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Note

Determination of methylhippuric acid in human urine by high-performance liquid chromatography and by isotachophoresis

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The commonly used xylene consists of a mixture of *o*-, *m*- and *p*-isomers. Xylenes taken up during occupational exposure are, to a large extent, biotransformed to the corresponding isomers of methylhippuric acid (MHA), which are excreted in the urine, and the determination of MHA in urine has been suggested for use in detecting occupational exposure to xylenes [1, 2]. Some analytical techniques for the determination of MHA have been described, such as gas chromatography (GC) [3], high-performance liquid chromatography (HPLC) [4, 5] and isotachophoresis (ITP) [6]. In this study, an HPLC and an ITP separation technique were compared on a series of human urine samples with *m*-MHA added over a concentration range of 0.39—25 mmol/l.

EXPERIMENTAL

A stock solution of *m*-MHA, synthesized according to Vogel [7], was prepared in one portion of pooled human urine collected from laboratory personnel without exposure to xylenes. Serial dilutions of this solution were prepared at concentrations of 25.0, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39

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mmol/l using the other portion of pooled urine as the diluent. Thymol was added as a preservative and the samples were kept at 4°C until taken for analysis.

An aliquot of each dilution was acidified and saturated with sodium chloride. A 1-ml volume of each acidified and salt-saturated urine sample was extracted with 5 ml of ethyl acetate by shaking for 10 min. A 2.5-ml volume of the organic layer containing MHA was evaporated to dryness in a gentle stream of nitrogen. The residue was dissolved in 0.5 ml of water and a portion of the solution was analysed by ITP. For HPLC analysis, a 0.2-ml aliquot of the ethyl aceate layer was evaporated to dryness and the residue dissolved in 0.2 ml of water.

ITP was performed as described by Sollenberg and Baldesten [6], but with some modifications. The leading electrolyte was 5 mmol/l hydrochloric acid in 0.4% hydroxypropylmethylcellulose, adjusted to pH 4.15 with β-alanine. The terminating electrolyte was 5 mmol/l caproic acid. The capillary length was 330 mm. Duplicate samples of each extract were analysed on an LKB 2127 Tachophor with an initial current of 250 μA, followed by 100 μA prior to detection of the zones. Ultraviolet detection at 254 nm was utilized. The instrument was calibrated with aqueous solutions of *m*-MHA.

The other aliquot of the urine extract was analysed in duplicate for *m*-MHA by an HPLC technique that combined the column procedure described by Matsui et al. [5] with a slightly modified elution solvent as described by Poggi et al. [4]. The Waters HPLC system consisted of a Model U6K injector, a Model 6000A solvent delivery module, a μBondapak C_{18} column (particle size 10 μm), a radial compression module and a Model 440 ultraviolet detector operated at 254 nm. A 5-μl volume of extract was injected. Peak heights were measured with a Hewlett-Packard Model 3390 integrator. The mobile phase was water—acetonitrile (90:10) containing 0.2 ml of acetic acid per litre. The flow-rate was 2 ml/min. The HPLC system was calibrated with *m*-MHA standards over the range 0—25 mmol/l.

RESULTS AND DISCUSSION

Fig. 1 shows the HPLC separation of *o*-MHA and *m*- + *p*-MHA aqueous standards. Aqueous standards of *m*-MHA (5 mmol/l) showed a within-day relative standard deviation (R.S.D.) of 2.4% and a between-day R.S.D. of 3.3%.

Fig. 2 shows ITP runs of the pooled urine with and without added *m*-MHA. The aqueous standards run each day to calibrate the ITP instrument showed an R.S.D. of up to 2.7% (n = 5). A standard solution analysed on five occasions during eight days showed an R.S.D. of 3.9%.

Fig. 3 shows the concentrations of *m*-MHA found in human urine samples with added *m*-MHA by HPLC and ITP. The HPLC results show some deviation from linearity at the highest *m*-MHA concentration. The R.S.D. averaged 2.9% over the range studied. The ITP results show good linearity and an average R.S.D. of 3.1%. The data are normally distributed for both methods.

Fig. 4 shows a comparison of HPLC and ITP for human urine samples with added *m*-MHA. The correlation coefficient is 0.997. A paired t-test on the means showed no significant differences between the methods. The plot

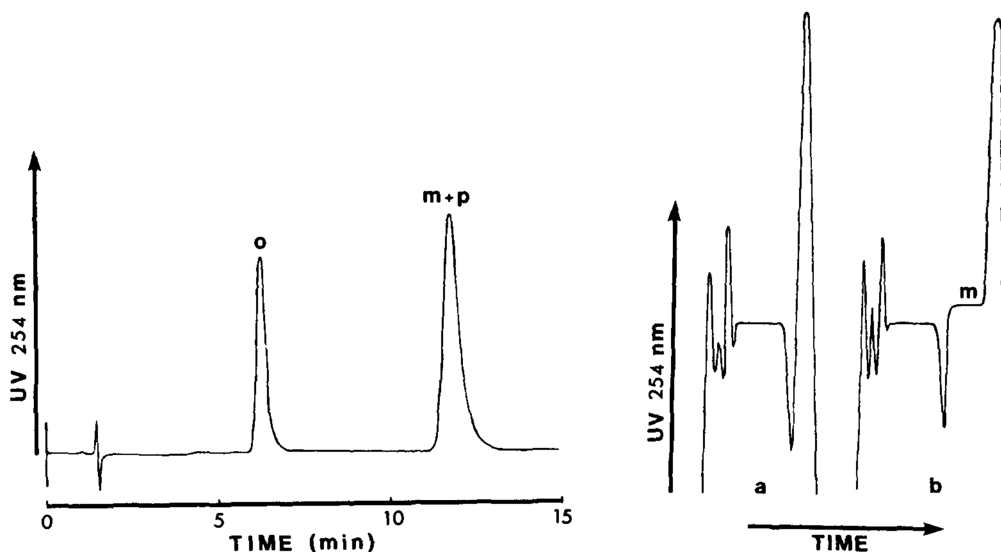

Fig. 1. HPLC separation of *o*-MHA and *m*- + *p*-MHA aqueous standards. Analytical conditions are described in the text.

Fig. 2. ITP run on pooled urine (a) before and (b) after the addition of *m*-MHA; m indicates the *m*-MHA zone.

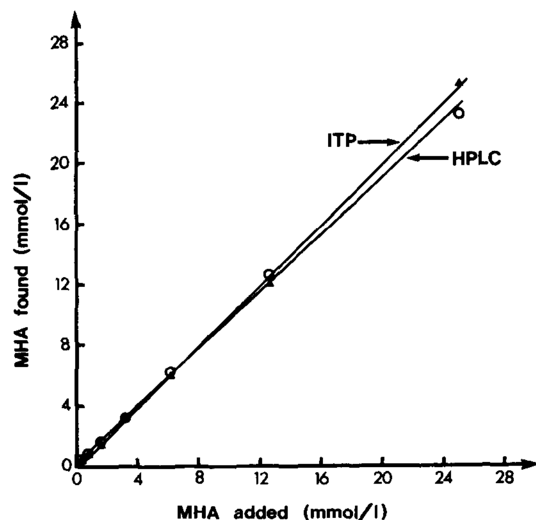

Fig. 3. Linear regression analysis of *m*-MHA found by HPLC and ITP versus concentration of *m*-MHA in human urine. ○, HPLC values; the linear regression equation for HPLC is HPLC = 0.242 + 0.937MHA with a correlation coefficient of 0.998. ▲, ITP values; the linear regression equation for ITP is ITP = −0.118 + 1.00MHA with a correlation coefficient of 0.999. Each plotted point represents the mean of ten determinations.

indicates a deviation from linearity with HPLC at the highest concentration, which reduces the slope of the calculated regression line.

The estimated limits of quantitation (defined as twice the limits of detection) for *m*-MHA in human urine were 0.2 mmol/l for both HPLC and ITP.

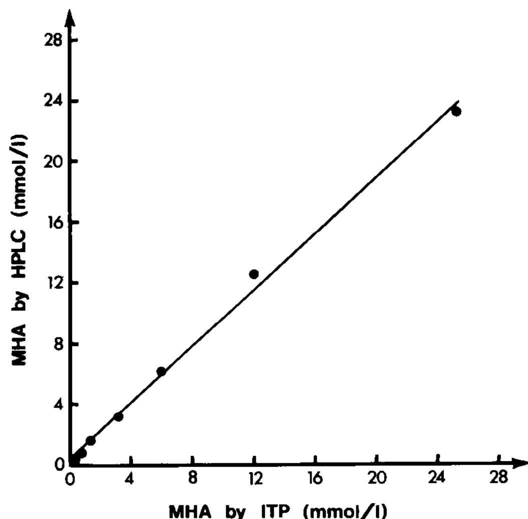

Fig. 4. Linear regression plot of *m*-MHA (mmol/l) by HPLC versus ITP. Each point represents the mean of ten determinations by each method. The linear regression equation is HPLC = 0.368 + 0.930ITP with a correlation coeficient of 0.997.

The average recovery of MHA extracted from urine was 102.0% (S.D. = 6.5%) for HPLC and 99.0% (S.D. = 4.9%) for ITP. The recoveries did not decrease at low concentrations of *m*-MHA. Urine samples with added *m*-MHA were stable at 4—6°C for at least four weeks.

The practical limits of quantitation of MHA in urine samples collected from persons exposed to xylenes were higher than for controls but were influenced by the types and amounts of interferents present in the urine. For example, when these two procedures were utilized to evaluate histology laboratory technologists for exposure to xylenes, the limits of quantitation varied from 0.2 to 0.8 mmol/l depending on the interferents present in the individual urine samples [8].

The two analytical techniques are complementary and give essentially identical data for urine samples with added *m*-MHA. The limits of detection vary depending on the presence of interferents present in the urine. HPLC has the advantage of being able to separate *o*- from *m*- + *p*-MHA, whereas ITP does not resolve the isomers of MHA. Commercial xylenes include mixtures of the isomers with *m*-MHA as the predominant isomer. *p*-Xylene represents the smallest component in the mixture. The choice of analytical methods is left to the investigator, depending on the instrumentation available and the purpose of the investigation.

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