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Note

A simple thin-layer chromatographic method for the estimation of hippuric acid: comparison with a photometric and a gas chromatographic method

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Hippuric acid is the main metabolite of toluene which is often used instead of benzene. Approximately 60% of inhaled toluene is expired. The toluene remaining in the body is oxidized to benzoic acid and after being conjugated, mainly to glycine, it is excreted in the urine as hippuric acid [1]. Off-set printers, for example, are affected by exposure to toluene. Furthermore, the estimation of hippuric acid is important in diagnosis for liver function [2] and other clinical [3,4] or pharmacological [5] problems. For the estimation of hippuric acid photometric [2,6], isotachophoretic [7] and chromatographic [8—10] methods have already been described. Photometric methods are unspecific, and isotachophoretic and gas chromatographic (GC) methods are expensive in both cost and time. Paper and thin-layer chromatographic (TLC) methods [9,10] render it possible to make a specific estimation with relatively small expense.

We have developed a routine TLC method, which allows simple, quick and specific, quantitative analysis of hippuric acid as well as application in the field of screening. Quantitation can be made either densitometrically in situ, or photometrically after elution, or by both methods.

The specificity and the importance in the field of occupational health of this TLC method has been compared with the modified GC method of Sedivec [11], and with photometric estimation after reaction with benzene sulphochloride [6].

METHODS

Thin-layer chromatography

Depending on the exposure, 1—5 μl urine (for semi-quantitative analyses, a millionth of the 24-h urine volume) are applied to silica gel (Silufol®; Kavalier, Sklárny, Czechoslovakia) and developed twice by the two-step procedure

up to 10 cm in chloroform—acetone—glacial acetic acid (40:10:5). After drying for 10 min at 150° the spots are detected with the help of 5% *p*-dimethylaminobenzaldehyde in acetic anhydride and heated again for 10 min at 150° (Fig. 1). When formic acid is used in the solvent instead of acetic acid, the colour intensity is reduced by about 20%. Contrary to alcohols ethyl acetate does not dissolve unreacted reagents. Estimation is done either semi-quantitatively by comparison of the colour intensity of standards and samples, or by quantitative densitometry across the chromatographic run with a variation coefficient of 6.50% for standards and 15.6% for urine samples, or by photometry at 468 nm after elution with 3 ml ethyl acetate with a coefficient of variation for standards and urine below 5%.

We used the electrophoresis scanner ERI 65 m® (Carl Zeiss, Jena, G.D.R.) in remission. No linear calibration curves have been obtained by means of this scanner.

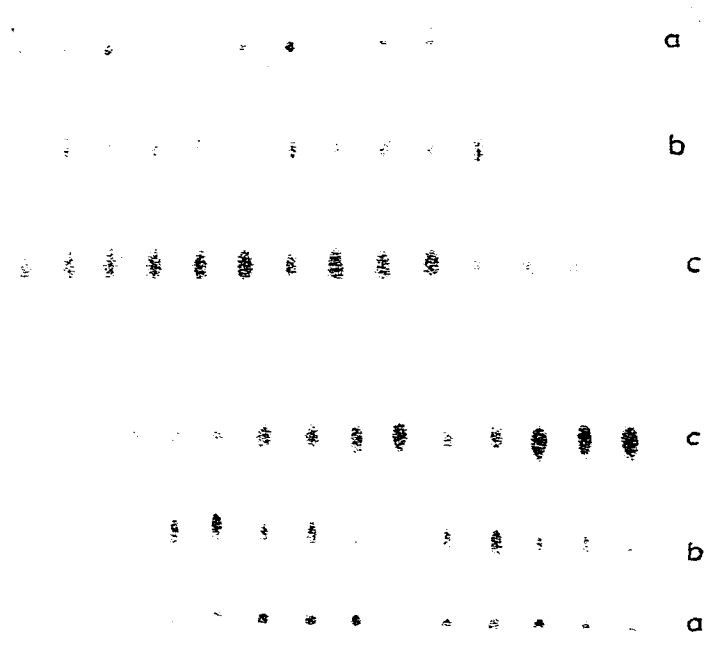

Fig. 1. Chromatogram of hippuric acid in urine on Silufol. Both sides of the plate were used for analyses. Spots: (a) unknown (violet); (b) unknown (green, not stable); (c) hippuric acid (red). Methylhippuric acid runs further than hippuric acid.

Gas chromatography

Five ml of urine were acidified with dilute sulphuric acid and saturated with ammonium sulphate. The extraction is made with ethyl acetate, containing phenacetin as internal standard, and derivatization is with diazomethane. After the addition of glacial acetic acid aliquots of this solution are chromatographed.

Technical data: Chromatograph Model GCHF 18.3® (VEB Chromatron, G.D.R.); steel column 1 m × 4 mm I.D. with 4.1% neopentylglycol succinate

on Chromosorb W AW, 80—100 mesh; carrier gas N_2 at a flow-rate of 40 ml/min; column temperature 197°; range 0—1000 μg hippuric acid. For the elimination of tailing by alteration of the column, it is better to make a hippuric acid standard daily. The photometric estimation was similar to that of Tomokuni and Ogata [6].

RESULTS

The regression equation of $y = 0.436 + 0.447x$ ($r = 0.971$, $n = 51$), which describes the connection between the values obtained densitometrically and photometrically after elution, as well as mean values of both estimations ($\bar{x}_{elution} = 1.62 \pm 1.15$, $\bar{y}_{densitometry} = 2.64 \pm 2.50$) show that at a good correlation the densitometrically estimated values are systematically higher. The linearity of the calibration curve of the estimation of hippuric acid is given at a sensitivity of about 5×10^{-8} g per spot up to 60 μg. In the measured concentration range of 0—17.6 μg hippuric acid per μl urine a very good correlation has been found between the GC and TLC estimations ($y_{TLC} = 0.996x_{GC} - 0.23$; $r = 0.998$, $n = 120$), but the correlation between the TLC method and the method of Tomokuni and Ogata was not satisfactory. It was found that the values above 10 μg hippuric acid when measured photometrically are systematically lower than when measured by TLC, and vice versa below 6 μg hippuric acid.

These results show that the TLC method represents a sufficiently exact and rational procedure for the estimation of hippuric acid. The number of samples per laboratory assistant and per day is approximately 200 analyses in the semiquantitative analysis and approximately 80 analyses in the quantitative analysis after elution. The reference range for the hippuric acid contents evaluated by means of the TLC method with a control group is 456 ± 213 mg/24 h. A strictly linear correlation has been found between exposure to toluene and the excretion of hippuric acid by means of this method.

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