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Note

Thin-layer chromatographic method for the quantitative analysis of paracetamol (N-acetyl-*p*-aminophenol) in blood plasma

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Paracetamol* is a commonly used non-prescription analgesic. It is potentially a hepato-toxic drug, and the determination of the plasma levels of the drug is helpful in the management of patients who have ingested an overdose of paracetamol [1]. Various methods for the estimation of paracetamol in body fluids have been critically reviewed recently [2]. Thin-layer chromatography (TLC) has been used to separate paracetamol from body constituents prior to its quantitation by ultraviolet absorptiometry [3].

This note describes a simple and rapid method for the estimation of "free" paracetamol in plasma. It consists of extraction and concentration of drug from a small volume of plasma. The drug is separated from other plasma constituents by TLC, and is made visible by spraying with Folin—Ciocalteu reagent which is specific for phenols. Quantitation is effected by measuring the intensity of coloured spots directly on the plate by a densitometer.

EXPERIMENTAL

All the chemicals used were of analytical grade, and were used without any further purification. Paracetamol was kindly supplied by McNeil Labs., Don Mills, Canada. Precoated silica gel plates 10 × 20 cm (EM Labs., Elmsford, N.Y., U.S.A.; Cat. No. 5610) were used. Premade Folin—Ciocalteu [4] reagent was purchased from BDH, Toronto, Canada. A Clifford densitometer (Model 445) was used to measure the intensity of coloured spots.

*In North America, paracetamol is known as acetaminophen.

Standard solutions

Paracetamol: 100 mg was weighed and dissolved in 100 ml of absolute methanol. Its concentration was checked by measuring its absorbance (λ_{max} 249 nm, $A^{1\%}_{1cm}$ 897) [5]. The solution was stored at 4°.

Plasma standards: 20 mg/l, 10 mg/l, and 5 mg/l were prepared by adding 2 ml, 1 ml and 0.5 ml of the above stock solution respectively to 100 ml of filtered plasma. These standards were divided into 2-ml aliquots and frozen.

Extraction

Samples of 0.5 ml of plasma (test, 20, 10 and 5 mg/l standards) were pipetted into PTFE-lined screw-capped 12 × 120 mm culture tubes. To each tube, 5 ml of dichloromethane was added and the tube was vortex mixed. About 2 g of anhydrous sodium sulphate were added to each tube, which was again vortex mixed. The tubes were centrifuged. Three ml of dichloromethane extract from each tube were transferred to correspondingly labelled 16 × 100 mm disposable glass tubes, and evaporated to dryness in a water-bath at 50°. The tubes were cooled and the residue was dissolved in 100 µl of methanol by vortex mixing. The tubes were kept tightly stoppered to prevent evaporation of methanol. From each tube, 10 µl of methanolic extract were applied to a thin-layer plate (along the length edge) in this sequence: S_{20}, S_{10}, S_5, x, x, S_5, S_{10}, S_{20}.

The plate was developed in chloroform—acetone (60:40) up to a height of about 8 cm. The plate was dried in the fume hood for 10 min, and was sprayed with 10% sodium carbonate followed by Folin—Ciocalteu reagent, diluted 1:2 with deionized water. The plate was allowed to dry in air for about 10 min and was then scanned in the densitometer for the measurement of colour intensity using 650-nm filter. A standard curve for the standards of 5, 10 and 20 mg/l versus their average peak heights was plotted. The concentration of the unknown was determined from the standard curve and its average peak height.

RESULTS AND DISCUSSION

Paracetamol has been recovered in 50% yield in the present procedure using a single extraction with dichloromethane. Extraction efficiency was somewhat better (70%) in a single extraction with diethyl ether. However, overall precision of the method was not so good when diethyl ether was used in place of dichloromethane to extract paracetamol from plasma. There was no improvement in recovery when the pH was lowered to 4 or 2 [6]. A number of solvents have been described for thin-layer separation of paracetamol on silica gel [5]. Paracetamol moves on silica gel plates without trailing in the developing solvent selected for this procedure (R_F = 0.42). Folin—Ciocalteu reagent proved to be the best visualisation agent for paracetamol among a large number of available reagents for the detection of paracetamol [5]. This reagent has adequate sensitivity to detect paracetamol at therapeutic concentrations. Extracts of drug-free plasma or plasma containing a number of drugs that are commonly ingested in overdose (barbiturates, salicylates, hydantoins, methylprylon, meprobamate) do not produce coloured spots after TLC and spraying with this detection reagent. However,

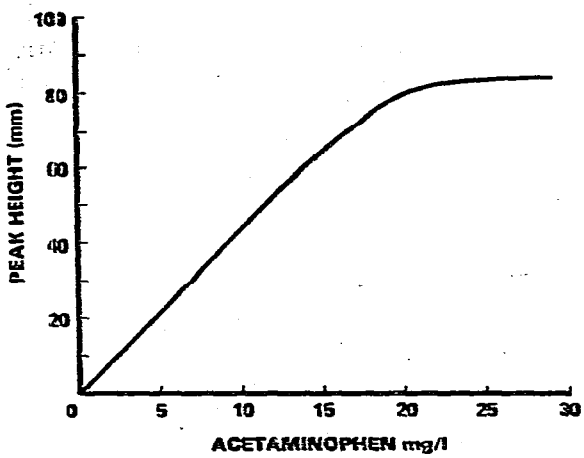

Fig. 1. Standard curve (peak height versus concentration) for the estimation of paracetamol (acetaminophen) in plasma.

the possibility of a phenolic metabolite of any of these drugs producing a spot with similar R_F value and colour to those of paracetamol cannot be ruled out.

This procedure is linear up to 20 mg/l (Fig. 1). In case a test specimen shows paracetamol concentration higher than 20 mg/l, TLC is repeated by spotting 10 μl of extracts of each of the standards, but only 5 μl of the extract of test specimens.

This procedure shows a within batch variation of 2.9% at a concentration of 5 mg/l ($n = 7$) and a between batch variation of 6.3% ($n = 9$) at a concentration of 8 mg/l.

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