Acetaminophen (Paracetamol) Detoxification HPLC Assay

Catalog Number: PDT34-H100
100 Tests
For Research Use Only. Not for use in diagnostic procedures.

v.1.0

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1. Intended purpose

The Eagle Biosciences Acetaminophen (Paracetamol) Detoxification HPLC Assay is intended for the quantitative determination of acetaminophen (paracetamol), acetaminophen sulfate (paracetamol sulfate), acetaminophen glucuronide (paracetamol glucuronide) and acetaminophen mercapturate (paracetamol mercapturate) in urine. This Acetaminophen (Paracetamol) Detoxification HPLC Assay is for research use only and is not for use in diagnostic procedures.

2. Introduction

Acetaminophen (Paracetamol) is a widely used drug. Its pain reducing and fever reducing ability was discovered at the end of the nineteenth century. When metabolized, mostly water soluble and consequently excretable compounds (sulfate and glucuronide) are synthesized. In case of a lack of conjugation partners (sulfate and glucuronide) the highly reactive compound N-acetyl-p-benzoquinone-imine (NAPQI) is built. This metabolite is then eliminated by addition of glutathione, resulting in acetaminophen (paracetamol) mercapturate and subsequent excretion with the urine. This metabolism process is only possible when the glutathione concentration is sufficient.

In case of a lack of glutathione, NAPQI reacts with amino acids of proteins and enzymes. NAPQI then binds to the surface of hepatocytes resulting in liver cell necrosis. It can also initiate a chain reaction in which even more glutathione molecules are used up and oxygen radicals are generated, which can cause lipid oxidation. With this Acetaminophen (Paracetamol) Detoxification HPLC Assay it is possible to determine the concentration of acetaminophen (paracetamol), acetaminophen sulfate (P- sulfate), acetaminophen glucuronide (P-glucuronide) and acetaminophen mercapturate (P- mercapturate) in urine. It is a suitable tool to investigate the individual detoxification of acetaminophen (paracetamol).

The Acetaminophen (Paracetamol) Detoxification HPLC Assay includes all reagents ready to use for preparation and separation of the samples with exception of the column (IC8300rp) and the controls (IC8300ko). Both can be supplied by Eagle Biosciences. Beside the complete test kit it is possible to order all components separately. Please request our single component price list.
3. Warnings and precautions

- All reagents of this The Acetaminophen (Paracetamol) Detoxification HPLC Assay are strictly intended for research use only.

- The Acetaminophen (Paracetamol) Detoxification HPLC Assay and column are concerted. Using alternative columns might cause in insufficient separation, resulting in false high results. The given test characteristics might not be fulfilled.

- Do not interchange the Eagle Biosciences The Acetaminophen (Paracetamol) Detoxification HPLC Assay components from different lots.

- Calibrator and controls contain human serum. It was tested and found negative for HBsAg, anti-HIV-1/2, and anti-HCV. No test can guarantee the absence of HBsAg or HIV, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.

- The mobile phase (ELU) and internal standard (IS) contain organic solvents and have to be handled carefully. Organic solvents are highly flammable and toxic if inhaled or contact the skin. They should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breath vapor and avoid inhalation. In case of an accident or indisposition contact a physician immediately.

- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

- Do not pipette by mouth.

- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

- The Acetaminophen (Paracetamol) Detoxification HPLC Assay reagents should not be used beyond the expiration date shown on kit label.

- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

4. Materials Provided

<table>
<thead>
<tr>
<th>Article no.</th>
<th>Component</th>
<th>Designation</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC8300lm</td>
<td>ELU</td>
<td>Mobile phase</td>
<td>1000 ml</td>
</tr>
<tr>
<td>IC8300ka</td>
<td>CAL</td>
<td>Calibrator, (lyoph. 250 µl)</td>
<td>3 vial</td>
</tr>
<tr>
<td>IC8300is</td>
<td>IS</td>
<td>Internal standard</td>
<td>20 ml</td>
</tr>
<tr>
<td>IC8300re</td>
<td>RECON</td>
<td>Reconstitution solution</td>
<td>5 ml</td>
</tr>
</tbody>
</table>
5. Additional Special Equipment

- Centrifuge
- 1.5 ml reaction tubes (Eppendorf)
- Various pipettes
- HPLC with UV-detector
- HPLC column Paracetamol (IC8300rp)
- Vortex mixer

6. Reagent preparation

- Reconstitute the **calibrator (CAL)** in 0.25 ml reconstitution solution (RECON), divide the calibrator in several portions and store them at -20 °C. Avoid repeated freeze-thaw circles. The exact concentrations are given on the Acetaminophen (Paracetamol) Detoxification HPLC Assay product specification sheet. The concentration of paracetamol and metabolites might have minor changes from lot to lot.
- All test reagents of the Acetaminophen (Paracetamol) Detoxification HPLC Assay are stable at 2-8 °C, the calibrator (CAL) and the internal standard (IS) at -20 °C up to the date of expiry stated on the label.

7. Specimen

- Urine should be used in this test system.
- The urine sample is taken 4 hours after the ingestion of a 500 mg paracetamol pill.
- For longer storage samples should be frozen at -20 °C.
8. Procedure

Principal of the method

For the determination of the paracetamol metabolites, an internal standard is added to the centrifuged sample. After thoroughly mixing it is injected into the HPLC system. The isocratic separation via HPLC at 30°C uses a "reversed phase" column. One run lasts 15 minutes. The chromatograms are recorded by a UV-detector at a wavelength of 254 nm. The quantification is performed with the delivered urine calibrator; the concentration is calculated by the "internal standard method" via integration of the peak heights resp. peak areas.

Sample preparation

1. Pipette into 1.5 ml Eppendorf cap:

   **10 µl** sample, CAL or CTRL
   
   +
   
   **200 µl** IS

2. Mix well on a vortex mixer (10 s).

3. Inject **20 µl** of the sample into the HPLC-system

Chromatographic settings

- **Column material:** Reversed phase C18 column, 5 µm
- **Column dimension:** 125 mm x 4 mm
- **Flow rate:** 1.2 ml/min
- **UV-detection:** 254 nm
- **Injection volume:** 20 µl
- **Running time:** 15 min
- **Temperature:** 30 °C

Treatment of the HPLC-column

After the analysis, the column should be flushed with 15 ml deionized water. Afterwards the column is rinsed with 85% acetonitrile in deionized water (approx. 15 min. flow 1.0 ml/min). The column should be tightened carefully. Before use, the system should be equilibrated with ca. 20 ml ELU.
9. Calculation of analytical results

Calculation

\[
\frac{\text{peak area patient} \cdot \text{concentration of the standard}}{\text{peak area IS patient}} \cdot F = \text{concentration patient sample}
\]

\[
F = \frac{\text{Peak area IS of the calibrator}}{\text{Peak area analyte of the calibrator}}
\]

Typical Chromatogram
10. Validation data

Precision and reproducibility

**Intra-Assay CV:**
- Paracetamol glucuronide: 0.2 % (705 A.U.) - 2.0 % (1960 A.U.) [n = 6]
- Paracetamol sulfate: 0.8 % (152 A.U.) - 1.8 % (351 A.U.) [n = 6]
- Paracetamol mercapturate: 1.3 % (11.0 A.U.) - 1.8 % (28.0 A.U.) [n = 6]
- Paracetamol: 1.0 % (57.1 A.U.) - 1.7 % (104 A.U.) [n = 6]

**Inter-Assay CV:**
- Paracetamol glucuronide: 1.7 % (719 A.U.) - 4.2 % (1940 A.U.) [n = 6]
- Paracetamol sulfate: 1.5 % (155 A.U.) - 4.0 % (348 A.U.) [n = 6]
- Paracetamol mercapturate: 2.2 % (11.4 A.U.) - 4.4 % (27.7 A.U.) [n = 6]
- Paracetamol: 1.5 % (58.7 A.U.) - 3.8 % (103 A.U.) [n = 6]

Detection limit

1.2 A.U.

Recovery

99.4 %

11. Limitations of the method

Serum, plasma and whole blood should not be measured

12. Disposal

The mobile phase (ELU) and internal standard (IS) must be disposed as non-halogenated solvent. Please refer to the appropriate national guidelines.
## 13. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal</td>
<td>No or defect connection to</td>
<td>Check signal cord and connection</td>
</tr>
<tr>
<td></td>
<td>evaluation system</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Detector lamp is altered</td>
<td>Change lamp</td>
</tr>
<tr>
<td>No peaks</td>
<td>Injector is congested</td>
<td>Check Injector</td>
</tr>
<tr>
<td>Double peaks</td>
<td>Dead volume in fittings and / or</td>
<td>Renew fittings and / or column</td>
</tr>
<tr>
<td>Contaminating peaks</td>
<td>Injector dirty</td>
<td>Clean injector</td>
</tr>
<tr>
<td></td>
<td>Contamination at the head of the</td>
<td>Change direction of the column and rinse for 30 min at low flow rate (0.2</td>
</tr>
<tr>
<td></td>
<td>column</td>
<td>ml/min) with mobile phase</td>
</tr>
<tr>
<td></td>
<td>Air in the system</td>
<td>Degas pump</td>
</tr>
<tr>
<td></td>
<td>Autosampler vials contaminated</td>
<td>Use new vials or clean them with methanol</td>
</tr>
<tr>
<td>Broad peaks, tailing</td>
<td>Precolumn / column exhausted</td>
<td>Use new precolumn / column</td>
</tr>
<tr>
<td>Variable retention times</td>
<td>Drift in temperature</td>
<td>Use a column oven</td>
</tr>
<tr>
<td></td>
<td>Pump delivers imprecise</td>
<td>Check pump, degas the system</td>
</tr>
<tr>
<td></td>
<td>System is not in steady state</td>
<td>Rinse system mobile phase for 15 min</td>
</tr>
<tr>
<td></td>
<td>yet</td>
<td></td>
</tr>
<tr>
<td>Baseline is drifting</td>
<td>Detector lamp did not reach</td>
<td>Wait</td>
</tr>
<tr>
<td></td>
<td>working temperature yet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Detector lamp is too old</td>
<td>Renew lamp</td>
</tr>
<tr>
<td>Continue baseline is</td>
<td>System is not in steady state</td>
<td>Rinse system mobile phase for 15 min</td>
</tr>
<tr>
<td>drifting</td>
<td>yet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pump delivers imprecise</td>
<td>Check pump, degas the system</td>
</tr>
<tr>
<td>Baseline is not smooth</td>
<td>Pump delivers imprecise</td>
<td>Check pump, degas the system</td>
</tr>
<tr>
<td></td>
<td>Detector flowcell is dirty</td>
<td>Clean flow cell</td>
</tr>
</tbody>
</table>

For further information about this kit, its application or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.