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Note

Thin-layer chromatographic evaluation of methylhippuric and hippuric acids in urine*

G. VAN KERCKHOVEN, V. BLATON, D. VANDAMME and H. PEETERS

Simon Stevin Instituut voor Wetenschappelijk Onderzoek, Jerusalemstraat 34, B-8000 Brugge (Belgium)

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Methods previously described for the assay of hippuric acid are titration[1], fluorimetry[2], colorimetry[3–5] and spectrophotometry[6–9]. These methods are inconvenient for mass screening of the urinary metabolites hippuric and methylhippuric acid, in manual workers exposed to toluene and xylene vapours.

The first paper chromatographic method for the detection of the urinary metabolites was introduced by Gaffney *et al.*[10], El Masry *et al.*[3] and Ogata *et al.*[11]. The separation of hippuric acid from urine extracts by thin-layer chromatography (TLC) was first described by Teuchy *et al.*[12], while Ogata *et al.*[13] detected hippuric and methylhippuric acids by TLC. The objective of our study was to develop a simple, rapid and direct method for the detection of hippuric and methylhippuric acids in urine on thin-layer plates and to introduce the method for mass screening.

EXPERIMENTAL AND RESULTS

Urine samples** were collected from manual workers in several industries around Belgium who had been working in a shift for at least 4 working hours. The samples were acidified to pH 2 with 6 *N* hydrochloric acid. Pre-coated plates (Schleicher and Schüll G. 1500 silica gel, 10 × 20 cm) were activated for 1 h at 120°, then 10-μl aliquots of the samples were spotted on the plates and air dried. Twelve urine samples can be applied on the same plate (Fig. 1) and are compared with 10-μl standard samples containing 1, 2 and 3 g/l (Fig. 2). The TLC plates were developed twice for about 20 min in toluene–acetic acid–water (60:40:2) until the solvent front reached the upper edge. In between, the plates were air dried. After final drying at 100° for 5 min and spraying with a 1.5% (w/v) solution of dimethylaminobenzaldehyde in acetic anhydride, the plates are heated at 120° for 10 min. The accompanying yellow background of the plate disappears when exposed to ammonia vapour. Both hippuric and methylhippuric acids are detected as orange spots, with R_F values of 0.28 ± 0.01 and 0.32 ± 0.01, respectively, after a single development. In contrast to the colorimetric analysis[5], there is no interference from urea, which gives a yellow spot on the TLC plate with an R_F value of 0.22 ± 0.01.

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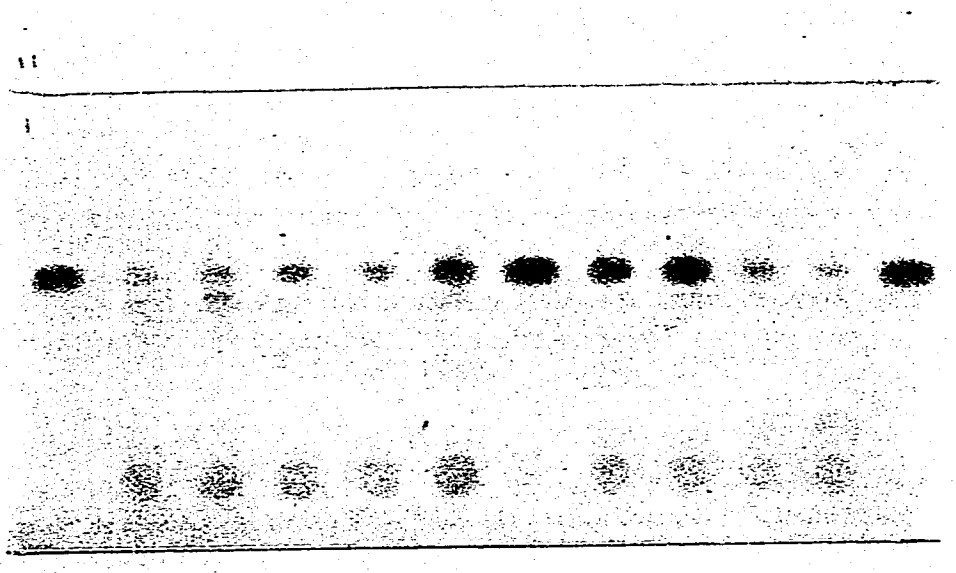

Fig. 1. Detection of hippuric acid in nine urine samples. Positions 1, 12 and 7 are standard samples of hippuric acid (1, 2 and 3 g/l, respectively).

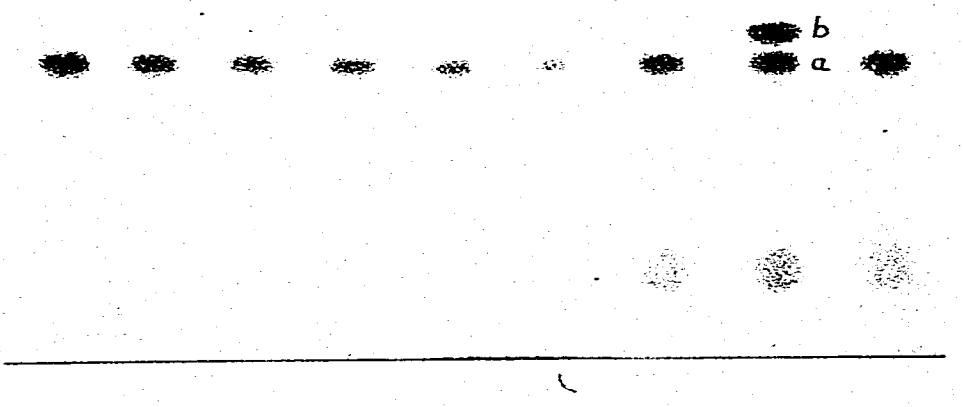

Fig. 2. Hippuric acid standards of 3, 2.5, 2, 1.5, 1 and 0.5 g/l. Positions 7, 8, 9 are urine samples. Position 8: (a) hippuric acid; (b) methylhippuric acid.

Semi-quantitative analysis can be performed by densitometry on a Kipp and Zonen densitometer (DD2). The results were compared with the colorimetric method using a 1.5% (w/v) solution of dimethylaminobenzaldehyde in acetic anhydride as colour reagent and a 0.16% (w/v) solution of iron(III) chloride in acetic anhydride as activator[5]. Thirty urine samples were used for comparison and from the results a correlation coefficient of 0.91 between the densitometric and colorimetric data was calculated (Fig. 3).

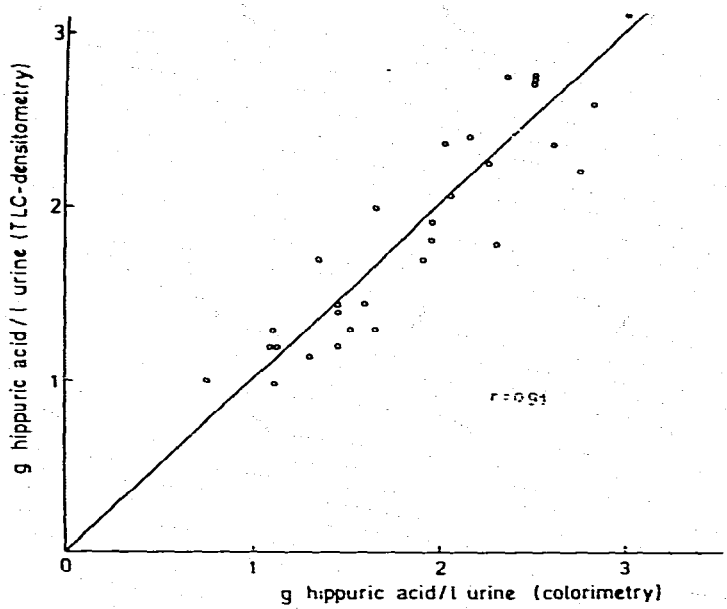

Fig. 3. Relationship between densitometric and colorimetric results for hippuric acid.

In conclusion, increased urinary hippuric and methylhippuric acid concentrations can be evaluated directly by TLC and evaluated by densitometry. Urea, which interferes in the colorimetric determination, does not influence the chromatographic evaluation of either metabolite.

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