

## Determination of hippuric-, methylhippuric-, mandelic- and phenylglyoxylic acid

**A-39.2**

### Key words

Instrumental HPTLC - quantitative analysis - densitometry - industrial medicine - toluene-, xylene-, ethylbenzene- and styrene-exposure

### Scope

Excessive exposure to toluene in the industrial environment leads to an increased presence of hippuric acid in the body. The analysis of hippuric acid in urine of exposed persons is suitable to monitor increased toluene-exposure. Likewise the finding of excessive amounts of methylhippuric acid can be used to monitor xylene exposure, mandelic acid to monitor ethylbenzene exposure and phenylglyoxylic acid to monitor styrene exposure. Using this method the mentioned substances in urine can be analyzed quantitatively without prior extraction. The limit of quantification is determined to be at 0,05 g/L, which correlates with the concentration in the urine of a non-exposed person.

### Advantages of performing this analysis by instrumental TLC

- Short analysis time
- High sample throughput at low operating cost
- Extremely simple sample preparation
- Analytical assurance by means of multi-level calibration and double determinations with little extra time and cost

## Chemicals

Water, dist.

Chloroform

Acetic acid

Methanol

Toluene

Standards: Hippuric acid (Aldrich Cat. No. 11,200-3), HA  
4-Methylhippuric acid 98% (Aldrich Cat. No. 32,802-2), MHA  
(R)(-)-Mandelic acid 98% (Aldrich Cat. No. M 220-9), MA  
Phenylglyoxylic acid 97% (Aldrich Cat. No. B 1,305-5), PGA

## Sample preparation

- Dispense 1 mL of urine sample in a centrifuge tube.
- Centrifuge the sample to precipitate suspended solids.
- An aliquot of the supernatant can be applied onto the HPTLC layer.

## Standard solution

Phenylglyoxylic acid 0,535 g/L aqueous solution

(R)(-)-Mandelic acid 1,620 g/L aqueous solution

Hippuric acid 0,525 g/L aqueous solution

4-Methylhippuric acid 0,600 g/L aqueous solution

Remark: Normally, it is recommended to dissolve the standards in the same matrix as the unknowns, i.e. analyte-free urine in this case. However, in a comparative study, no difference was found between standards dissolved in water and in blank urine.

## Chromatogram layer

HPTLC plate silica gel Merck 60 F<sub>254</sub>, 20x10 cm

## Sample application

Apply bandwise with Automatic TLC Sampler III, bandlength 6 mm, track distance 10 mm, distance from lower edge 8 mm, distance from left side 15 mm, delivery speed 100 nL/s (= 18 applications per plate); 1, 2, 4, 6, 8 and 10 µL of the standard, 1 µL of the unknowns, all in duplicate, according to the application pattern.

Note, when a Linomat is used instead of the Automatic TLC Sampler III, it is recommended to dilute unknowns and standards 1:1 and apply double the volumes, in order to ensure volume precision.

Application pattern:

track	1	2	3	4	5	6	7	8	9	...
	S1	a	b	S2	c	d	S3	e	f	...
μL	1	1	1	2	1	1	4	1	1	...
PGA mg/mL	0,535			1,070			2,140			...
MA mg/mL	1,620			3,240			6,480		1	...
HA mg/mL	0,525			1,050			2,100			...
MHA mg/mL	0,600			1,200			2,400			...

## Chromatography

In CAMAG twin-trough chamber 20x10 cm with 10 min chamber saturation with chloroform - methanol - acetic acid - water 80:20:8:2; migration distance 70 mm;  $R_f$ -values: PGA = 28, MA = 40, HA = 50, MHA = 55.

## Densitometric evaluation

CAMAG TLC Scanner with CATS Software, scanning by absorbance at 233 nm with deuterium lamp, evaluation via peak area by polynomial regression.

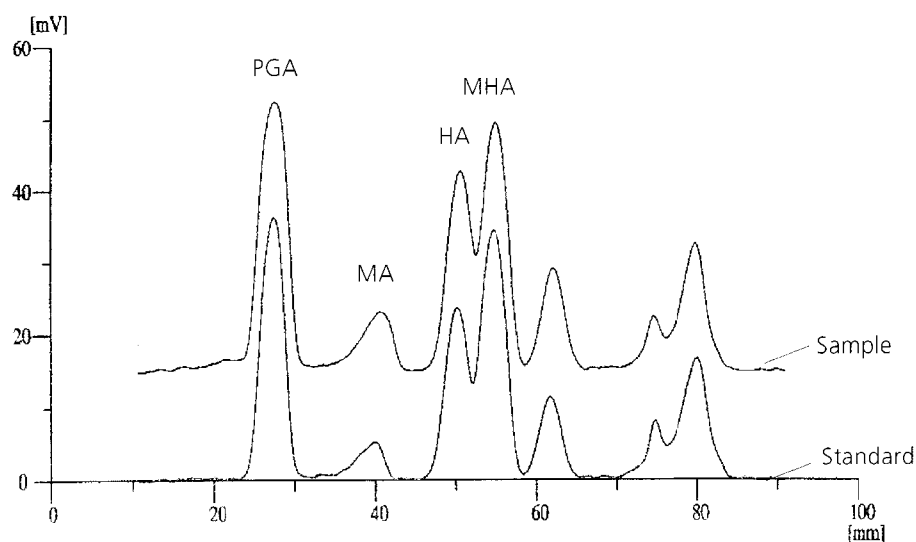


Fig. 1 Densitogram of sample and standards

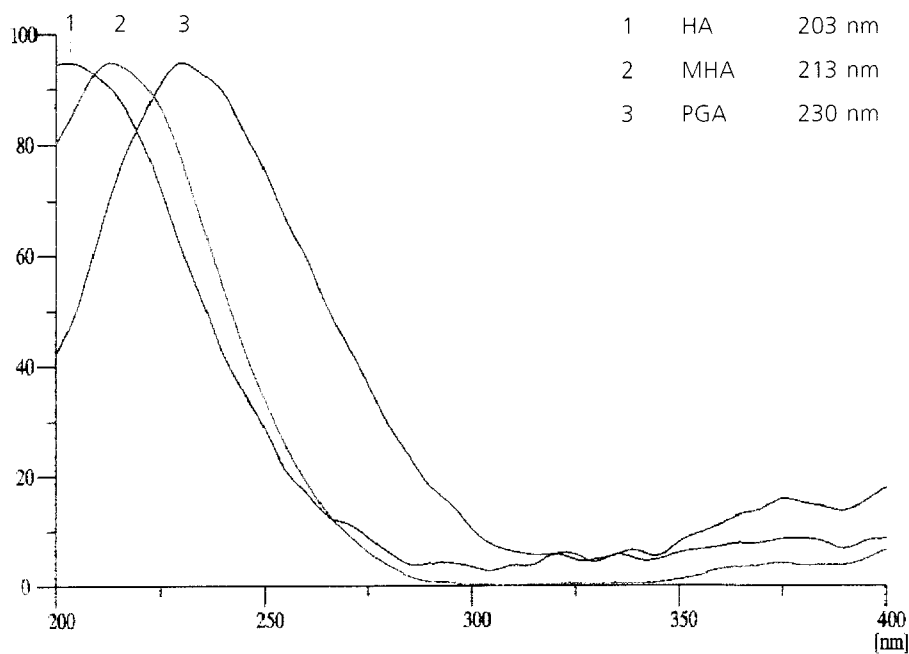


Fig. 2 In-situ spectra (200-400 nm) of 4-methylhippuric acid, phenylglyoxylic acid and hippuric acid.

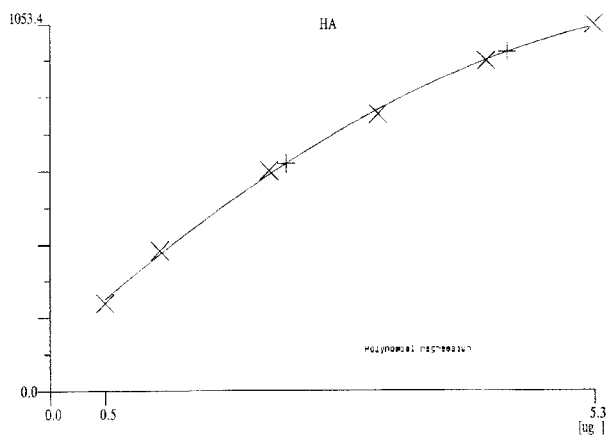


Fig. 3 Calibration curve of hippuric acid

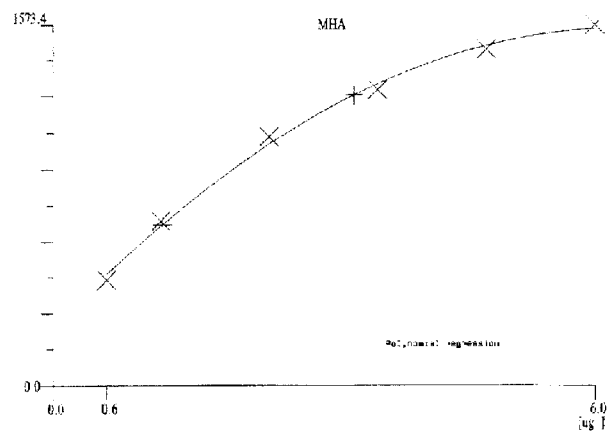


Fig. 4 Calibration curve of 4-methylhippuric acid

## Results

The limit of quantification is determined to be at 0,05 g/L which is well suitable to monitor the exposure of PGA, HA and MHA. The concentration in the urine of an non-exposed person lies between 0,1-0,4 g/L. Concentrations of 3,0 g/L are considered as warning limit and concentrations of 5,0 g/L as action limit. The values for MA are 3 times higher.