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DETERMINATION OF PAPAVERINE IN BLOOD SAMPLES BY GAS CHROMATOGRAPHY USING A FLAME-IONIZATION AND A NITROGEN–PHOSPHORUS DETECTOR

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SUMMARY

The determination of papaverine in blood samples using a flame-ionization and a nitrogen–phosphorus detector is described. The method is quite simple and permits the determination of papaverine at blood levels at 5–500 ng/ml.

INTRODUCTION

Papaverine is therapeutically used as a peripheral vasodilator. The drug is generally administered either in solution or as a rapid release or sustained release tablet/capsule. In order to establish the efficacy and bioavailability of the experimental dosage forms, a sensitive and accurate method for the determination of papaverine in plasma is necessary.

Two recently published gas chromatographic (GC) methods for the determination of papaverine have the sensitivity necessary to determine the concentration in blood or plasma, which often is as low as 10 ng per ml, after injection of a therapeutic dose. According to the method by Guttman *et al.*[1], a selective ion-pair extraction step to clean up the sample from interfering peaks is necessary. Investigation of this method in our laboratory produced unsatisfactory results. In the method of Mussini and Marzo[2], the internal standard was added only after the extraction steps were completed. Also, the blood levels measured using an electron-capture detector were those achieved after administering twice the therapeutic dose level. In another study, De Graeve *et al.*[3] describe two methods: one, good only under certain conditions, using a capillary column system; the other, using mass fragmentography. Both methods, however, require unconventional instrumentation not suitable for routine use.

In the method described below, the internal standard was added to the sample prior to the extraction step. High sensitivity was achieved by the use of a conventional flame-ionization detector (FID) or a nitrogen–phosphorus detector (NPD).

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EXPERIMENTAL

Reagents

Diethyl ether (Mallinckrodt-nanograde) was purified by refluxing for 24 h over ferrous sulfate and sulfuric acid followed by distillation. Only the amount needed for the day was distilled. Anhydrous sodium sulfate was purified by the method of Street[4]. All other reagents and solvents were used as received without further purification.

Equipment

A Hewlett-Packard Model No. 7610 high efficiency gas chromatograph equipped with FID was used. A 1.2 m × 3 mm I.D. glass column was packed with 3% OV-17 on Gas-Chrom Q (80–100 mesh; pretested type from Applied Science Labs., State College, Pa., U.S.A.) and operated at 265°. The original injection port of the instrument was substituted with that of an F & M Model 810 gas chromatograph. This injection block allows the insertion of the column directly into the septum area to permit heated, on-column injection. The injector and detector temperatures were 300 and 320°, respectively. Flow-rates of carrier gas (nitrogen), hydrogen and air were 80, 40 and 300 ml/min, respectively. The electrometer sensitivity was $8 \cdot 10^{-11}$ A/mV. The column was silylated *in situ* by injecting 15 μl of Sylon-HTP (Supelco, Bellefonte, Pa., U.S.A.) and also treated with a concentrated alcoholic solution of papaverine and strychnine prior to any series of analyses.

A Hewlett-Packard Model 5730 gas chromatograph equipped with an NPD was also used. This detector and its applications have been described recently by Burgett *et al.*[5]. A 1.2 m × 2 mm I.D. glass column was packed with 2% OV-101 on Chromosorb W HP (100–120 mesh; Hewlett-Packard, Avondale, Pa., U.S.A.) and operated at 275°. The injector and detector temperatures were 300°. The electrometer sensitivity was $8 \cdot 10^{-11}$ A/mV. The carrier gas (helium) flow-rate was 30 ml/min, plasma gases flow-rate (8% hydrogen in helium) was 30 ml/min, and the air flow-rate was 50 ml/min. The voltage for the alkali metal bead was set at 16. The column was treated with a solution of papaverine and strychnine as described above, but the use of a silylating agent was avoided.

Glassware cleanliness was achieved by baking in an inexpensive kiln (Model A88-B; Paragon Industries, Dallas, Texas, U.S.A.) set at 600°. After cooling, the glassware was also silylated using Sylon-CT (Supelco).

Preparation of standards

Using the flame-ionization detector

An amount of papaverine HCl equivalent to 50 mg of free base was dissolved in 50 ml water. This stock solution was then diluted with water to yield concentrations of 250, 500 and 1000 ng/ml (working standard solutions). The internal standard solution of strychnine was prepared by dissolving 40 mg of the compound in 5 ml of ethanol and diluting to 50 ml with 0.1 *N* hydrochloric acid. This solution was further diluted with water to yield a concentration of 2 μg/ml.

Using the nitrogen–phosphorous detector

Above stock solution of papaverine HCl was diluted with water to yield concentrations of 20, 40, 80 and 160 ng/ml of free base (working standard solutions). Similarly, the internal standard solution was diluted to a concentration of 100 ng/ml.

Assay procedure

Blood or plasma (3.0 ml), internal standard (1.0 ml), water (1.0 ml) and 10.0 *N* KOH (1 ml) were pipetted into a 50-ml centrifuge tube equipped with a PTFE stopper and shaken for about 30 sec on a vortex mixer. Toluene (10 ml) was added and the tube shaken vigorously for 10 min on a reciprocating shaker set at high speed (Eberbach, Ann Arbor, Mich., U.S.A.). The tube was centrifuged and the toluene extract transferred to a second 50-ml centrifuge tube. The extraction was repeated with a second 10-ml portion of toluene. The two toluene extracts were combined, 1.0 *N* hydrochloric acid (3 ml) was added and the toluene was extracted by shaking for 10 min and then centrifuged. The organic phase was removed carefully, 1 ml of 10.0 *N* KOH was added and mixed. After cooling, 4 ml of ether was added and the tube was shaken for 2 min on a vortex mixer, then centrifuged. The ether extract was transferred into a 15-ml centrifuge tube and treated with 0.5 g of anhydrous sodium sulfate. The dried extract was decanted into a 5-ml Reacti-Vial (Pierce, Rockford, Ill., U.S.A.) and the solvent was evaporated either by leaving the samples at room temperature overnight or using a water bath at 40° and a stream of dry nitrogen. The residue was dissolved in 20 μl of isopropyl alcohol and 3.0 μl (for the FID) or 1.0 μl (for the NPD) were injected into the gas chromatograph. Standards were prepared by adding 1.0 ml of each working standard solution to 3.0 ml of blood or plasma. These standards were processed concurrently with the samples.

CALCULATIONS

A standard curve was prepared for each series of analyses by plotting on linear graph paper the ratio of the peak height of papaverine to peak height of internal standard *versus* known amount (ng) of papaverine. Unknown sample concentrations were calculated from the standard curve.

RESULTS AND DISCUSSION

GC of alkaloids, amines, etc. often suffers from the adsorption problems resulting in the late eluting unsymmetrical peaks. The problems are generally overcome by silanization of column and support materials as well as derivatization of the compound to be analyzed. We minimized the adsorption effects by *in situ* silanization of the column and injection of a concentrated solution of papaverine and the internal standard prior to routine analysis. Fig. 1A shows the elution of papaverine and the internal standard with a symmetrical peak shape and Fig. 1B shows that there is no interference from plasma endogenous materials in the elution region of the drug or the internal standard.

The recovery study was carried out by spiking known amounts of papaverine in plasma. The extraction efficiency is shown in Table I.

The standard curve was plotted daily and a typical curve was found to be linear between 0–1000 ng. The results demonstrate that the accuracy and precision of the method are excellent.

In earlier work, the purified ethyl ether was used in the initial extraction step. Although satisfactory results were obtained, the elution of a peak just ahead of the papaverine peak was limiting the precision at high sensitivity when using the FID.

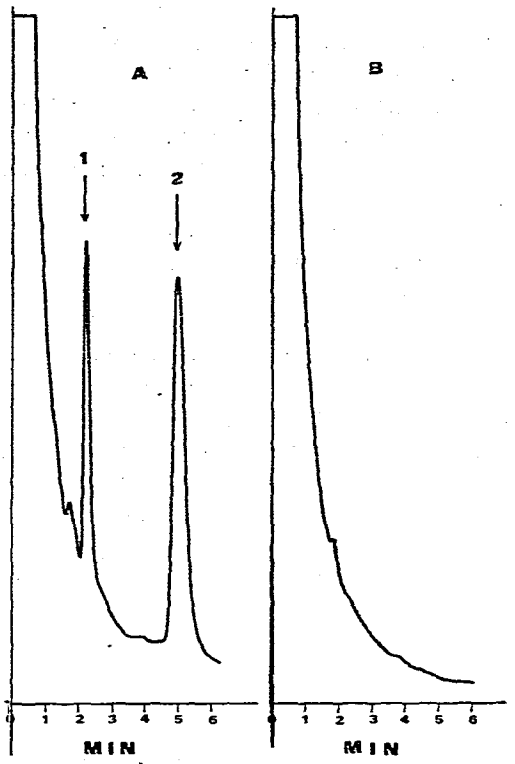

Fig. 1. Gas chromatograms obtained from 3 ml of blood containing 1000 ng of papaverine and 2000 ng of strychnine (A) and from 2 ml of control blood (B). The arrows 1 and 2 indicate the retention time of papaverine and strychnine, respectively. Detector: FID.

TABLE I

PRECISION AND ACCURACY OF PAPAVERINE ANALYSIS IN BLOOD

Each result is the mean of four determinations. S.D. = standard deviation; R.S.D. = relative standard deviation.

Nanograms added	*Nanograms found*	*S.D.*	*R.S.D. (%)*	*Detector*
20.0	19.8	2.5	12.7	NPD
40.0	40.0	2.7	6.7	NPD
80.0	79.5	3.9	4.9	NPD
160.0	159.5	4.0	2.6	NPD
100.0	100.3	10.5	10.5	FID
250.0	250.0	26.9	10.8	FID
500.0	502.5	20.6	4.1	FID
1000.0	995.5	56.9	5.7	FID

The elimination of this interfering impurity was accomplished by using toluene in place of ether. The FID was used at blood levels higher than 30 ng/ml, but at lower levels between 5–30 ng/ml the NPD gave excellent results. Once the voltage applied to the bead and its position were optimized, no other adjustments were needed for

several weeks. The method was used to analyze blood samples from human subjects who received a single dose of 150 mg of papaverine HCl as an elixir. Results from two subjects are illustrated in Fig. 2. Peak blood levels were 443 and 454 ng/ml, respectively at 0.5 and 1 h.

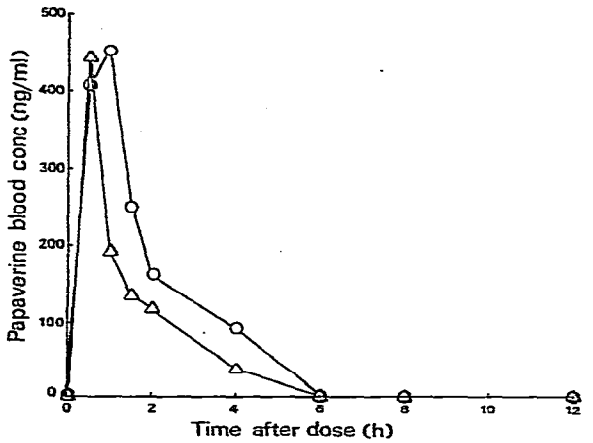

Fig. 2. Time course of blood papaverine concentration in two human subjects given a single dose of 150 mg of papaverine HCl as an elixir. △, Subject A; ○, Subject B.

In conclusion, the method described here was found to be accurate and reliable for the determination of papaverine in blood/plasma. The use of selective NPD enhanced the sensitivity of the method.

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