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Note

Measurement of exposure to xylenes by separate determination of *m*- and *p*-methylhippuric acids in urine

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Hippuric acid occurs as a metabolite of toluene after inhalation of large doses, and *m*- and *p*-methylhippuric acids (*m*- and *p*-MHA) are urinary metabolites of *m*- and *p*-xylene. These acids are rapidly excreted in urine over a period of time that increases with increasing level of exposure[1]. The determination of these acids is therefore a valuable index of exposure to toluene and also to xylenes, which often occur as contaminants of toluene.

Many methods have been described for the determination of these metabolites, the first based on colorimetry. These have now been supplanted by various chromatographic techniques: thin-layer chromatography[2], gas chromatography (GC)[3–5], high-performance liquid chromatography[6–8] and isotachophoresis[9]. GC procedures have not yet produced a simple separation of the various urinary metabolites of xylene, especially *m*- and *p*-MHA.

The aim of this work was to develop a simpler routine technique for hippuric acids; this was achieved with high sensitivity by GC using a thermionic detector and an original internal standard.

EXPERIMENTAL

Reagents

The following analytical-reagent grade chemicals were used: concentrated hydrochloric acid, sodium chloride, ethyl acetate, methanol, diazomethane–diethyl ether solution, benzoylproline (Fluka, Buchs, Switzerland) (internal standard), hippuric acid (Fluka), *m*-MHA* and *p*-MHA*.

Gas chromatographic system

A Hewlett-Packard 5710A gas chromatograph with a 2 m × 2 mm I.D. glass column packed with 2% OV-225 was used, equipped with a nitrogen–phosphorus flame-ionization detector. The carrier gas was nitrogen at a flow-rate of 20 ml/min.

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The flame was air (50 ml/min)–hydrogen (3 ml/min). The following temperatures were maintained: column, 210°C; injector, 250°C; and detector, 300°C.

Method

A mixture of 0.5 ml urine, 0.5 ml of concentrated hydrochloric acid, 0.3 g of sodium chloride, 1 ml of 150 mg/l aqueous benzoylproline solution (internal standard) and 3 ml of ethyl acetate was shaken vigorously in a glass screw-capped test-tube for 10 min, and 2 ml of the supernatant ethyl acetate were transferred into another test-tube with 1 ml of methanol. Methylation was effected with diazomethane–diethyl ether solution. A 1-μl volume of the solution obtained was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Table I shows the extraction recovery of the internal standard and Tables II–IV show the results obtained with known amounts of the various hippuric acids as the methyl esters. Fig. 1 shows a chromatogram obtained for a mixture of compounds.

The extraction technique used here is that of Ogata *et al.*[10], which provides at least 99% recoveries of the various hippuric acids. However, with high levels of hippuric acids, the detector becomes saturated and the results are not reproducible. A ten-fold dilution of the sample will prevent this from occurring.

The GC separation of *m*- and *p*-MHA is very satisfactory. The specificity of OV-225 is much better than that of SE-30 as used by Kira[5] for the same order of sensitivity; the latter type of packing, even when used at 10% concentration, does not separate the two isomers. Moreover, salicyluric acid and its monomethyl ester inter-

TABLE I

RECOVERY OF BENZOYLPROLINE FROM WATER

	Amount added to water (mg/l)	*Amount found (mg/l) (n = 5)*	*Recovery (%)*	*S.D.*	*Coefficient of variation (%)*
Benzoylproline (mg/l)	100	100.28	100.28	2.91	2.9

TABLE II

RECOVERY OF HIPPURIC ACID FROM URINE

	Amount added to urine (mg/l)	*Amount found (mg/l) (n = 5)*	*Recovery (%)*	*S.D.*	*Coefficient of variation (%)*
Urine (U)	0	120	–	4.8	4
U + hippuric acid (mg/l)	250	303.6	73.1	8.0	2.6
	500	522.1	80.2	9.8	1.9
	1000	885.1	77.4	20.1	2.3

TABLE III

RECOVERY OF *m*-METHYLHIPPURIC ACID FROM URINE

	Amount added to urine (mg/l)	*Amount found (mg/l) (n = 5)*	*Recovery (%)*	*S.D.*	*Coefficient of variation (%)*
Urine (U)	0	0	–	–	–
U + *m*-MHA (mg/l)	28.37	26.98	95	1.30	4.8
	56.75	50.37	88.7	3.09	6.1
	113.5	102.86	90.6	2.04	2

TABLE IV

RECOVERY OF *p*-METHYLHIPPURIC ACID FROM URINE

	Amount added to urine (mg/l)	*Amount found (mg/l) (n = 5)*	*Recovery (%)*	*S.D.*	*Coefficient of variation (%)*
Urine (U)	0	0	–	–	–
U + *p*-MHA (mg/l)	34.25	27.88	81.4	1.43	5.1
	68.5	54.35	79.3	2.90	5.3
	137	112.3	82	2.56	2.3

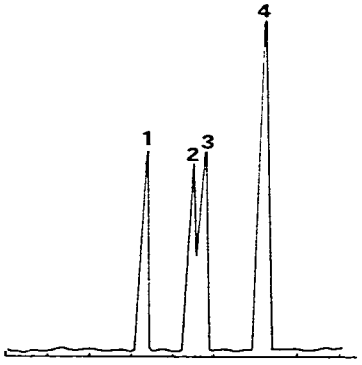

Fig. 1. Chromatogram of hippuric acid (1), *m*-methylhippuric acid (2), *p*-methylhippuric acid (3) and benzoylproline (internal standard) (4).

fere with this peak, whereas on the OV-225 column there is no interference, the retention times being hippuric acid 3.39 min, *m*-MHA 4.51 min, *p*-MHA 4.76 min, internal standard 6.16 min and methyl salicylurate 8.73 min.

We have compared the GC method with that of Engström *et al.*[4], by which hippuric acids were determined as their alkaline hydrolytic products. The correlation between exposure to toluene and urinary concentrations of benzoic and toluic acids

was better with the latter method for high levels corresponding to a heavy inhalation, but this was not our concern as we were more interested in long-term, low-level, exposure. It would seem more useful to evaluate the increase in the levels of the metabolites to achieve a better sensitivity together with a good resolution. This is what we propose here with the OV-225 separation and the thermionic detector, the sensitivity of which averages 1 mg/l with a signal-to-noise ratio of 5.

The relative response of the detector to hippuric acid and benzoylproline (internal standard) was about 1.01, *i.e.* the sensitivity of the detector is almost identical for the two compounds.

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