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Letter to the Editor

Determination of isoniazid in plasma by liquid chromatography

Sir,

The determination of plasma concentrations of isoniazid (INH) is useful to monitor and optimize therapy with this drug [1]. Selective extraction of INH from biological fluids and separation of the drug from coextracted impurities by liquid chromatography (LC) with reversed-phase columns has been difficult because of the highly polar nature of INH. A simple LC procedure for the determination of INH in plasma after derivatization with cinnamaldehyde has been described [2]. However, cinnamaldehyde has a limited shelf life as it tends to darken. We evaluated a number of aromatic aldehydes (*o*-anisaldehyde, *p*-anisaldehyde, benzaldehyde, cinnamaldehyde, *m*-hydroxybenzaldehyde, *p*-hydroxybenzaldehyde, salicylaldehyde and vanillin) to optimize the hydrazone formation for the determination of INH by LC. Aliquots of a 4 μg/ml plasma INH standard were treated with solutions of different aldehydes of the same concentrations and a solution of trichloroacetic acid according to the procedure described below. Supernatants were chromatographed on an octyl column using mobile phases of different methanol content and different flow-rates so that the retention times of different INH hydrazones were similar and each hydrazone was well separated from its reagent aldehyde. *p*-Hydroxybenzaldehyde, a pleasant smelling crystalline solid, proved to be the most efficient aldehyde with respect to the ratio of absorbance of INH hydrazone/reagent at a given wavelength and the time for the separation of the hydrazone from the corresponding aldehyde. Cinnamaldehyde has been considered as the ideal aldehyde for the spectrophotometric determination of INH [3]. However, as seen in Fig. 1, *p*-hydroxybenzaldehyde shows a similar INH response but shows a smaller reagent response than cinnamaldehyde allowing sensitive detection of INH.

A 0.25-ml aliquot of the sample was mixed with 0.05 ml of a 1.5% methanolic solution of *p*-hydroxybenzaldehyde and 0.1 ml of a 10% aqueous solution of trichloroacetic acid with a vortex mixer for 1 min. The tubes were centrifuged at 3000 *g* for 10 min. A 20-μl aliquot of the supernatant was injected onto a 15 cm × 4.6 mm I.D., 5-μm Ultrasphere-octyl column (Beckman) proceted by a 7-μm RP-8 guard cartridge (Pierce Chemical) and eluted at ambient temperature at a flow-rate of 1 ml/min with a mobile phase of 200 ml of methanol, 800 ml of water, 0.5 ml of 70% perchloric acid and 0.5 ml of a 20% methanolic solution of tetra-

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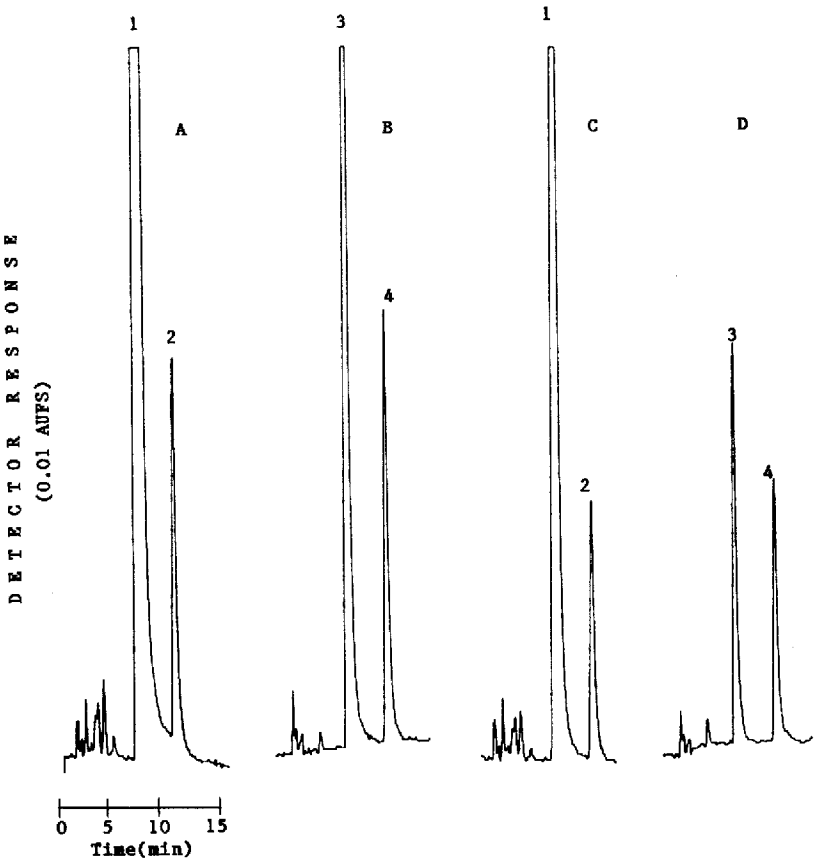

Fig. 1. Chromatograms of INH (4 μg/ml in plasma) hydrazone. A 15 cm×4.6 mm I.D., 5-μm Ultrasphere-octyl column and ambient temperature were used for all chromatograms. Cinnamaldehyde: (A) 340 nm; (C) 350 nm; mobile phase, 400 ml of methanol, 600 ml of water, 0.5 ml of 70% perchloric acid and 0.5 ml of 20% methanolic tetramethylammonium hydroxide; flow-rate, 1.4 ml/min. *p*-Hydoxybenzaldehyde: (B) 340 nm; (D) 350 nm; mobile phase, 180 ml of methanol, 820 ml of water, 0.5 ml of 70% perchloric acid and 0.5 ml of 20% methanolic tetramethylammonium hydroxide; flow-rate, 1.1 ml/min. Peaks: 1 = cinnamaldehyde; 2 = INH cinnamaldehyde; 3 = *p*-hydroxybenzaldehyde; 4 = INH *p*-hydroxybenzaldehyde.

methylammonium hydroxide. The peaks were detected at 350 nm with a Shimadzu 6AV detector set at 0.002 a.u.f.s.

The reaction of INH with *p*-hydroxybenzaldehyde is complete at room temperature instantly as there is no increase in the peak height of INH when the reaction mixture is heated at 60°C for 20 min or allowed to stand at room temperature for 2 h. However, the yield of INH hydrazone depends upon the concentration of the reagent. There is a 25% reduction of the INH hydrazone peak when the concentration of *p*-hydroxybenzaldehyde is reduced to half of the concentration described in the present procedure.

The recovery of INH from plasma is 80% when compared to an aqueous standard of the same concentration. The procedure is linear for the range tested (0.2–10 μg/ml). The within-batch coefficients of variation (C.V.) of the proce-

dure were 8.4% ($n=10$, mean $=0.44\ \mu g/ml$) and 6.7% ($n=10$, mean $=5.0\ \mu g/ml$) and the between-batch C.V. were 12.7% ($n=15$, mean $=0.50\ \mu g/ml$) and 7.2% ($n=15$, mean $=5.0\ \mu g/ml$). N-Acetylisoniazid does not show any response under the described conditions.

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