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Note

Measurement of strychnine by high-performance liquid chromatography

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No high-performance liquid chromatographic (HPLC) method has been described for the measurement of strychnine in biological media. Such a method would be of use in toxicology and in monitoring strychnine levels in the blood of non-ketotic hyperglycinemic infants in whom the drug is used clinically [1]. In addition, it would be of use in the elucidation of the metabolism and pharmacokinetic characteristics of strychnine. Achari and co-workers described an HPLC method which measured different basic drugs [2] especially quinidine in human plasma, using strychnine as an internal standard [3]. We have adapted their method to strychnine using quinine as an internal standard.

MATERIALS AND METHODS

Reagents

Strychnine sulfate and quinine were obtained from Sigma (St. Louis, MO, U.S.A.). All solvents were glass-distilled grade from J.T. Baker (Phillipsburg, NJ, U.S.A.). Other reagents were analytical grade.

Extraction procedure

Strychnine is a dibasic alkaloid showing almost identical solubility properties as quinidine and we therefore used the extraction procedure described by Achari et al. [3]: extraction by chloroform at basic pH, evaporation of the chloroformic extract, and reconstitution of the residue in methanol. This procedure resulted in the recovery of 83 ± 5% of added strychnine to plasma. The internal standard, quinine (10 μg/ml), is added with the chloroform.

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High-performance liquid chromatography

The apparatus is composed of a Beckman Model 110A pump, an Altex Model 210 injector and a Beckman Model 153 UV detector set at 254 nm. The chromatographic parameters were controlled by a Beckman Model 421 microprocessor. A Perkin-Elmer silica gel column (10 μm partical size), 25 × 0.26 cm, was used. The eluent, concentrated ammonium hydroxide (28.0—30.0% NH_3) in methanol (0.75:99.25, v/v) was pumped at a flow-rate of 1.1 ml/min.

RESULTS

For each set of measurements, a calibration curve is established. In the case of plasma and urine samples the calibration curve is obtained by taking known amounts of strychnine and extracting them from plasma or urine. In the case of simple solutions, the calibration curve is obtained directly by adding known amounts of strychnine dissolved in methanol. The concentrations of strychnine base used are from 20 to 0.625 μg/ml, the lower limit of sensitivity of the assay. The volume injected is 20 μl. Under those conditions quinine and then strychnine are eluted in less than 6 min, the two peaks being well separated (Fig. 1).

The calibration curve, which is linear (r = 0.999) over the concentration range employed, is established by plotting the peak height ratio of strychnine base to quinine against the concentration of strychnine base. The strychnine concentration in unknown samples is derived directly from the calibration curve. The intra-assay variance is between 3% and 5%; the inter-assay variance is less than 10%.

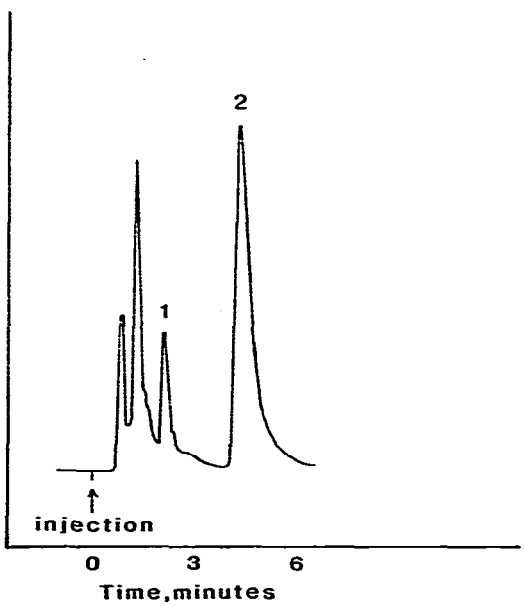

Fig. 1. Chromatogram showing the separation of strychnine base (peak 2) and the internal standard, quinine (peak 1). Chromatographic conditions were as follows: 25 × 0.26 cm silica gel column with a mobile phase of ammonium hydroxide in methanol (0.75%, v/v), flow-rate 1.1 ml/min and the range set at 0.02 a.u.f.s.; detection was at 254 nm.

We have used the above method to analyse the amount of strychnine remaining in Alzet mini-osmotic pumps after 19 days of administration of subconvulsive doses in rats [4]. This method is a definite improvement in sensitivity, accuracy and rapidity over the thin-layer chromatographic method formerly used [5] and is easily capable of monitoring plasma levels of strychnine either as used in pediatric therapy of non-ketotic hyperglycinemia [6] or as encountered in strychnine intoxication [7].

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