CHROMSYSTEMS®

DIAGNOSTICS BY HPLC & LC-MS/MS

ARBEITSMEDIZIN
OCCUPATIONAL MEDICINE
MÉDECINE DU TRAVAIL
MEDICINA DEL LAVORO
MEDICINA OCUPACIONAL


Instruction Manual for the HPLC Analysis of

# Hippuric Acid, Methylhippuric Acids, Mandelic Acid, Phenylglyoxylic Acid in Urine

Order Number 43000

CE IVD

Chromsystems Instruments & Chemicals GmbH is certified according to DIN EN ISO 9001, DIN EN ISO 13485 and ISO 13485 CMDR. Products are produced and put into circulation according to IVD guideline 98/79/EC.



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# Contents .....Page

# 1 Ordering information

| Order no. | Product | |
|---|---|---|
| **43000** | **HPLC Reagent kit** | |
| | **Hippuric Acid, Methylhippuric Acids, Mandelic Acid, Phenylglyoxylic Acid in urine** | |
| | Contents for 100 analyses: | |
| | Mobile Phase | 1000 ml |
| | Urine Calibration Standard | 5 x 0.5 ml (lyoph.) |
| | Internal Standard | 100 ml |
| | Reaction vials | 100 pcs. |
| | **Components available separately** | |
| 43001 | Mobile Phase | 1000 ml |
| 43002 | Mobile Phase | 10 x 1000 ml |
| 43003 | Urine Calibration Standard | 5 x 0.5 ml (lyoph.) |
| 43004 | Internal Standard | 100 ml |
| 3006 | Reaction vials | 100 pcs. |
| | **Accessories** | |
| 43100 | HPLC column (equilibrated, with test chromatogram) | 1 pc. |
| 15009 | PEEK-encased prefilter, 5µm | 5 pcs. |
| 15010 | PEEK prefilter housing | 1 pc. |
| 18001 | Precolumn cartridge holder 4/10 | 1 pc. |
| 18043 | Precolumn cartridge 4/10 | 1 pc. |
| | **Chromsystems calibrator and controls for hippuric acid, methylhippuric acids, mandelic acid, phenylglyoxylic acid in urine** | |
| 43003 | Urine Calibration Standard | 5 x 0.5 ml (lyoph.) |
| 0141 | Urine Control Bi-Level (I + II) | 2 x 5 x 0.5 ml (lyoph.) |
| 0142 | Urine Control Level I | 5 x 0.5 ml (lyoph.) |
| 0143 | Urine Control Level II | 5 x 0.5 ml (lyoph.) |

# 2 Introduction

Styrene is an important monomer used in the synthesis of plastics. Toluene and xylene are of great importance as organic solvents for oils, paint and printing inks, adhesives, dry cleaning, and as ingredients in fuels as anti-knocking agents. These compounds are also abused in "glue sniffing". Because these substances are highly volatile they are mainly taken up by inhalation, being rapidly absorbed by the lungs. Absorption through the skin, too, is possible but much slower. As they are highly lipophilic, highest concentrations will be found in body fat and in nervous tissue. For urinary excretion metabolisation into water soluble molecules is necessary:

Toluene firstly is oxidized following conjugation with the amino acid glycine thus forming hippuric acid. Xylene is metabolised in the same way forming the corresponding ortho-, meta- and para-methylhippuric acids (see figure 1).

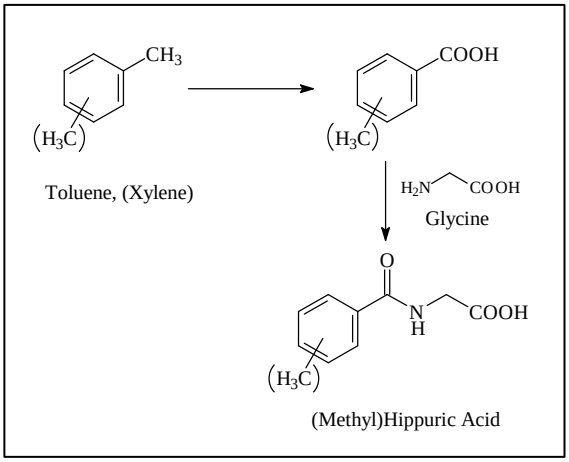



Figure 1:

Metabolism of Toluene and Xylene

Styrene, too, is oxidized but is not conjugated and excreted as mandelic acid (85 %) and phenylglyoxylic acid (10 %) (see figure 2).

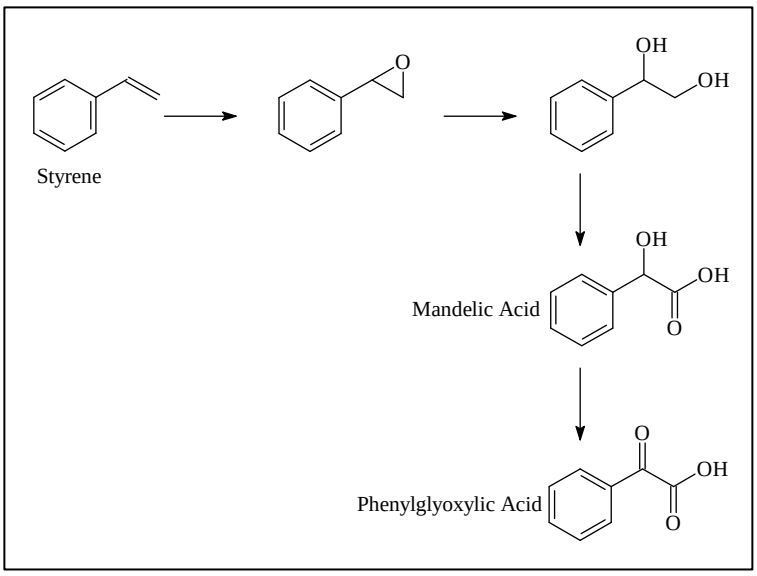



Figure 2:

Metabolism of Styrene

In acute intoxication, euphoria and hallucinations are the main symptoms; chronic poisoning leads to damage in the central nervous and peripheral system, in the liver, and the kidneys. As an indication of exposure the Threshold Limit Value (TLV) is commonly used. It is defined as the airborne concentration to which an individual may be repeatedly exposed eight hours per day, five days per week without adverse effect.

Convincing evidence that toluene, xylene or styrene have carcinogenic or teratogenic effects has not yet been found. Frequently, however, they are contaminated with benzene since large-scale production of benzene free toluene, xylene or tyrene is difficult and/or too expensive.

The aim of the **"Occupational Medicine"** is the biological monitoring of the individual's exposure to assess the possible risk for damage of health. For this, determination of the metabolites in urine gives the most satisfactory data. Even after exposure to toluene, xylene or styrene, the concentrations of these substances in blood are very low due to their very rapid metabolism; 95 % of the absorbed amounts are excreted within 24 h. Measurement of the airborne concentrations ignores the time and pharmacokinetics of absorption, metabolism, and clearance, and other routes into the body e.g. through the skin. The level of the metabolites in urine, however, correlates directly with exposure giving the most convincing data.
To assess whether exposure levels are within acceptable limits, the biological monitoring results must be compared with defined reference standards. For this the so called Biological Exposure Index (BEI) has been developed. It is defined as the concentration of a substance or its metabolite in biological material (e.g. blood, urine) which does not normally affect the individual's health.

**Intended use:**
The Chromsystems reagent kit Hippuric Acid, Methylhippuric Acids, Mandelic Acid, Phenylglyoxylic Acid in urine is an *in vitro* diagnostic device to be used in clinical laboratories for the quantitative determination of hippuric acid, ortho-, meta-, and para –methylhippuric acid, mandelic acid as well as phenylglyoxylic acid in patient urine samples via high performance liquid chromatography (HPLC). It is intended as a test for patients whose levels of metabolites in urine act as a marker of styrene, toluene or xylene are exposed following an occupational medicine investigation should be monitored.

**Principle of the reagent kit:**
This Chromsystems reagent kit allows specific monitoring of hippuric acid, methylhippuric acids, mandelic acid, and phenylglyoxylic acid in a single HPLC run. During sample preparation the urine is stabilised and particulates in the urine are removed by centrifugation. The sample can by analysed with any isocratic HPLC system with UV detection. Inclusion of an internal standard minimizes analytical variations and guarantees high precision and reliability of the results.

# 3 HPLC system

| | |
|---|---|
| **Caution**: | When using the reagents, please note the recommended hazards information in appendix I. |

## 3.1 Equipment and instrument parameters

The analysis of hippuric acid, methylhippuric acids, mandelic acid, and phenylglyoxylic acid requires a simple, isocratic HPLC system with a pump, injector and UV detector. Vacuum or any other form of degassing should not be used as this could result in changes in the mobile phase. To prevent this, the mobile phase should be kept capped, even during use. The use of a thermostatted column oven will avoid temperature variations and ensure optimal stability and reproducibility of the chromatographic separation.

**Instrument settings:**

| | |
|---|---|
| Injection volume: | 20 µl |
| Run time: | 20 min |
| Flow rate: | 1.0 ml/min |
| Column temperature: | ambient (approx. 25 °C) |
| UV detector: | Wavelength 207 nm |
| Needle rinsing solution for the injector: | pure HPLC water |

## 3.2 HPLC column

The HPLC column for the analysis of occupational medicine parameters is supplied equilibrated and tested, and is ready to use. **It must not be rinsed with any other solutions**. The backpressure of a new column at a flow rate of 1.0 ml/min is about 100 bar and may increase with column age and/or use. As long as the separations are satisfactory, a raised backpressure is of no consequence. To lengthen column life, a PEEK prefilter (order no. 15009 and 15010) or a precolumn (order no. 18001 and 18043) should be used.

**Before beginning an analysis:**

1. Rinse the system with approx. 30 ml mobile phase at a flow rate of 1.0 ml/min before installing the HPLC column;
2. Install the column and equilibrate the system at a flow rate of 1.0 ml/min for about 15 – 20 min, until the baseline has stabilized;
3. Inject the prepared calibrator repeatedly until two successive chromatograms show identical retention times and peak areas/heights.
4. Thereafter, the mobile phase can be recirculated.

## 3.3 Shut-down

For periods of disuse up to 3 days, pump the mobile phase at a low flow rate (approx. 0.2 ml/min) through the system. The HPLC column remains connected, but to lengthen the life of the detector lamp, the detector should be turned off. For longer periods of disuse, the HPLC column should be disconnected. Rinsing or conservation is not necessary. Store the column in mobile phase (at **room temperature**). The column should be replaced by a union and the HPLC system rinsed with about 50 ml $H_2O$/methanol (80/20 vol/vol).

# 4 Chromatographic separation

The following table shows the retention times of the analytes and the internal standard at a flow rate of 1.0 ml/min:

| Substance | Retention time (approx. min) |
|---|---|
| Mandelic Acid | 3.7 |
| Hippuric Acid | 6.2 |
| Phenylglyoxylic Acid | 7.0 |
| o-Methylhippuric Acid | 8.7 |
| Internal Standard | 13.1 |
| p-Methylhippuric Acid | 16.0 |
| m-Methylhippuric Acid | 17.0 |

The chromatographic separation takes about 20 min (see chromatograms chapter 12). Minor variations in the retention times may be due to e.g. temperature changes. If a new lot of the mobile phase is used or the HPLC column is replaced, the retention times may change slightly. Therefore, the retention times should be adapted appropriately.

# 5 Sample preparation

**Caution**: When using the reagents, please note the recommended hazards information in appendix I.

## 5.1 Collection and storage of patient specimens

Urine collected after end of shift is used for analysis. Specimens must be kept cool for transport. Storage life is up to 5 days at +2 to +8 °C. For longer storage (up to a maximum of 3 months) deep freeze below –18 °C. **Avoid repeated freeze/thaw cycles!**

**Note**: It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

## 5.2 Reconstitution of the urine calibrator

The calibrator (order no. 43003) is traceable to reference substances purchased from a certified supplier. After reconstitution, the calibrator is subjected to the entire sample preparation procedure, analogous to patient specimens. The standard, so prepared, is used to calibrate the HPLC system. **To reconstitute the lyophilised urine calibrator, pipette exactly 0.5 ml distilled water into the vial.** Let the vial stand at room temperature for about 10 - 15 min, shake occasionally and gently until the vial contents are clear. Avoid exposure to direct sunlight! The actual concentrations depend on the batch and will be found on the information leaflet accompanying the calibrator.

**Caution:**
This product has been manufactured from pooled human urine. Each donor contributing to this product is constantly subjected to medical control and judged as free of infectious diseases. Due to the fact that there is no test method giving absolute assurance that products containing human source materials will be free of infectious agents, a possible danger of infection should be taken into account. This product may also contain unknown agents or other pathogens for which there are no approved tests. We therefore recommend considering all products containing human source material as potentially infectious. As a consequence exercise the same care in the handling of this product as in the handling of potentially infectious patient samples.

**Storage life of the reconstituted calibrator:**
The reconstituted calibrator can be kept for up to 4 days if stored tightly sealed, light protected and cool (+2 to +8 °C). For longer periods of storage (up to a maximum of 3 months), aliquot and deep freeze (below –18 °C).

## 5.3 Reconstitution of the urine controls

The urine controls level I (order no. 0142) and level II (order no. 0143) are subjected to the entire sample preparation, analogous to patient specimens. The prepared controls are included in every analytical series to monitor accuracy and precision within the system. **To reconstitute the lyophilised urine controls, pipette exactly 0.5 ml distilled water into the vial.** Let the vial stand at room temperature for about 10 - 15 min, shake occasionally and gently until the vial contents are clear. Avoid exposure to direct sunlight! The actual concentrations depend on the batch and will be found on the information leaflet accompanying the controls.

**Caution:**
This product has been manufactured from pooled human urine. Each donor contributing to this product is constantly subjected to medical control and judged as free of infectious diseases. Due to the fact that there is no test method giving absolute assurance that products containing human source materials will be free of infectious agents, a possible danger of infection should be taken into account. This product may also contain unknown agents or other pathogens for which there are no approved tests. We therefore recommend considering all products containing human source material as potentially infectious. As a consequence exercise the same care in the handling of this product as in the handling of potentially infectious patient samples.

**Storage life of the reconstituted urine controls:**
The reconstituted urine controls can be kept for up to 4 days if stored tightly sealed, light protected and cool (+2 to +8 °C). For longer periods of storage (up to a maximum of 3 months), aliquot and deep freeze (below –18 °C).

## 5.4 Sample preparation procedure

1. Place 1000 µl Internal Standard into a labelled reaction vial;

2. Add 10 µl urine (calibrator, controls, specimens) and mix briefly (vortex), rinse the pipette tip several times;

3. Centrifuge 5 min at 9000 x g;

4. Inject 20 µl of supernatant into the HPLC system.

Precision and accuracy of the analyses should be monitored by the inclusion of additional controls in each analytical run.

## 5.5 Storage life of samples

The prepared samples can be kept for at least 24 hours at room temperature, at +2 to +8 °C up to 5 days. For longer periods of storage (up to 14 days), deep-freeze the sample (below –18 °C).

# 6 Data acquisition and evaluation

## 6.1 Calibration of the data analysis system

The actual concentration of the analytes in the calibrator depends on the batch and will be found on the information leaflet accompanying the calibrators. Before beginning the quantitative analysis of patient samples, it is recommended that a calibration chromatogram should be run. For this purpose, inject the prepared calibrator repeatedly, until two successive chromatograms show practically identical retention times, peak resolution and peak areas/heights. These chromatograms can be used to set the integration parameters correctly. The chromatogram of the last test injection is used to calibrate the data analysis system (PC software, integrator).

Enter the retention times obtained and the concentrations (see information sheet) of the calibrator in the analysis table:

| Peak no. | Analyte | Retention time (approx. min) | Concentration [mg/l] |
|---|---|---|---|
| 1 | Mandelic Acid | 3.7 | see information sheet |
| 2 | Hippuric Acid | 6.2 | see information sheet |
| 3 | Phenylglyoxylic Acid | 7.0 | see information sheet |
| 4 | o-Methylhippuric Acid | 8.7 | see information sheet |
| 5 | Internal Standard | 13.1 | 1 |
| 6 | p-Methylhippuric Acid | 16.0 | see information sheet |
| 7 | m-Methylhippuric Acid | 17.0 | see information sheet |

To ensure that neither the calibration nor the HPLC conditions (retention times etc.) have changed in the course of an analytical run, the prepared calibrator should be injected during the run and again at the end. For evaluation select "internal standard method".

## 6.2 Quantitative evaluation with internal standard

The use of an Internal Standard allows potential losses during sample preparation to be compensated for. A known amount of the Internal Standard is added to every specimen (calibrator, controls, patient specimen). The integrator is given the appropriate peak (see sample chromatograms in chapter 13) from the calibration run as the Internal Standard in the component table. **Since the same amount of Internal Standard is added to the urine calibrator and to the patient samples, the concentration of the Internal Standard can be entered as "1".**

# 7 Quality control

Precision and accuracy of the analyses can be monitored by the inclusion of additional controls in each analytical run (Chromsystems urine controls, order no. 0142, 0143).

If the analysis of these controls yields values outside the range given on the accompanying information leaflet the system must be checked and, if necessary, recalibrated.

# 8 Biological Exposure Indices (BEI)

| Substance | BEI [5] (mg/l Urine) |
|---|---|
| Hippuric Acid | no values available |
| Methylhippuric Acids | 2000 mg/l |
| Mandelic Acid plus Phenylglyoxylic Acid | 600 mg/g Creatinine or rather 300 mg/l |

# 9 Conversion factors

The following table lists conversion factors between mass and molar concentrations and conversely.

| Substance | µmol/l to mg/l | mg/l to µmol/l |
|---|---|---|
| Hippuric Acid | 0.179 | 5.58 |
| Methylhippuric Acids | 0.193 | 5.18 |
| Mandelic Acid | 0.152 | 6.57 |
| Phenylglyoxylic Acid | 0.150 | 6.66 |

# 10 Storage and lifetime of the reagents

Unopened reagents can last up until the expiry date stipulated on the label, provided that the storage conditions indicated on the label are complied with.

**Storage conditions of the reagents:**

| Product | Storage |
|---|---|
| Mobile Phase | +18 to +30 °C |
| Internal Standard | +2 to +8 °C |
| Urine Calibrator | below –18 °C |
| Urine Controls, level I and II | below –18 °C |

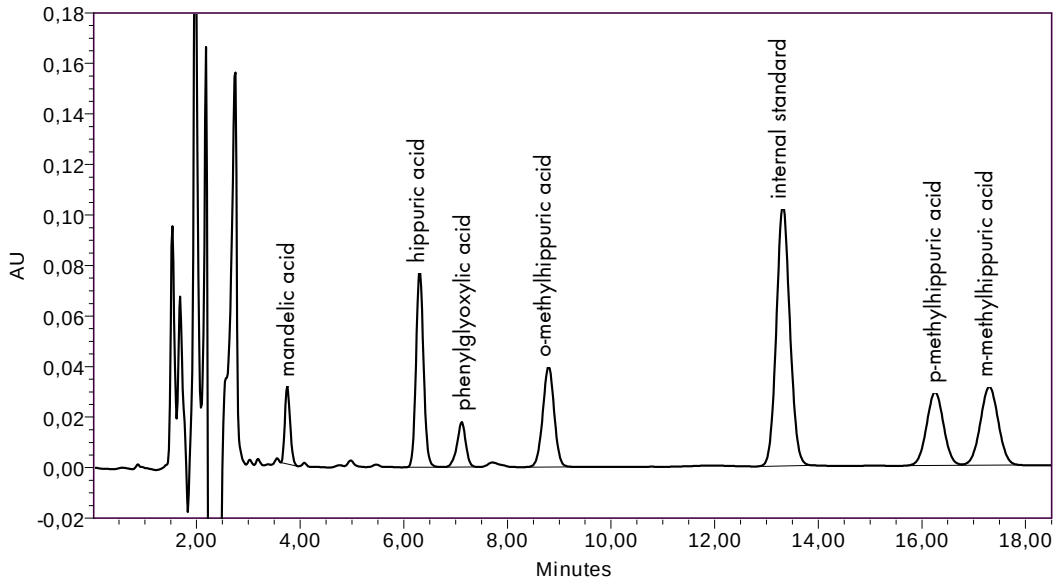
The reagents must be properly closed and stored directly after use. Provided that nothing else has been stipulated, the lifetime would then amount to one year after the date of opening, but will not exceed the expiry date. For calibrator and controls refer to chapter 5.2 and 5.3.

# 11 Waste disposal

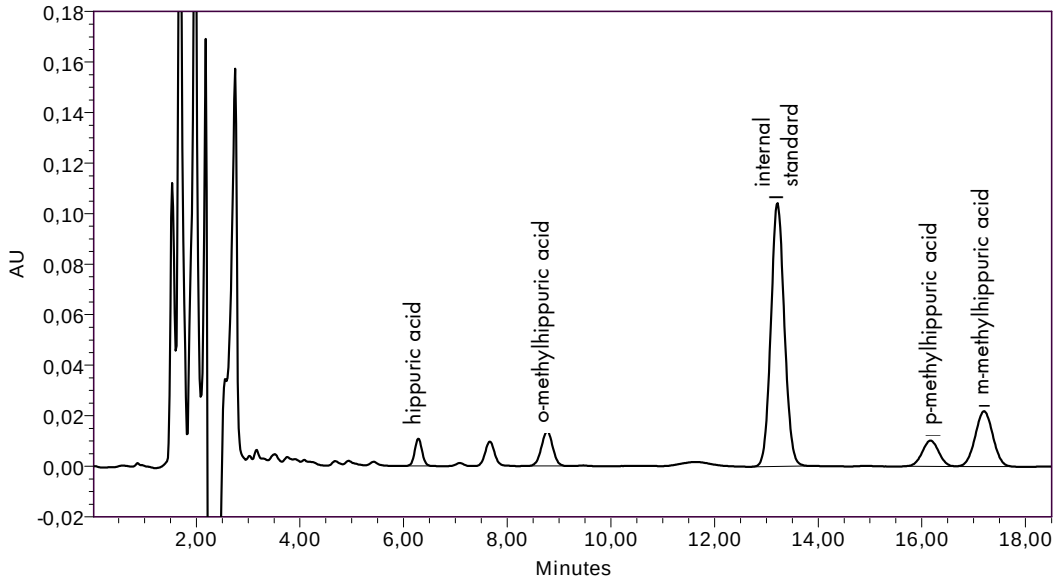
The Mobile Phase and residues of the prepared specimens contain organic solvents. Dispose product residues into a container for organic halogen-free solvents. They must not be disposed together with domestic waste. Do not circulate into the main water supply. Dispose of in compliance with Directive 2008/98/EC on Waste and national and local requirements. The waste containers must be stored appropriately and only access permitted to authorized parties.

# 12 Examples of chromatograms

## 12.1 Chromatogram of a urine calibrator




## 12.2 Chromatogram of a patient exposed to Xylene




| | | |
|---|---|---|
| Hippuric Acid: | 200 mg/l | (1120 µmol/l) |
| o-Methylhippuric Acid: | 240 mg/l | (1240 µmol/l) |
| p-Methylhippuric Acid: | 240 mg/l | (1240 µmol/l) |
| m-Methylhippuric Acid: | 490 mg/l | (2540 µmol/l) |

# 13 Trouble shooting

| Problem | Possible cause | Remedy |
|---|---|---|
| **Interference peaks** | Air in the system | Purge HPLC system. |
| | HPLC column contaminated | Rinse column with 30 ml HPLC water and acetonitrile each and inject water and acetonitrile several times (approx. 10 times). |
| | | Replace column. |
| | Injection system contaminated | Clean with methanol or acetonitrile and inject mobile phase several times. |
| | Injector contaminated | Clean injector. |
| | Autosampler vials contaminated | Use new vials, or clean vials with methanol. |
| **Broad peaks, tailing** | HPLC column too old | Renew column. |
| **Baseline drifts** | Detector lamp still cold | Wait. |
| | Detector lamp too old | Replace lamp. |
| | System not yet in equilibrium | Inject calibrator repeatedly, until two successive chromatograms are identical. |
| | Temperature drift | Use column oven. |
| | Flow rate not constant | Check pump. |
| **Baseline unstable** | HPLC pump | Check pump (air, leaks). |
| | Air in the system | Purge HPLC system. |
| | Detector cell contaminated | Clean detector cell. |
| **Double peaks** | Dead volume in fittings | Renew fittings. |
| | Dead volume in HPLC column | Renew column. |
| **No peaks** | Injector leaks | Check injector. |
| **Reduced sensitivity** | Detector lamp ageing | Replace lamp. |
| | Detector cell contaminated | Clean cell. |
| | Defective injection valve | Check injector. |

| Problem | Possible cause | Remedy |
|---|---|---|
| **Retention times change** | Temperature drift | Use column oven. |
| | Irregular flow rate | Check HPLC pump, adjust flow rate. |
| | System not yet in equilibrium | Pump mobile phase for about 15 min through the system; inject calibrator repeatedly. |
| **No signal** | Connection to integrator/recorder defective or interrupted | Check signal cable and connection. |
| | Detector lamp | Check supply voltage; renew lamp if necessary. |

# 14 Literature


1. Flanagan RJ, Ruprah M, Meredith TJ, Ramsey JD. (1990) An introduction to the clinical toxicology of volatile substances. *Drug Saf* **5**(5): 359–83.

2. Marczynski B, Peel M, Baur X. (2000) New aspects in genotoxic risk assessment of styrene exposure – a working hypothesis. *Med Hypotheses* **54**(4): 619–23.

3. Langman JL. (1994) Xylene: its toxicity, measurement of exposure levels, absorption, metabolism and clearance. *Pathology* **26**(3): 301–9.

4. Ikeda M. (1995) Exposure to complex mixtures: implications for biological monitoring. *Toxicol Lett* **77**(1-3): 85–91.

5. Deutsche Forschungsgemeinschaft (Hrsg). MAK- und BAT-Werte-Liste 2013: Maximale Arbeitsplatzkonzentrationen und Biologische Arbeitsstofftoleranzwerte. Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Mitteilung 49, Wiley-VCH Verlag (2013).

# Appendix I: Hazardous substance information

The following information must be noted and the relevant safety measures taken. More information can be gathered from the respective material safety data sheets. These are available upon request or can be downloaded from our website www.chromsystems.com.

| Pictograms | Hazard and precautionary statements |
|---|---|
| **Mobile Phase (order no. 43001, 43002)** | |
|  | **Warning**<br><br>H226 Flammable liquid and vapour.<br>H351 Suspected of causing cancer.<br><br>P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.<br>P280 Wear protective gloves/protective clothing/eye protection/face protection.<br>P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. |
| **These components are not classified as dangerous according to European Union legislation:**<br><br>Urine Calibration Standard (order no. 43003)<br>Internal Standard (order no. 43004)<br>Urine Controls (order no. 0141, 0142, 0143) | |

# Appendix II: Notes on manual calculation

For the manual calculation the following data are required:

- Peak area/height of substance A in the chromatogram of the sample = $A_{Sample}$
- Peak area/height of substance A in the chromatogram of the calibrator = $A_{Calibrator}$
- Peak area/height of the internal standard in the chromatogram of the sample = $IS_{Sample}$
- Peak area/height of the internal standard in the chromatogram of the calibrator = $IS_{Calibrator}$
- The concentration of the substance A in the calibrator = $C_{Calibrator}$

The concentration of the substance A in the sample ($C_{Sample}$) is then calculated as follows:

$$C_{Sample}\ [mg/l] = \frac{A_{Sample} \times IS_{Calibrator}}{A_{Calibrator} \times IS_{Sample}} \times C_{Calibrator}$$

# Appendix III: Validation

To check the linearity and to validate the method, urine specimens were spiked with defined amounts of hippuric acid, o-, m- and p-methylhippuric acid, mandelic acid, and phenylglyoxylic acid. Multiple aliquots from these preparations were subjected to the sample preparation procedure.

**Recovery:**
The analytical recovery was determined from the slope of the calibration curves of spiked urine samples and diluted standard solutions. The following table show the recovery rates of the analytes and the Internal Standard:

| | Recovery [%] |
|---|---|
| Mandelic Acid | 103 |
| Hippuric Acid | 103 |
| Phenylglyoxylic Acid | 109 |
| o-Methylhippuric Acid | 103 |
| p-Methylhippuric Acid | 104 |
| m-Methylhippuric Acid | 102 |
| Internal Standard | 102 |

**Linearity / limit of quantification:**
The method is linear from the designated limit of quantification up to the upper limit.

| | Limit of quantification * [mg/l] | Linear range (up to at least mg/l) |
|---|---|---|
| Mandelic Acid | 10 | 4000 |
| Hippuric Acid | 11 | 18000 |
| Phenylglyoxylic Acid | 6 | 1650 |
| o-Methylhippuric Acid | 5 | 7000 |
| p-Methylhippuric Acid | 4 | 7100 |
| m-Methylhippuric Acid | 6 | 7000 |

*The limit of quantification depends on the detector employed.

**Intra-assay precision:**
Determination of the intra-assay precision was done by means of multiple clean up (n = 10) and determination of the analyte concentrations of the same specimen at 3 different concentrations:

| | Coefficient of variation [%] (concentration mg/l) | | |
|---|---|---|---|
| | n = 10 | n = 10 | n = 10 |
| Mandelic Acid | 1,0 (242) | 0,7 (647) | 0,8 (425) |
| Hippuric Acid | 1,0 (951) | 0,7 (1930) | 0,8 (1460) |
| Phenylglyoxylic Acid | 1,1 (98,0) | 0,8 (394) | 0,8 (228) |
| o-Methylhippuric Acid | 0,9 (371) | 0,7 (1231) | 0,8 (710) |
| p-Methylhippuric Acid | 1,1 (400) | 0,9 (1237) | 0,8 (719) |
| m-Methylhippuric Acid | 1,1 (392) | 0,9 (1218) | 0,8 (708) |

**Inter-assay precision:**

Determination of the inter-assay precision was done by tenfold clean up and determination of the analyte concentration in 3 urine pools in 10 different test series:

| | Coefficient of variation [%] (concentration mg/l) | | |
|---|---|---|---|
| | n = 100 | n = 100 | n = 96 |
| Mandelic Acid | 3,3 (225) | 4,3 (634) | 1,7 (428) |
| Hippuric Acid | 3,5 (888) | 4,1 (1872) | 0,9 (1459) |
| Phenylglyoxylic Acid | 3,7 (91,5) | 3,9 (380) | 0,9 (227) |
| o-Methylhippuric Acid | 3,6 (346) | 4,1 (1192) | 0,9 (709) |
| p-Methylhippuric Acid | 3,5 (373) | 4,6 (1209) | 0,9 (718) |
| m-Methylhippuric Acid | 3,5 (367) | 4,5 (1191) | 1,0 (708) |

CHROMSYSTEMS
DIAGNOSTICS BY HPLC & LC-MS/MS



# EC-Declaration of Conformity

according to directive 98/79 EC on in vitro diagnostic medical devices

We, as manufacturer

*Chromsystems Instruments & Chemicals GmbH*
*Am Haag 12*
*D-82166 Gräfelfing, Germany*

declare on our own responsibility, that herein after called in vitro diagnostic medical devices for the HPLC determination of:

Nomenclature term: Hippuric Acid
Nomenclature code: 12-09-02-08-00
Classification: *other product*

Product name: **Hippuric acid, Methylhippuric acid, Mandelic acid and Phenylglyoxylic Acid in Urine**
Controls: **Occupational Medicine Urine Control**

meets all applicable requirements of the directive 98/79/EC

Conformity assessment procedure:
Annex III of the directive 98/79/EC

Applied harmonized standards:
*EN ISO 9001, EN ISO 13485, EN ISO 14971, EN 18113-2, EN 980, EN 13640, EN 13641*

Notified body: –

Munich, February 02, 2012

Michael Meier
Managing Director

Vers. 2.0

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