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DETERMINATION OF PAPAVERINE IN BLOOD SAMPLES BY GAS–LIQUID CHROMATOGRAPHY AND MASS FRAGMENTOGRAPHY

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SUMMARY

The measurement of papaverine in blood samples by using either a glass capillary column with a flame-ionization detector or a packed column with mass fragmentographic detection is described. The two methods permit the determination of the normal range of concentrations of papaverine in blood (2–500 ng/ml). Owing to its high specificity, mass fragmentography is greatly superior to capillary chromatography, which is sometimes subject to interferences by solvent impurities.

INTRODUCTION

Although papaverine is a commonly used drug in man, very few methods[1,2] are available for its measurement in biological fluids. Most of the published studies were directed to the determination of this alkaloid in plant extracts or pharmaceutical preparations and used techniques such as volumetric titration[3,4], UV[5] and visible[6] spectrophotometry, spot tests on thin-layer chromatograms[7,8], spectrofluorimetry[9] and gas–liquid chromatography (GLC)[10]. The blood levels of papaverine in patients receiving the usual dose (50–100 mg three times daily) are normally below 500 ng/ml so that, except for GLC or if large amounts of blood are withdrawn, none of the above methods are suitable for this purpose (Table I).

To measure the biological half-life of orally administered papaverine, more sensitive and specific methods are required in order to determine as little as 10 ng/ml of papaverine in blood. This level could not be measured with the spectrophotometric method described by Axelrod *et al.*[1]. The other available method[2], using GLC with an electron-capture detector, seemed more accurate and applicable for our study but it was developed on blood samples taken from man and dogs receiving higher doses of papaverine (4 and 5 mg/kg, *i.e.*, about twice the therapeutic dose).

In this paper, we describe the difficulties of applying a conventional GLC technique to the measurement of blood papaverine concentrations and then present two new methods for this assay. The first method uses GLC on glass capillary columns and is applicable only under certain conditions; the second method uses mass frag-

TABLE I

ANALYTICAL METHODS FOR THE DETERMINATION OF PAPAVERINE IN BLOOD

Pharmacological blood levels of papaverine: $< 0.5\ \mu g/ml$.

Method	*Detection limit (µg/ml)*	*Reference*
TLC with spot tests	5–7	7, 8
UV spectrophotometry	2–5	5
Spectrofluorimetry	2.5	9
Visible-light spectrophotometry	2.5	6
Gas–liquid chromatography (packed column) with:		
Flame-ionization detector	5	10
Electron-capture detector	0.2	2

mentography and gives very accurate results. In addition to its high sensitivity, the latter method is also the only one that is absolutely specific.

MATERIALS AND METHODS

Reagents

All solvents and reagents, including the papaveraldine (Aldrich, Milwaukee, Wisc., U.S.A.) which served as the working standard, were of analytical-reagent grade and used without further purification.

Extraction of blood samples

The method followed was essentially that of Mussini and Marzo[2]. A 3-ml volume of heparinized whole blood was treated with 5 ml of 0.12 *N* sodium hydroxide solution and the papaverine was extracted quantitatively with two 10-ml volumes of diethyl ether. The drugs were back-extracted in 5 ml of 0.1 *N* hydrochloric acid and 4 ml of this phase made alkaline with 0.5 ml of 1 *N* sodium hydroxide solution was extracted with two 10-ml volumes of diethyl ether. Papaveraldine (400 ng), the working standard chosen for its structural similarity to papaverine, was then added to the organic phase. The solvent was slowly evaporated to dryness and the residue, re-dissolved in 3 ml of diethyl ether, was then transferred into a capillary tube sheltered from light. The evaporation was carried out carefully under a stream of nitrogen.

The drug residue was finally re-dissolved in 50 µl of chloroform and 1–5 µl of the solution obtained was injected into the chromatographic column.

Gas–liquid chromatography on a packed column

We used a Packard Model 846 chromatograph equipped with a flame-ionization detector. The glass chromatographic column (2 m × 3 mm I.D.) was packed with OV-17 (3%) on 100–120-mesh Gas-Chrom P (AW-DMCS). The gas vector flow was set at 40 ml/min and the oven temperature at 272°. The temperature of the injector and the detector was 300°.

Gas–liquid chromatography on a glass capillary column

An Intersmat Model IGC-12 DFL chromatograph equipped with a flame-ionization detector was used. The Pyrex capillary column, (30 m × 1 mm O.D. × 0.25 mm I.D.) was treated and deactivated according to the technique developed by Rutten and Luyten[11,12]. A static coating containing 0.8% SE-30 in *n*-hexane according to Bouche and Verzele[13] was applied. The introduction of the samples into the column was achieved by means of a solid injector device described by Van den Berg and Cox[14]. The temperature of the chromatographic oven was 225° and that of the injector and detector cell was 280°. Nitrogen, serving as the carrier gas, was regulated at *ca.* 1.5 ml/min for an inlet pressure of 1.3 bar. In order to use the flame-ionization detector under the optimal conditions, 30 ml/min of make-up nitrogen was introduced at the base of the detector.

Mass fragmentography

Specific ions of papaverine and papaveraldine were detected on an LKB 9000 S mass spectrometer equipped with a multiple ion detector. The glass chromatographic column was filled with 1% OV-1 on 100–120-mesh Gas-Chrom P and used under the same temperature conditions as those for the glass capillary column. The temperature of the separator was set at 290° and that of the source at 310°. The helium flow-rate was 32 ml/min, which corresponds to the minimum of the Van Deemter curve. The ionization current was 60 μA and the electron energy 20 eV.

RESULTS

Gas–liquid chromatography on a packed column

Under the above conditions, the analysis of organic extracts obtained from a 1-ml sample of blood serum loaded with 50 μg of papaverine gave a chromatogram such as that shown in Fig. 1. The peak corresponding to papaverine was obscured by a larger peak due to one of the numerous non-volatile impurities in the diethyl

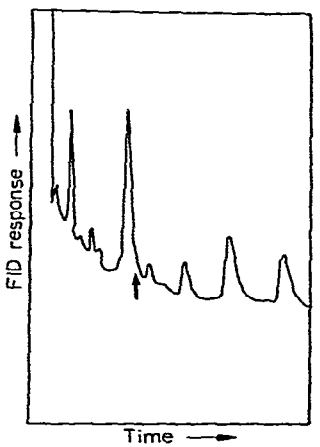


Fig. 1. Chromatography on a packed column. A 3-ml volume of blood containing 50 ng of papaverine was extracted as described. The arrow indicates the position corresponding to the retention time of papaverine.

ether. The signal caused by this impurity was considerably amplified by the experimental conditions, which necessitated a 400-fold concentration of the organic phase (20 ml of diethyl ether evaporated to dryness and the residue re-dissolved into 50 μl of chloroform). Different techniques of solvent clean-up (re-distillation, liquid chromatography on aluminium oxide in the acidic form) did not improve the elimination of the impurities.

Gas–liquid chromatography on a capillary column

Fig. 2 demonstrates that papaverine can be resolved from the impurities contained in the diethyl ether by high-resolution GLC on a glass capillary column.

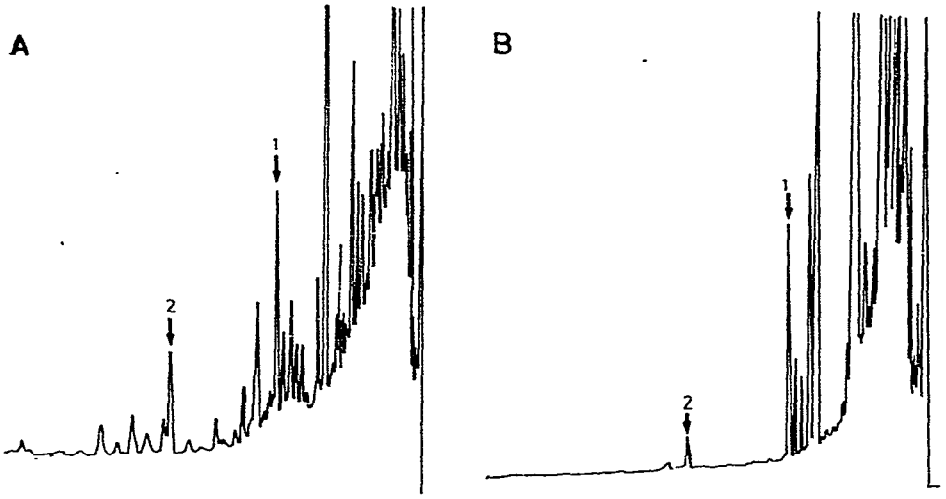


Fig. 2. Chromatography on a glass capillary column. A 3-ml volume of blood containing 400 ng of papaveraldine and (A) 50 ng or (B) 200 ng of papaverine was extracted as described. Volumes of 2 μl of the final organic solutions were injected into the chromatograph. Peaks 1 and 2 correspond to papaverine and papaveraldine, respectively.

Under these conditions, a linear relationship between the peak height ratio of papaverine to papaveraldine and the amount of papaverine injected, ranging from 50 to 200 ng, was obtained (Fig. 3). The line passed through the origin, indicating that no adsorption on the chromatographic columns occurred. The overall reproducibility of the measurements is excellent ($\pm$ 5%) and the method is sensitive enough for 10 ng of papaverine to be determined accurately in the injected sample. The yield of the extraction procedure was about 70%.

Nevertheless, the nature and importance of the impurities varied greatly with the batch number of the diethyl ether (Fig. 4A and 4B), which makes it difficult to apply such a method for routine laboratory purposes as the use of a new batch of solvent could lead to complex erroneous results.

Other organic solvents were also tested but gave even worse results. Fig. 4C and 4D illustrates the types of impurities present in the evaporation residue from identical volumes of dichloromethane and chloroform. These traces were recorded

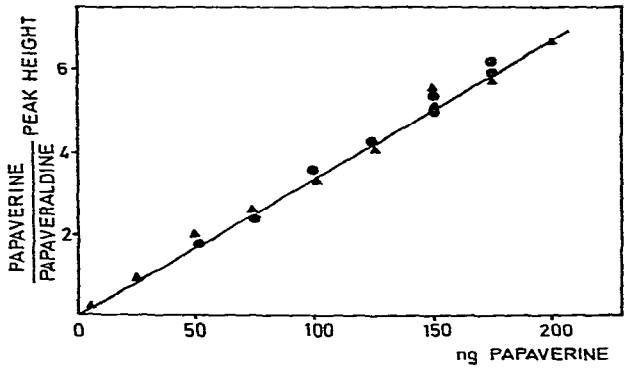


Fig. 3. Calibration graph for papaverine obtained by GLC on a glass capillary column. ● and ▲ represent data recorded at different dates.

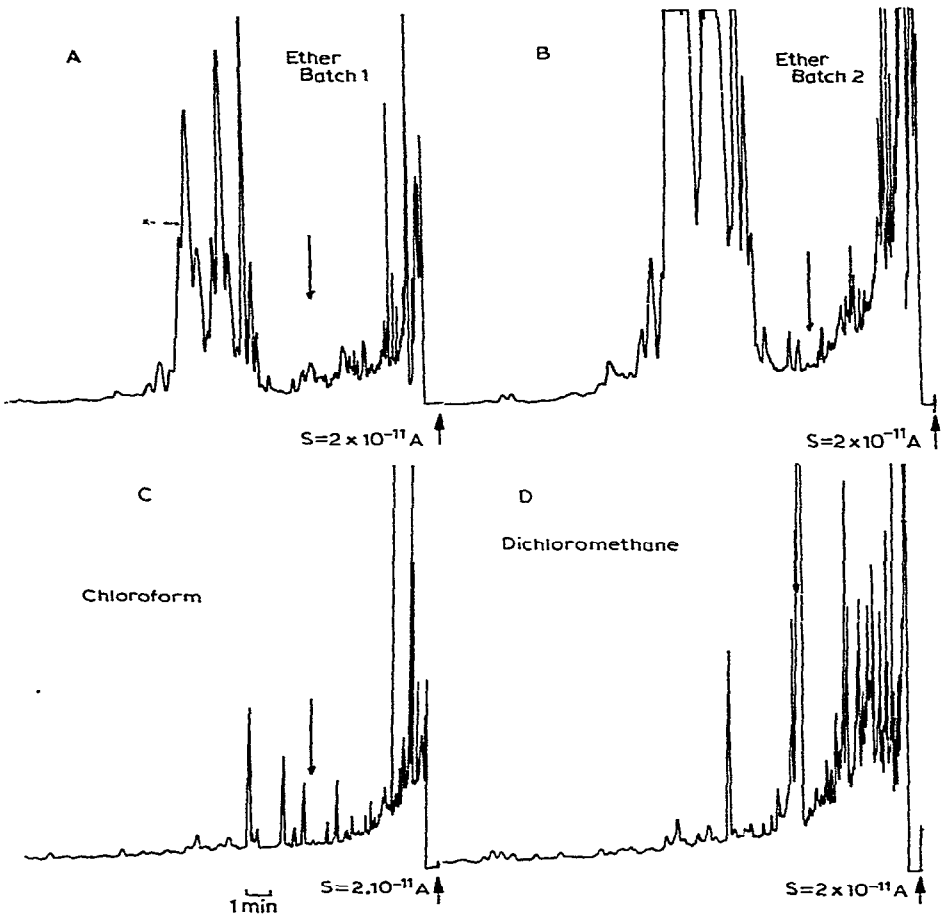


Fig. 4. Chromatography on a glass capillary column. Volumes of 20 ml of pure solvents were evaporated to dryness; the residues were dissolved in 3 ml of diethyl ether, the solutions transferred to a capillary tube, evaporated to dryness and the final residues dissolved in 50 μl of chloroform. Volumes of 2 μl of this solution were injected into the chromatograph. The recording sensitivity was similar to that in Fig. 2 for the ether residues, but was five times lower for chloroform and dichloromethane.

at a five time slower sensitivity than the chromatogram shown in Fig. 2B. Hence, the use of such solvents would have completely masked the papaverine peak. In addition, the yield of papaverine extraction with those two solvents was even lower (*ca.* 50%).

Gas–liquid chromatography coupled with a multiple ion detector

In this technique, the mass spectrometer serves as a specific detector for the compounds studied[15]. The mass spectra of papaverine and papaveraldine, recorded at 20 eV, on the OV-1 chromatographic column are shown in Fig. 5. The papaverine spectrum contains a very intense molecular ion at *m/e* = 339. The fragmentation pattern of papaverine gives rise to three principle ions at *m/e* 324 ($M-CH_3$), 308 ($M-OCH_3$) and 293 ($M-OCH_3-CH_3$). The same holds for papaveraldine, the molecular ion of which is at *m/e* 353.

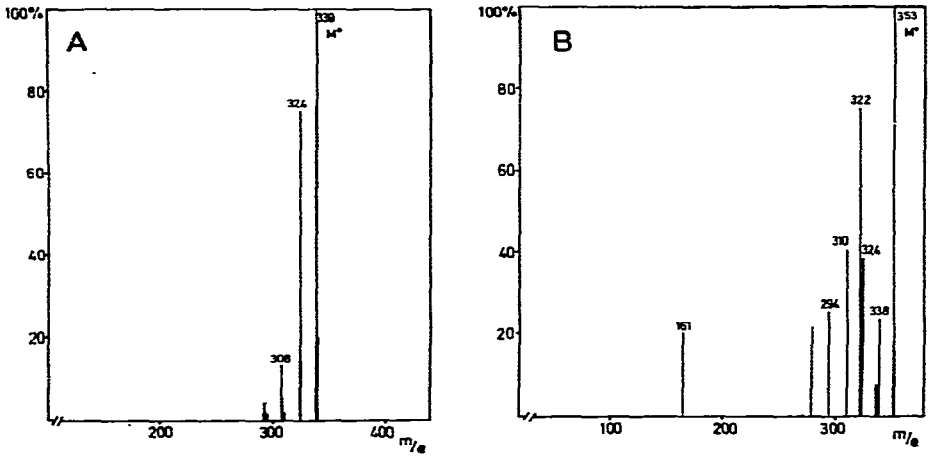
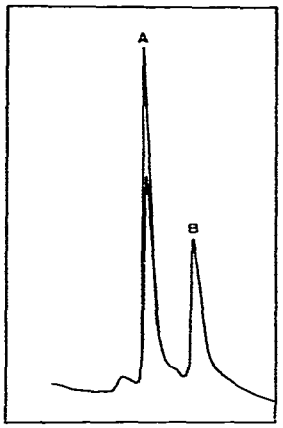


Fig. 5. Mass spectra of (A) papaverine and (B) papaveraldine.

The apparatus was focused on the *m/e* 324 ion of papaverine. The accelerating voltages were calculated and set to record the *m/e* 339 and 324 ions of papaverine and the *m/e* 353 ion of papaveraldine was utilized as the internal standard. Fig. 6 shows a typical fragmentogram. A linear relationship was obtained between the *m/e* 339/353 and 324/353 peak height ratios and the amount of papaverine (up to 100 ng of injected papaverine) (Fig. 7). The precision of the measurement was 2% and the reproducibility of the overall method was 10%. The limit of detection in blood was about 5 ng/ml, corresponding to that obtained with an electron-capture detector[2].

Plasma half-life of papaverine

This method of measurement was easily applied to determinations of blood levels of papaverine, as shown by a pharmacokinetic study on three volunteers receiving the same oral dose. The results of these measurements are shown in Fig. 8. A great variation can be seen in the plasma level of papaverine for the three subjects. In particular, the maximal levels observed were 185, 65 and 395 ng/ml for a single dose of 150 mg per person, and always appeared between the first and second hour



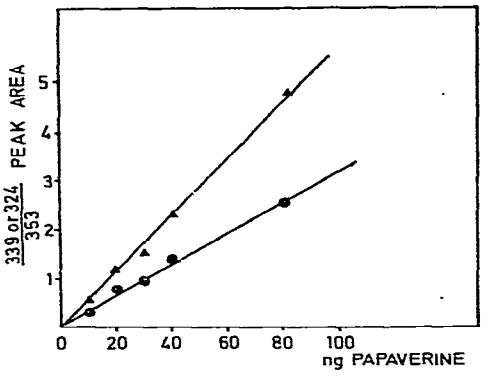


Fig. 6. Mass fragmentogram of (A) papaverine (*m/e* 324 and 339) and (B) papaveraldine (*m/e* 353).

Fig. 7. Calibration graph for papaverine obtained by mass fragmentography. ▲ = 339/353 ratio; ● = 324/353 ratio.

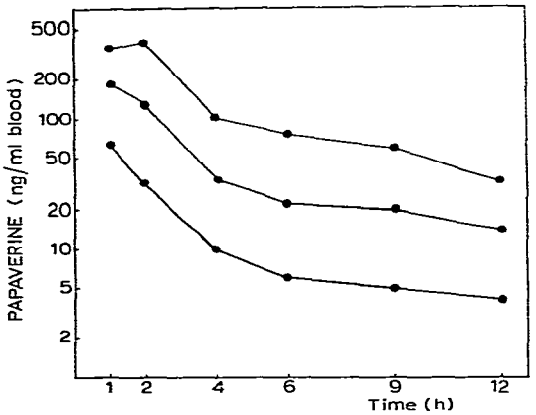


Fig. 8. Time course of papaverine level in the blood of three volunteers receiving a single 15-mg dose of the drug.

following administration. Even taking into account the body weight, the variability of the maximal papaverine concentration in the blood is still very important (86, 36 and 113 ng/ml for a single dose of 1 mg/kg).

The graphical representation of these results in semi-logarithmic coordinates (Fig. 8) shows that the elimination of papaverine is not a simple phenomenon and occurs in two steps, *viz.*, a rapid elimination during the first 6 h followed by a slower elimination during the next 6 h. The plasma half-lives of papaverine in the three volunteers during the first rapid phase were 60,65 and 58 min.

CONCLUSION

Most methods that have been proposed for monitoring papaverine are not compatible with the evaluation of therapeutic levels of the drug in human plasma.

Only chromatographic methods offer the required qualities of precision, specificity and sensitivity. We investigated GLC using a packed column and a high-resolution glass capillary column.

Measurements with a flame-ionization detector on a packed column were impossible because the papaverine peak was obscured by a large non-volatile impurity peak resulting from the extracting solvent.

High-resolution capillary column chromatography allowed the separation of the papaverine peak from those of the impurities. Nevertheless, the number and the importance of the background peaks and their qualitative and quantitative variations as a function of the solvent batch interfered with the measurements. Extraction with chloroform or dichloromethane instead of diethyl ether did not solve the problem; impurities present in those solvents almost completely masked the papaverine peak on the chromatogram. This obliged us to resort to combined gas–liquid chromatography–mass spectrometry. Using a multiple ion detection device, we were able to solve the technical problems. Under selected conditions, a precision of about 2% was obtained. The limit of detection for papaverine was largely compatible with the therapeutic levels of this compound in blood.

The pharmacokinetic experiment carried out on three volunteers showed that the mass fragmentographic method was particularly well adapted for this study. The specificity and precision of the method were clearly improved in comparison with the classical GLC method. For identical drug administrations we observed highly significant individual variations in the blood levels of papaverine.

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