic acid, and 0.005 *M* 1-heptanesulfonic acid sodium. Prior to use, the mobile phase was degassed by vacuum filtration through a 5-μm filter[9].

Procedure—An aliquot of plasma, 1.0 ml, in a 15-ml glass-stoppered centrifuge tube was spiked with 2 μg of chlorpheniramine maleate as the internal standard (100 μl of an aqueous solution) and vortexed. A 0.2-ml aliquot of 7 *N* NaOH and 10.0 ml of ether were added. The sample was shaken mechanically for 15 min and centrifuged to separate the phases.

The ether phase was transferred with a Pasteur pipet to another centrifuge tube and extracted with 0.2 ml of 0.3 *N* HCl by shaking and centrifuging as described previously. The ether phase was aspirated, and 100 μl of the aqueous phase was injected.

The ratio of the peak height of papaverine to that of the internal standard was used to calculate the papaverine concentration, based on a calibration curve prepared from spiked plasma samples.

Plasma Level Study—To demonstrate the applicability of this procedure to the determination of plasma papaverine levels from a bioavailability study, 150 mg of papaverine hydrochloride[10] was administered orally with 100 ml of water to two healthy male volunteers who had fasted overnight. Blood samples, 10 ml, were withdrawn from the cubital vein with heparinized evacuated tubes and centrifuged, and the plasma was transferred to screw-capped culture tubes before storage at −25°.

Table I—Standard Curve for Papaverine in Plasma [a]

Plasma Papaverine Concentration (as Free Base), ng/ml	Peak Height Ratio, Papaverine to Internal Standard	Response Factor[b]
5	0.054	10.8
10	0.119	11.9
25	0.304	12.2
50	0.591	11.8

[a] Linear regression equation: $y = 84.0813x + 0.0402$, $r = 0.9998$. [b] Peak height ratio divided by papaverine concentration in micrograms per milliliter.

Table II—Mean Coefficient of Variation Calculated from Duplicate Analyses of Samples

Number of Samples, *n*	Concentration (Range of Means), ng/ml	Mean *CV*, %
12	0–25	4.5
17	25–100	3.5
20	100–200	3.0
26	200–500	3.7
20	500–1000	1.5
12	1000–3000	3.0

RESULTS AND DISCUSSION

A UV scan of a solution of 1 μg of papaverine/ml in the mobile phase was performed to determine the optimum wavelength for detection. The absorption maximum was a broad band at ~250 nm, demonstrating the suitability of using a 254-nm fixed-wavelength UV detector.

The use of 10 ml of ether in the initial extraction, followed by back-extraction into dilute acid, yielded excellent recovery (~95–100%), which was independent of the concentration with essentially no interference

[1] Eastman Kodak, Rochester, N.Y.
[2] Marion Laboratories, Kansas City, Kans.
[3] Series 3, Perkin-Elmer, Norwalk, Conn.
[4] Model 7105, Rheodyne, Berkeley, Calif.
[5] CO:PELL ODS, Whatman, Clifton, N.J.
[6] Partisil 10-ODS, ISCO, Lincoln, Neb.
[7] Model 220, Chromatronix Inc., Berkeley, Calif.
[8] Omniscribe, Houston Instrument, Austin, Tex.
[9] FHUP 47 mm, Millipore Corp., Bedford, Mass.
[10] PAVABID Plateau CAPS, Marion Laboratories, Kansas City, Kans.

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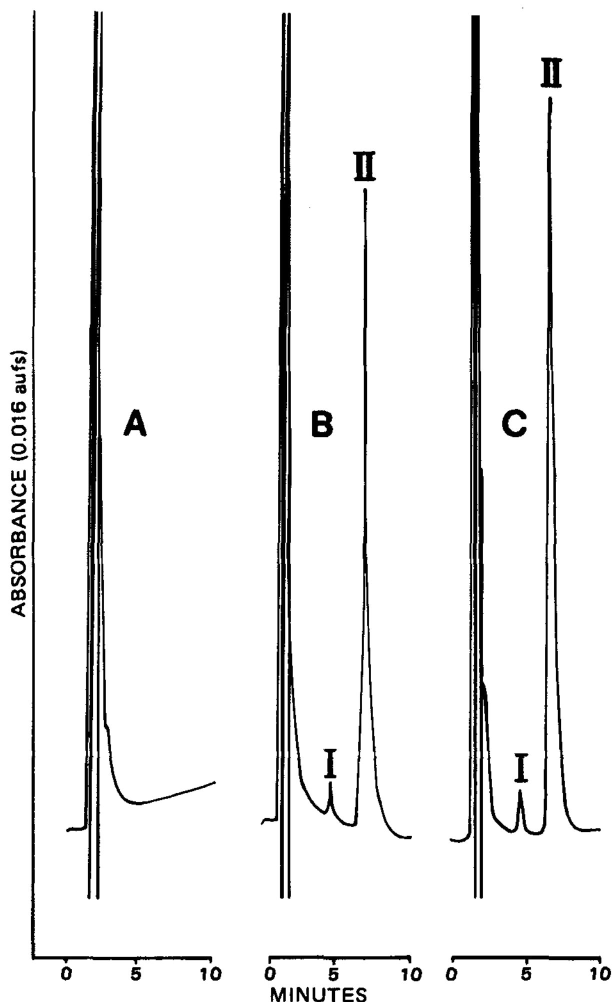

Figure 1—*Chromatogram of a human plasma blank (A), spiked plasma containing 5 ng of papaverine free base/ml and 2 µg of the internal standard/ml (B), and plasma from a dosed volunteer (1 hr) containing 6.0 ng of papaverine free base/ml and 2 µg of the internal standard/ml (C). Key: I, papaverine (free base); and II, internal standard.*

from the plasma. Good reproducibility is indicated by coefficients of variation ($n = 12$) of 4.0, 1.7, and 2.0% at 25, 75, and 500 ng of papaverine/ml, respectively.

Minor modifications in the extraction procedure (use of 4 µg of internal standard/ml and back-extraction into 1.5 ml of acid) were implemented in this laboratory for papaverine analysis in plasma obtained from multiple-dose bioavailability studies. These modifications facilitated the use of an autosampler[11], resulting in increased efficiency and reduced technician time.

Standard curves based on the ratio of the peak height of papaverine to that of the internal standard were linear over a wide range (2.0 ng/ml–1.0 µg/ml) and had intercepts near the origin. The data used to establish a typical standard curve for the plasma level study are summarized in Table I. The correlation coefficient of 0.9998 and the consistency of the response factors indicate the good linearity.

Daily standard curves showed a similar degree of linearity, reproducibility, and sensitivity. The average slope for seven standard curves using 2 µg of internal standard/ml was 0.01285 ± 0.00059 (mean ± *SD*), with an average correlation coefficient of 0.9997 ± 0.0003 (mean ± *SD*) and a detection limit (*i.e.*, three times baseline noise) of 2 ng/ml. Over 5 months with three different columns and various internal standard

[11] LC-420, Perkin-Elmer, Norwalk, Conn.

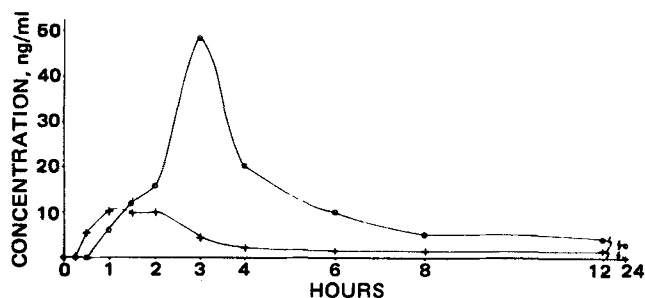

Figure 2—*Human plasma concentration of papaverine free base after administration of a single oral 150-mg dose of papaverine hydrochloride. Key: +, Subject 1; and O, Subject 2.*

concentrations, the average correlation coefficient was 0.9997 ± 0.0003 (mean ± *SD*, $n = 24$), demonstrating the linearity of this method over an extended period. In Table II, the mean coefficient of variation, as calculated from replicate samples from subjects in four bioavailability studies, indicates the reproducibility that may be expected from actual samples rather than from spiked plasma.

Typical chromatograms of blank human plasma, plasma spiked with papaverine and the internal standard, and plasma collected 60 min after administration of a 150-mg oral papaverine dose and spiked with internal standard are shown in Fig. 1. Papaverine and the internal standard were well resolved and eluted with retention times of 5.0 and 7.2 min, respectively.

The plasma papaverine time courses for the two subjects in the bioavailability study are shown in Fig. 2. The significant subject variability noted by others (3, 8) also was found in this study, as shown by the differences in the concentration maxima and time to peak values for the two subjects.

Current methods for papaverine analysis in plasma are time consuming and do not provide the sensitivity often required as demonstrated by the data for Subject 1 (Fig. 2). These problems were overcome with the present HPLC method, which has a detection limit of 2 ng/ml, response linearity over a wide range, and a simplified extraction procedure. Moreover, it uses only 1 ml of plasma and is adaptable to automatic sampling systems.

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