Trans 1,2 CICLOHEXANEDIOL in urine by GC/FID and GC/MS
Code GC19310

BIOCHEMISTRY

The trans 1-2 cycloexanediol is one of the products (with 1-4 cycloexanediol) of cyclohexane metabolism and it is present in free form or in conjugated form (70%) in the urine of subjects exposed to the solvent (cyclohexane) but also substances such as cyclohexanol and cyclohexanone.

The metabolism of cyclohexane, by liver microsomal enzymes, leading to the formation of diols. In a survey of subjects exposed to cyclohexane, the various urinary metabolites were present in the following proportions: cyclohexanol 0.5%, 23.4% trans 1-2cyclohexanediol, 1-4 cyclohexanediol 11.3%. The metabolic pattern is practically the same for exposure to cyclohexanol and cyclohexanone. The elimination half-life of the diols as a result of exposure to cyclohexane by inhalation is of 14-18h.

The instrumental technique used for the determination of trans 1-2 cyclohexanediol is gas chromatography with flame ionization detector (FID). This method is routinely applicable to biological monitoring of subjects occupationally exposed to cyclohexane. It must be clearly assessed the possibility of simultaneous exposure to cyclohexanol and cyclohexanone, which lead to the excretion of these metabolites. The analysis of the correlation line between the environmental data of cyclohexane and the urinary concentrations of metabolites showed that the most reliable indicator is the trans 1-2 cyclohexanediol because more sensitive, specific and well correlated with the external dose of the solvent.

Using the sum of the two indicators, trans 1-2 and 1-4 cyclohexanediol not produce the best results (Vol. 6 "Environmental and biological monitoring of occupational exposure to xenobiotics).
TECHNICAL FEATURES

Principle of the Method:
The present method determines the trans 1-2 Cyclohexanediol in urine using column chromatography with FID or MS detector after acid hydrolysis of urine and liquid-liquid extraction.

Recovery: 98%

Sensitivity: 1 mg/l

Dynamic Range of the Method: 1 - 300 mg/l

Reference Values:
< 170 mg/g creatinine exposed subjects
< 1,4 mg/g creatinine note exposed subjects

Ref. List of MAK and BAT Values 2007. DFG

CV%: 1,39

Components of the kit:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Quantity</th>
<th>Storage Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>Diluting Solution</td>
<td>1 x 50 ml</td>
<td>≤ 20°C</td>
</tr>
<tr>
<td>Reagent B</td>
<td>Hydrolysis Reagent</td>
<td>1 x 50 ml</td>
<td></td>
</tr>
<tr>
<td>Reagent C</td>
<td>Test Solution</td>
<td>1 x 10 ml</td>
<td>Store at 2-8 °C</td>
</tr>
<tr>
<td>Reagent D</td>
<td>Neutralization Solution</td>
<td>1 x 100 ml</td>
<td></td>
</tr>
<tr>
<td>Reagent E</td>
<td>Stabilization Solution</td>
<td>1 x 250 gr</td>
<td></td>
</tr>
<tr>
<td>Reagent F</td>
<td>Estraent Solution with Internal Standard</td>
<td>1 x 150 ml</td>
<td></td>
</tr>
<tr>
<td>Reagent G</td>
<td>Calibrator lyophil.</td>
<td>2 x 10 ml</td>
<td>See Warnings</td>
</tr>
</tbody>
</table>

Minimum Instrumental equipment required:

GC/FID or GC/MS Instrument
Operational Computer

Optional Equipment:

Autosampler

Urine Collection Procedure:
At the end of the shift, at the end of the week, collect urine in plastic containers (polyethylene or polycarbonate) to 150 ml. If the exposure is constant, there are no significant differences between the urine of those Thursday and Friday. The urine samples should be stored at - 20 ° C until analysis. In case you make the analysis within 3-4 days from the time of collection, samples can be stored at 2-8 ° C.
IMPORTANT: USE ONLY IN GLASS TUBE EVERY STEP - DO NOT USE PLASTIC TUBES

PREANALYTICAL PROCEDURE

Preparation of Test Solution.

Dispense in a glass tube of 10ml:

- 900 µl of Reagent A – Diluting Solution
- 100 µl of Reagent C – Test Solution

**Vortex for 20 sec.**

Inject 1 µl of solution into gas chromatograph GC. Verify that the trans-1,2 Cyclohexanediol has retention time and massa spectrum similar to fig. below. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

ANALYTICAL PROCEDURE

STEP 1: Dispense in a pyrex tube with teflon cap:

<table>
<thead>
<tr>
<th></th>
<th>Calibrator</th>
<th>Control</th>
<th>Sample</th>
<th>Blanck</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G - Calibrator</td>
<td>2500 µl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2500 µl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td>2500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanck</td>
<td></td>
<td></td>
<td>2500 µl</td>
<td></td>
</tr>
<tr>
<td>Reagent B – Hydrolisis Reagent</td>
<td>500 µl</td>
<td>500 µl</td>
<td>500 µl</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

STEP 2: Close the tubes and incubate at 110 °C for 1 hour

*Cooling at Room Temperature*

STEP 3: Add 1 ml of Reagent D – Neutralization Solution

**Vortex for 20 sec – WARNING: develops heat!**

*Cooling at Room Temperature*

STEP 4: Add about 2,5 gr of Reagent E – Stabilization Solution

*WARNING: a precipitate appears!*

STEP 5: Add 1,5 ml of Reagent F – Estraent Solution with Internal Standard

*Close vials and mix at Vortex*

*Mix for 10 minutes on a mechanical rotator*

*Centrifuge at 3000 rpm for 10 minutes*

Release N° 006 Trans 1,2 Cyclohexanediol in urine by GC/FID or GC/MS October 2011
• The samples are injected directly transferring the supernatant into vials.

_Mix at Vortex_

_N.B: The prepared sample is injected immediately_

Place them in the Sampler – Inject 1 µl in GC

_Prepares work list and start the serie according to the system’s specifications_
Reagent C: Test Solution

Trans 1,2 Cyclohexanediol

Reagent G: Urine Calibrator Lyophil. – Lot n°

Use and Reconstitution: Urine Calibrators are used for calibration of the HPLC system. This lyophilised calibrator has to be prepared like a patient sample. Add exactly 10.0 ml HPLC water to the vial and mix for 15 min. When all material is dissolved, the solution is ready to use.

Stability: 36 months if stored at 2-8 °C. After reconstitution the stability of the analytes is at least 24 hours when stored at +20°C, at least 7 days at +4°C and at least 3 months at -20°C. Don’t use after expiry date.

Packaging: 2 x 10 ml

Warning: The calibrator derives from human urine, so it could be potentially infected. It must be handled with care.

Set of Gas-Chromatograph for Injection in FID:

- VF-WAXms Column 30 m x 0.25 mm, 0.25 µm (conditioned)
- Injection Temperature 220 °C
- Split Report Start: In ON 20
  - 0° OFF
  - 0.02° ON 60
  - 3° ON 20
- Temperature 80 °C x 6 minutes + 10 °C/min till up 220 °C + 20 °C/min till up 290 °C for 2.5 min (run 27 minutes)
- Helium Gas 1 ml/min
- Temperature FID: 250 °C
- Range (sensitivity): 12 (max)

Set of Gas-Chromatograph for Injection in MS:

- VF-WAXms Column 30 m x 0.25 mm, 0.25 µm (conditioned)
- Injection Temperature 220 °C
- Split Report: 1:20
- Temperature 80 °C x 6 minutes + 10 °C/min till up 220 °C for 1 minute + 20 °C/min till up 250 °C for 2.5 min (run 25 minutes)
- Helium Gas 1 ml/min
- MS Temperature: 180 °C
- Manifold Temperature: 80 °C
- Transfer Line Temperature: 270 °C
- Operative Mode in Electronic Impact (EI)
- Massa Range: 28-250
- Filament on: 8 minutes

Conditioning of VF-WAX Column and Syringe:

Before injection wash the syringe with ACETONE only for Preanalytical Phase and with ETHYL ACETATE only for Analytical Phase.

Follow the manufacturer prescription.

Cleaning of the Column

Disconnect the detector. Keep the column at the highest temperature for the recommended time. (See the manufacturer’s instructions)

Operational Computer Parameters

In conformity with the specification of Operational Computer Software
Trans 1,2 CYCLOHEXANEDIOL IN URINE
(Chromatograms / Reference Spectra GC-FID)

**Fig. 1:** Test Solution
R.T. 10.9  INTERNAL STANDARD
R.T. 17.6  trans 1,2 CYCLOHEXANEDIOL

**Fig. 2:** Calibration Standard
R.T. 10.5  INTERNAL STANDARD
R.T. 17.4  trans 1,2 CYCLOHEXANEDIOL  25 mg/l
Trans 1,2 CYCLOHEXANEDIOL IN URINE
(Chromatograms / Reference Spectra GC-FID)

Fig. 3: Calibration Standard
<table>
<thead>
<tr>
<th>R.T.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.5</td>
<td>INTERNAL STANDARD</td>
</tr>
<tr>
<td>17.4</td>
<td>trans 1,2 CYCLOHEXANEDIOL 50 mg/l</td>
</tr>
</tbody>
</table>
Trans 1,2 CYCLOHEXANEDIOL IN URINE
(Chromatograms / Reference Spectra GC-MS)

**Fig. 4**: Test Solution
R.T. 15.9  trans 1,2 CYCLOHEXANEDIOL

**Fig. 5**: Massa Spectrum of trans 1,2 Cyclohexanediol
MOLECULAR IONS: 70-57-98
Trans 1,2 CYCLOHEXANEDIOL IN URINE
(Chromatograms / Reference Spectra GC-MS)

Fig. 6: Calibration Standard
R.T. 8.7 STANDARD INTERNO
R.T. 15.9 trans 1,2 CICLOESANDIOLO 66,8 mg/l

Fig. 7: Massa Spectrum of Internal Standard
MOLECULAR IONS: 146-148-111