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Note

Rapid determination of pyrazinamide in biological fluids by high-performance liquid chromatography

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Pyrazinamide is the amide of pyrazinic acid, derived from pyrazine (1,4-diazine), with the structural formula shown in Fig. 1. It is an old drug (useful in pulmonary tuberculosis) which has actually a new interest, particulary in the resistance to other major anti-tuberculous drugs.

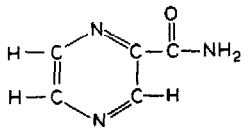

Fig. 1. Structural formula of pyrazinamide.

Determination of pyrazinamide levels in biological fluids is described essentially by spectrophotometric methods [1, 2]. However, Roboz et al. [3] reported a gas chromatographic—mass spectrometric technique and Ratti et al. [4] published a high-performance liquid chromatographic (HPLC) technique that had a very long and difficult extraction.

The present method involves reversed-phase HPLC with UV detection of pyrazinamide. Its advantage is the precipitation of proteins without extraction. This method is suitable for monitoring pyrazinamide levels in routine clinical

situations and allows the determination of pyrazinamide levels if this antituberculous drug is given alone or with isoniazide.

EXPERIMENTAL

Reagents

The following reagents were used: sodium hydroxide (Normapur Prolabo, Paris, France), $Na_2HPO_4 \cdot 2H_2O$ (Merck, Darmstadt, F.R.G.), KH_2PO_4 (Prolabo, Paris, France), perchloric acid (UCB, Leuven, Belgium), methanol (Normapur Prolabo), distilled water (P.C.H., Paris, France) and pyrazinamide, which was provided graciously by the Lepetit-Merrell Laboratory (Neuilly-sur-Seine, France).

The pH 7.4 buffer was prepared with 19.7 ml of 0.02 *M* KH_2PO_4 and 80.3 ml of 0.02 *M* $Na_2HPO_4 \cdot 2H_2O$.

Apparatus and techniques

The chromatographic system consisted of a Chromatem 38 solvent delivery pump (Touzart et Matignon, France) and a Rheodyne injector fitted with a 50-μl loop. The column was a μBondapak C_{18} (30 cm × 4.6 mm I.D.; 10 μm) (Waters Assoc., Milford, MA, U.S.A.), equilibrated at room temperature. The detector was a variable-wavelength Schoeffel spectrophotometer. The detection wavelength was 268 nm (0.2 a.u.f.s.). All chromatograms were recorded on a CSA 10-mV recorder (Lirec, France) at a chart speed of 5 mm/min.

The mobile phase consisted of pH 7.4 buffer—methanol (98:2), at a flow-rate of 1 ml/min. The mobile phase was thoroughly degassed for 15 min.

Sample preparation

A 1-ml aliquot of 0.7 *M* perchloric acid was added to a 5-ml glass tube containing 1 ml of plasma and thoroughly mixed for 10 s. After centrifugation at 1500 *g* for 10 min, 1 ml of the upper phase was removed and neutralized with 0.2 ml of 1 *M* sodium hydroxide; 50 μl of this phase were injected.

Calibration curve

The standard curve was established in the following way: to 0.9 ml of drug-free plasma were added 100 μl of an aqueous solution of pyrazinamide ranging between 50 and 800 μg/ml to obtain a calibration between 5 and 80 μg/ml. Standard samples were then treated as for plasma samples.

RESULTS

Fig. 2 shows the chromatograms of plasma from a healthy volunteer (blank sample and 2 h after oral administration of 30 mg/kg pyrazinamide). The calibration curve was linear from 5 to 80 μg/ml. Its equation was as follows: $y = -4.7 + 2.9x$; $r = 0.9996$, where y is the peak height of pyrazinamide, x is the concentration of pyrazinamide in μg/ml and r is the correlation coefficient.

The limit of quantitation was below 2 μg/ml of plasma. The reproducibility of the method can be seen in Table I. The precision of the method is represented by the relative standard deviation of the mean of replicate assays of

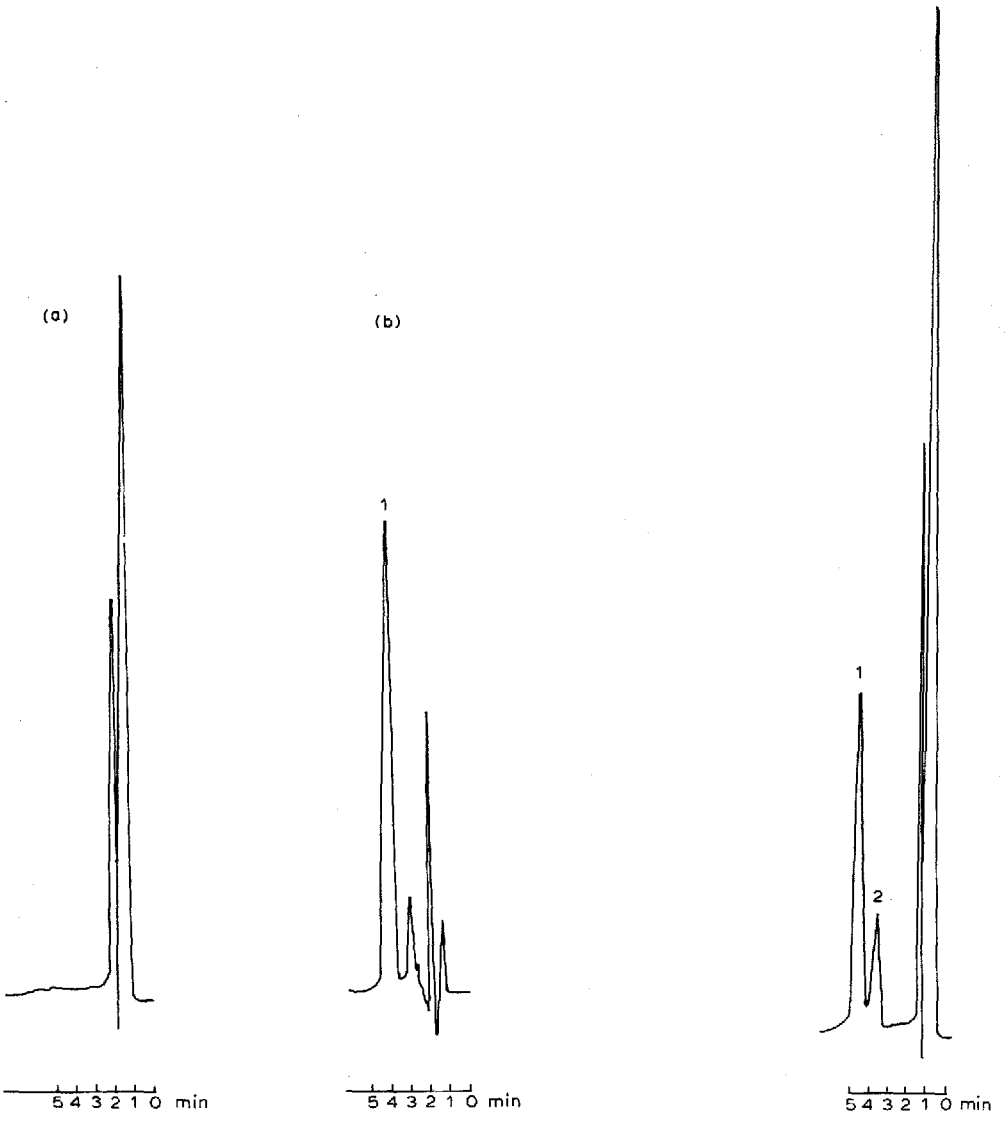

Fig. 2. Chromatograms of a healthy volunteer's plasma extract. (a) Blank plasma; (b) sample plasma containing 38 μg/ml pyrazinamide (1) (flow-rate 1 ml/min; retention time 4.6 min).

Fig. 3. Typical chromatograms of a healthy volunteer's plasma extract containing 29 μg/ml pyrazinamide and 10 μg/ml isoniazide (flow-rate 1 ml/min; retention time of pyrazinamide 4.6 min and isoniazide 4.0 min). Peaks: 1 = pyrazinamide; 2 = isoniazide.

the same sample. The precision of the method was checked for three plasma concentrations: 5, 40 and 80 μg/ml. The coefficients of variation were 10, 1.5 and 5.1%, respectively.

The recovery of the method, defined as the difference between the amount added to standard plasma and the amount found (expressed as a percentage of the amount added), is summarized in Table II. The recovery of this method

TABLE I

REPRODUCIBILITY OF PYRAZINAMIDE FROM HUMAN PLASMA PERFORMED ON TEN DETERMINATIONS AT EACH CONCENTRATION

Concentration (μg/ml)	Coefficient of variation (%)
5	11
10	9
20	4
40	6
80	5

TABLE II

RECOVERY OF PYRAZINAMIDE FROM HUMAN PLASMA PERFORMED ON TEN DETERMINATIONS AT EACH CONCENTRATION

Concentration added (μg/ml)	Concentration found (μg/ml)	Concentration found expressed as percentage of concentration added (%)
10	9.2	92
20	18.4	92
40	36.4	91

TABLE III

RESULTS OF PYRAZINAMIDE LEVELS IN PATIENTS WITH NORMAL OR PATHOLOGICAL RENAL FUNCTION

Renal function	Oral administration (mg/kg)	Levels of pyrazinamide 2 h after oral administration (μg/ml)
Normal	30	37
	30	36
	30	55
Pathological	15	36 (before haemodilasyis)
		23.8 (after haemodialysis)

(> 90%) and the precipitation of proteins without sample extraction allowed us to dispense with an internal standard.

The method developed has been applied to the determination of pyrazinamide in human plasma of tuberculous patients with normal or pathologic renal function after oral administration. The results are shown in Table III. Fig. 3 shows peaks of isoniazide and pyrazinamide, well separated, at a flow-rate of 1 ml/min. This technique is also adapted to the determination of pyrazinamide levels in the presence of isoniazide.

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