GLC Determination of Papaverine in Biological Fluids

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Abstract □ A procedure for the determination of papaverine in blood plasma or urine, based on ion-pair extraction with diethylhexyl phosphate and GLC, permits specific determination of papaverine at levels as low as 0.01 μg/ml. The applicability of this method for bioavailability studies of papaverine after acute administration of oral dosage forms is demonstrated.

Keyphrases □ Papaverine—GLC determination in biological fluids □ GLC—analysis, papaverine in biological fluids

Few methods for the estimation of papaverine in biological fluids such as blood, plasma, and urine have been described. The turbidimetric method utilizing phosphomolybdic acid (1) is not specific for papaverine in the presence of its metabolites. Specificity has been demonstrated for the differential spectrophotometric method combined with a heptane extraction procedure (2) but, unfortunately, the sensitivity of this method (about 0.5 μg) precludes its use for the measurement of papaverine concentrations present in biological fluids after acute oral administration.

A procedure based upon ion-pair extraction and GLC is reported here; it is specific and permits the measurement of papaverine levels down to 0.01 μg/ml in a 5-ml sample. The suitability of the method for evaluating the acute bioavailability of orally administered dosage forms is also described.

Because papaverine is a weak base (pKa ≈ 6.4), its extraction from an aqueous solution by an organic solvent would be expected to be optimal under alkaline conditions. Preliminary studies supported this prediction since an extraction greater than 90% was achieved with a single partition from a pH 12 solution into an equal volume of ether. However, subsequent concentration and GLC of the extract yielded gross interfering peaks with respect to both papaverine and the internal standard. Attempts to reduce this interference by traditional techniques such as buffer washing and/or repeated back-extraction were unsuccessful. However, the inclusion of an ion-pair extraction step with diethylhexyl phosphate and extraction of papaverine as its protonated species from an aqueous phase adjusted to pH 4.0 yielded chromatograms free from interfering peaks.

EXPERIMENTAL

To 5 ml of plasma in a 13-ml centrifuge tube were added 1 ml of internal standard solution (dibucaine hydrochloride, 5 μg of base/ml in distilled water) and 1 ml of 5 *N* NaOH. The solution was extracted with 2 × 5 ml of freshly distilled ether (analytical grade) by gently shaking for 5 min and then centrifuging for the same time. The ether extracts were transferred and combined in a separate centrifuge tube containing 4 ml of 0.2 *M* acetate buffer (pH 4.0).

After extracting, under the same time conditions, the ether was discarded and the aqueous phase was shaken with 2 × 5 ml of ethereal 0.1 *N* diethylhexyl phosphate solution. The ethereal phase was aspirated off, shaken with 3 ml of 1 *N* HCl, and then discarded. The aqueous phase was made alkaline with 1 ml of 5 *N* NaOH and extracted with 3 ml of ether. The final extract was evaporated to dryness in a 10-ml centrifuge tube, using a water bath maintained at 42°, and the residue was reconstituted with 4–12 μl of analytical grade carbon disulfide. Depending upon the expected concentration of papaverine, from 1 to 2 μl of this solution was injected into the gas chromatograph.

The relatively high molecular weight of papaverine necessitated the use of a lightly loaded column and high temperature conditions if the analysis was to be completed in a reasonable time. A gas chromatograph[1] equipped with a flame-ionization detector was employed. The column was Pyrex glass, 1.8 m (6 ft) × 2 mm i.d., packed with 80–100-mesh, dimethyldichlorosilane-treated, calcined diatomaceous earth coated with 1% phenyl (50%) methyl sili-

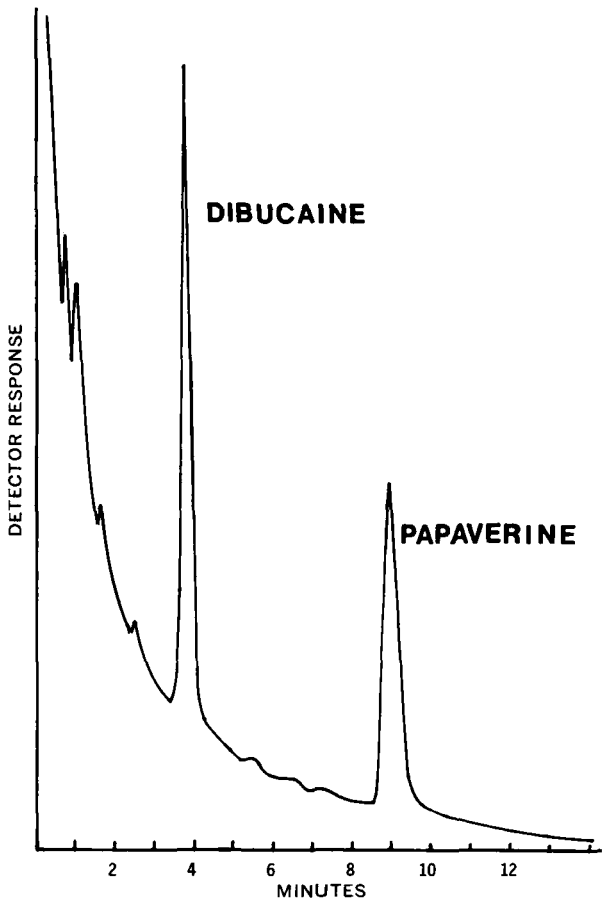

Figure 1—*Chromatogram obtained by spiking blank plasma with dibucaine hydrochloride at 1.0 μg/ml and papaverine hydrochloride at 1.0 μg/ml.*

[1] Varian Aerograph, model 1740, Varian Instrument Division, Palo Alto, Calif.

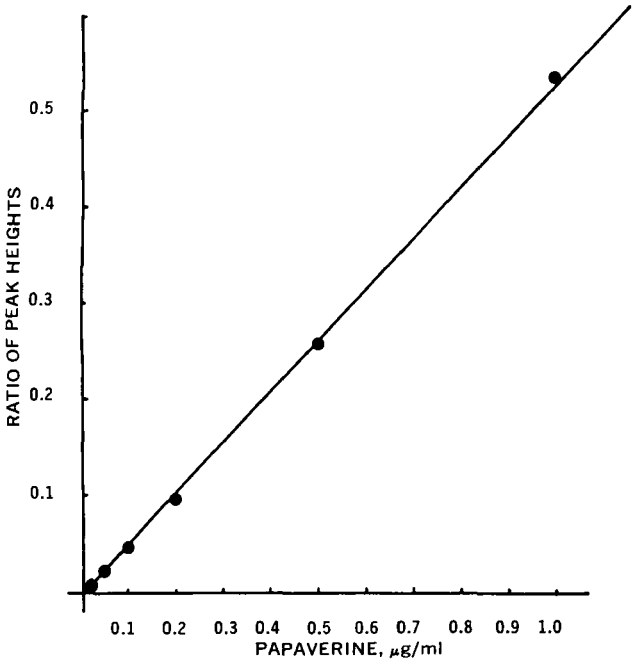

Figure 2—*Typical standard curve obtained from spiked plasma samples.*

cone fluid[2]. The column was conditioned at 300° with a nitrogen carrier gas flow for 8 hr and then overnight under the following operating conditions: oven temperature, 275°; injection port temperature, 285°; detector oven temperature, 300°; carrier gas flow rate, 30 ml/min; hydrogen flow rate, 30 ml/min; and air flow rate, 300 ml/min. The column was silanized[3] *in situ* initially and then periodically.

To reduce further any potential adsorption of papaverine by the column, three injections of a concentrated ethereal solution of papaverine and dibucaine were made prior to any series of analyses. The plasma concentration of papaverine was obtained by calculating the peak height ratio of the drug to that of the internal standard and relating this to a standard curve, which was prepared daily over the concentration range of 0.01–2 μg of papaverine/ml.

RESULTS AND DISCUSSION

Well-resolved, sharp, and symmetrical peaks were obtained for both dibucaine and papaverine with retention times of 3.9 and 9.1 min, respectively (Fig. 1). Extraction of blank plasma samples revealed no interfering peaks in the region of the retention time of either papaverine or dibucaine. A typical standard curve is shown in Fig. 2, and excellent day-to-day reproducibility of the slope of the curve was obtained. Data for five replicate determinations on spiked plasma samples indicate a relative standard deviation of 5.1% at 0.5 μg/ml and 14.4% at 0.05 μg/ml.

The plasma concentrations obtained after the oral administration of 2 × 150-mg papaverine hydrochloride capsules to two male volunteer subjects are shown in Fig. 3 and indicate that the drug is rapidly absorbed since peak levels were seen within 2 hr. Terminal phase half-lives for these two subjects were 1.0 and 1.4 hr. Assay results on urine samples were in agreement with the previous observation (2) that less than 1% of the dose is recoverable in urine as

[2] Gas Chrom Q coated with 1% OV-17, Applied Science Laboratories, State College, Pa.

[3] Silyl 8, Pierce Chemical Co., Rockford, Ill.

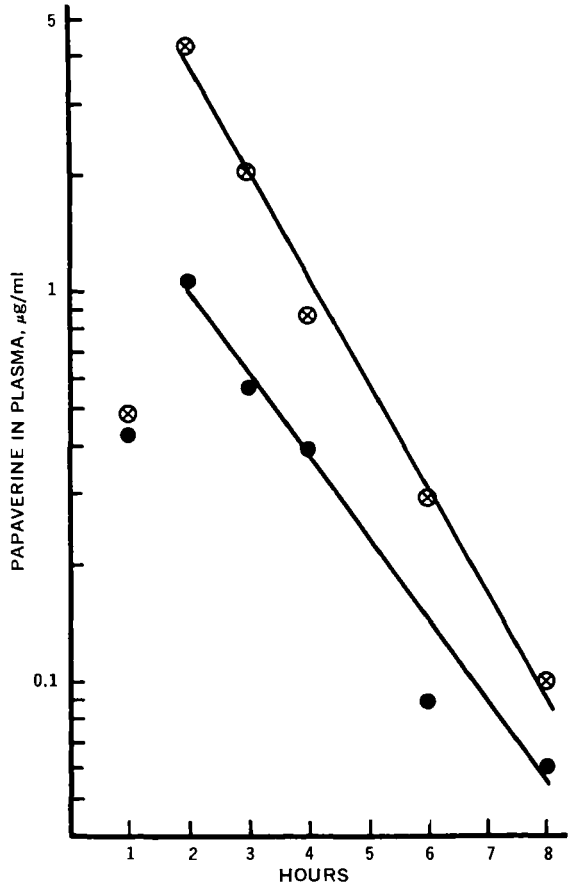

Figure 3—*Plasma papaverine levels, expressed as papaverine hydrochloride, in two human subjects following oral administration of 2 × 150-mg papaverine hydrochloride capsules.*

intact papaverine. The almost negligible urinary recovery of intact papaverine indicates that phenolic metabolites of papaverine do not interfere with the described assay procedure.

This assay procedure was found suitable for determination of papaverine plasma levels up to 12 hr after administration of 300 mg of papaverine hydrochloride in timed-release form. These studies will be reported separately.

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