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Assay of fusidic acid in plasma by high-performance liquid chromatography

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Summary

An accurate and selective method for the assay of fusidic acid in human plasma has been developed. The column used was stainless steel, 30 cm × 4 mm i.d., packed with microparticulate silica. The mobile phase consisted of hexane–methylene chloride–ethanol (69 : 25 : 6), and the flow rate was 1.8 ml/min. Absorbance was measured at 254 nm, and quantitation was carried out by measuring peak area. Plasma, 500 μl, was extracted with 10 ml methylene chloride, centrifuged, 6 ml was evaporated under N_2, and the residue was redissolved in 500 μl of the mobile phase. A linear relationship ($r = 0.9898$) was obtained between peak area and amount of drug added to plasma. This method could detect 250 ng of drug in 500 μl of plasma. The average recovery rate was 75.5 ± 3.9% in the range 0.5–100 μg/ml. This method has been applied to determine fusidic acid in plasma following the oral administration of 500 mg to a healthy volunteer. The selectivity of the method is demonstrated by the appearance of peaks which could be assigned to fusidic acid metabolite(s).

Introduction

Fusidic acid is an antibiotic produced by the growth of certain strains of *Fusidium coccinium* (R. Tabaki), and it possesses a very high antistaphylococcal activity (Scowen and Garrod, 1962; Dodson, 1963). At the present time, the only available method for assaying fusidic acid in biological fluids is a microbiological procedure requiring considerable time and specialized technique (Williamson et al., 1970). In a previous report (Hikal et al., 1982) a method was described for the determination of fusidic acid or its sodium salt in dosage forms by HPLC. The present work describes a selective and accurate procedure for assaying fusidic acid in human plasma.

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Experimental

Reagents

Fusidic acid was prepared from sodium fusidate, B.P., (Leo Pharmaceutical Products, Ballerup, Switzerland) by acidification, filtration, and recrystallization from ethanol. All other reagents were B.P., U.S.P., or Analytical Reagent grade and were used as obtained. Hexane, methylene chloride and ethanol (Fluka AG, Chemische Fabrik, Buchs) were 'Spectroscopic' grade. Water was double-distilled in an all-glass still, and normal procedure for purification of the mobile phase was adhered to.

Apparatus

The liquid chromatograph used (Model ALC/GPC 244U, Waters Associates, Milford, MA, U.S.A.) was equipped with a low-volume positive-displacement pump (Model 6000A, Waters Associates), a universal injector (Model U6K, Waters Associates), a single wavelength detector (254 nm, Model 440, Waters Associates), and a data module (model 730, Waters Associates). The column was stainless steel, 30 cm × 4 mm i.d., packed with microparticulate silica (Microporasil, Waters Associates). The mobile phase consisted of hexane–methylene chloride–ethanol (69 : 25 : 6), and the flow rate was 1.8 ml/min. Experiments were conducted at ambient temperature (25°C).

Extraction of fusidic acid from plasma

To 500 μl of plasma, 10 ml of methylene chloride was added and vortexed (Scientific Instruments, Bohemia, NY, U.S.A.) for 2 min. The mixture was then centrifuged (Labsystems, Helsinki, Finland) at 2500 rpm for 5 min. 6 ml of the methylene chloride layer was transferred to a conical-bottomed test tube, and the solvent was evaporated under a stream of nitrogen at room temperature. The residue was redissolved in 500 μl of the mobile phase, and 80 μl was injected onto the column, in duplicate.

Preparation of calibration curve

Freshly harvested human plasma was used to prepare the calibration curve. To 500 μl aliquots of plasma, 5–50 μl of standard solutions of fusidic acid in ethanol were added to yield concentrations of 0.5, 1, 5, 10, 20, 40, 60, 80 and 100 μg/ml. The spiked plasma was extracted as described above. The observed peak area was plotted against the concentration, and the least-squares line was calculated by linear regression.

To determine percent recovery, standard solutions of the drug in the mobile phase were prepared covering the same range of concentrations as used for spiking plasma. Duplicate injections of 80 μl were made unto the column, and peak area was plotted against concentration. Percent recovery was calculated by comparing points on the linear regression line prepared from this plot to corresponding points on the line calculated in the previous paragraph.

In vivo experiment

The subject was male, Indian, 28 years of age, and weighed 63 kg. He fasted overnight and until 4 h post-administration of drug, water being allowed ad libitum. The drug was administered in the form of a commercial suspension (Fucidin, Leo Pharmaceutical Products, lot. no B06AA) containing 50 mg/ml. After shaking the bottle thoroughly, 10 ml was withdrawn into a wide-mouthed pipette and directly swallowed, followed by 250 ml of water. Immediately preceding administration of the drug, 10 ml of blood was withdrawn, plasma was separated and used to prepare the calibration curve as described above. Following administration, 5 ml blood samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6 and 12 h. Plasma was immediately separated, and 500 μl was transferred to a glass-stoppered tube and stored at 5°C until all samples were ready for assay. All samples and spiked plasma were assayed on the same day.

Results and discussion

Under the experimental conditions described, fusidic acid showed a single peak with a retention time of 4.5 min. When standard solutions of the drug in the mobile phase were assayed, a linear relationship was observed between concentration and peak area ($r = 0.9999$). When spiked human plasma was assayed as described, a linear relationship was also obtained between amount of drug added and peak area ($r = 0.9898$). The average rate of recovery in the range 0.5–100 μg/ml was 75.5 $\pm$ 3.9%. The limit of sensitivity was 250 ng in 500 μl of plasma; lower concentrations did not produce a consistent response. Other extraction procedures, namely, the use of ethyl acetate, or acidification with trichloroacetic acid followed by chloroform, did not lead to any improvement in percent recovery or sensitivity.

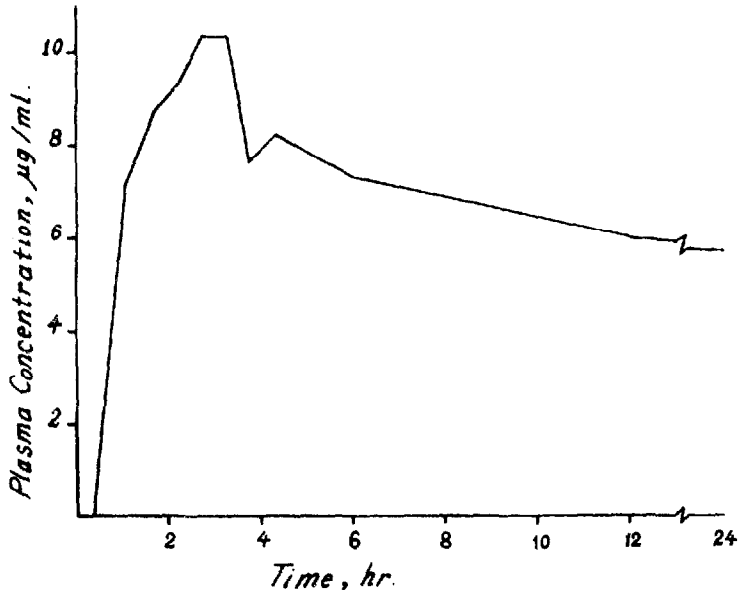

Fig. 1. Plasma concentration of fusidic acid following a single oral dose of 500 mg.

Fig. 1 depicts the relationship between plasma concentration and time following the oral administration of a single dose of 500 mg as a suspension in an aqueous vehicle. The drug appeared in plasma within 1 h after administration and reached a peak of about 10.3 μg/ml in 3 h. A measurable level was still detectable 24 h after administration. Using the part of the plasma concentration vs time curve which represents the elimination phase, a half-life of 32.2 h was calculated. In a report dealing with sodium fusidate (Wade, 1979), the time to reach a maximum concentration is given as 2–4 h, and the peak concentration reached after a single 500 mg dose is about 30 μg/ml. There is, furthermore, an increase in plasma concentration with repeated administration, indicating a slow elimination. The present results seem to agree fairly well with published data, especially when we consider that only a single subject was used.

Fig. 2A–D shows typical chromatograms obtained from blank plasma, spiked plasma, and plasma obtained from a human volunteer following the oral administration of the drug. No interfering peaks are observed in the region where the drug peak elutes. Additional peaks are observed in the plasma obtained from the subject, which can be assigned to metabolic product(s). This assignment is supported by the observation that one of these additional peaks increases in size as the drug peak gets smaller with the lapse of time (Fig. 2C and D).

The present procedure for the assay of fusidic acid in plasma was demonstrated

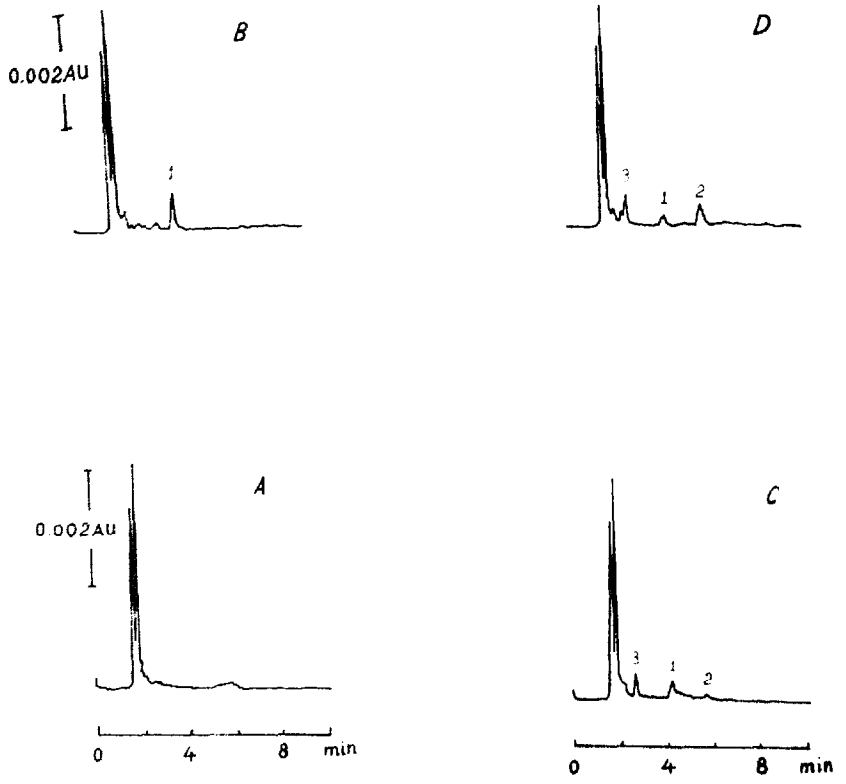

Fig. 2. Typical chromatograms. A: blank plasma; B: plasma spiked with drug; C: plasma obtained from volunteer after 6 h; D: plasma obtained after 12 h. (1), fusidic acid, (2) and (3), metabolic product(s).

to be accurate, sensitive and selective. It represents a considerable saving of time when compared to the microbiological assay.

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