The analysis of glyburide and metabolites in bovine urine with LC-MS
1 Introduction
This method describes a liquid chromatography - mass spectrometry (LC-MS-MS) method for the analysis of glyburide (CAS# 10238-21-8) and metabolites in bovine urine. Sample clean up is based on solid phase extraction (SPE). The LC-MS/MS method was validated following SOP ARO/475.

2 Apparatus
Standard laboratory glassware and equipment is used, with addition of:
- Glass tubes 10 ml.
- pH-meter, Schott.
- HPLC-vials.
- HPLC column C18 5μ Length 150mm ID 2.1mm, Alltech.
- The MS system is a Thermo LCQ Deca XP-system equipped with an ESI+ interface. The LC system consists of an Alliance 2695 autosampler system.

4 Chemicals and reagents
Reference to a company and/or product is for purposes of identification and information only and does not imply approval or recommendation of the company and/or the product by the RIVM to the exclusion of the others which might also be suitable.

All chemicals including standards and solutions are of defined quality. Pure chemicals are of “Pro Analyse” quality or better, used water is of bidest or better quality.
- Glyburide, Stephim.
- Glyburide-d11, Cambridge Isotopes
- Helix Pomatia juice, Biosepra, France.
- Acetonitril, Biosolve.
- Ethylacetate, Merck.
- Acetic Acid, J.T. Baker.
- Methanol, Biosolve.
- Ethanol, Biosolve.
- Ammonium acetate, Merck.
- 2 Mol/l acetic acid buffer pH 5.2. Dissolve 25.2 g acetic acid and 129.5 g sodium acetate in 1000 ml of water. The pH is adjusted with acetic acid to 5.2 ± 0.1.
- Methanol/water 20:80 v/v. Mix 200 ml of methanol and 800 ml of water.
- Acetonitril/water 30:70 v/v. Mix 300 ml of methanol and 700 ml of water.
- Formic acid/methanol 2:98 v/v. Mix 20 ml formic acid and 980 ml of methanol.
- Ethanol/water 40:60 v/v. Mix 10 ml of methanol with 90 ml of water.
- LC Solvent A,10:90 v/v methanol/water 5 mMol ammonium acetate. Mix 100 ml of methanol and 900 ml of water and 40 mg ammonium acetate
- LC Solvent B, 90:10 v/v methanol/water 5 mMol ammonium acetate. Mix 900 ml of methanol and 100 ml of water and 40 mg ammonium acetate.

5 Procedure
1. Homogenize the urine sample.
2. Pipette 5 ml of urine in a centrifuge tube of 50 ml.
3. 1 sample of 5 ml blank urine is spiked with 50 μl of 0.1 ng/μl of glyburide (control sample).
4. All samples are spiked with 50 μl of 0.1 ng/ml of glyburide-d11.
5. Add 2 ml of acetate buffer pH 5.2.
6. Add 50 μl of suc d’Helix Pomatia.
7. Adjust the pH of the sample between 4.8 to 5.5 with diluted acetic acid or 0.1 ml/l sodium hydroxide.
8. Incubate overnight at 370C.
9. Cool the sample to room temperature.
10. Condition a SPE C18-column with 5 ml of methanol and 5 ml of water.
11. Apply the sample to the column.
12. Wash the column with 5 ml of 30:70 v/v acetonitril/water.
13. Wash the column with 5 ml 20:80 v/v methanol/water.
14. Elute the analytes from the column with 5 ml 80:20 v/v methanol/water.
15. Condition a SPE NH2-column with 5 ml of methanol and 5 ml of water.
16. Apply the eluate to the column.
17. Wash the column with 5 ml of water.
18. Wash the column with 5 ml of methanol.
19. Elute the analytes from the column with 5 ml 98:2 v/v methanol/formic acid.
20. Evaporate the solvent under a stream of nitrogen at 500C.
21. Redissolve the residue in 125 μl of methanol/water 40/60; v/v %, and vortex for 30 seconds.
22. Make a standard calibration line with 6 standards (Table 1).
23. Performance check and analytical control.
24. Inject 100 μl of the final sample extract and the standard solutions into the LC-MS.

Table 1 Standards for calibration line

<table>
<thead>
<tr>
<th>Name</th>
<th>Glyburide</th>
<th>Glyburide-d11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0 ng</td>
<td>0.0 ng</td>
<td>5.0 ng</td>
</tr>
<tr>
<td>Standard 2.5 ng</td>
<td>2.5 ng</td>
<td>5.0 ng</td>
</tr>
<tr>
<td>Standard 5 ng</td>
<td>5.0 ng</td>
<td>5.0 ng</td>
</tr>
<tr>
<td>Standard 7.5 ng</td>
<td>7.5 ng</td>
<td>5.0 ng</td>
</tr>
<tr>
<td>Standard 10 ng</td>
<td>10.0 ng</td>
<td>5.0 ng</td>
</tr>
<tr>
<td>Standard 15 ng</td>
<td>15.0 ng</td>
<td>5.0 ng</td>
</tr>
</tbody>
</table>

Table 2 LC-Gradient

<table>
<thead>
<tr>
<th>Time</th>
<th>Flow</th>
<th>Solvent A(%)</th>
<th>Solvent B(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.30</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>10.00</td>
<td>0.30</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>11.00</td>
<td>0.30</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>15.00</td>
<td>0.30</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>15.01</td>
<td>0.30</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>22.00</td>
<td>0.30</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3 Scan event details

<table>
<thead>
<tr>
<th>Name</th>
<th>Pseudo molecular ion m/z</th>
<th>Collision energy (%)</th>
<th>Second transition ion m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyburide (Gly)</td>
<td>494-496</td>
<td>23</td>
<td>369-371</td>
</tr>
<tr>
<td>Glyburide-d11 (Gly-d11)</td>
<td>505-507</td>
<td>25</td>
<td>369-371</td>
</tr>
</tbody>
</table>
Metabolites* 510-512 23 369-371

*Note: There are no reference standards available for these compounds

Quantification
Quantitative results are obtained by constructing a calibration curve based on the linear regression of the corrected response or ratio Gly/Gly-d11 versus the concentration. For this purpose the software program CALWER is used (5.1).

Quantification is only valid if:
- The maximum of the signal originated form the analyte in the suspected sample has a S/N ratio ≥ 3.
- The coefficient of the correlation of the constructed curve is > 0.96.

LC-MS2 confirmation analysis
The results of the analysis can only be mentioned "non-compliant" if the presence of the analyte in the sample is confirmed according to the criteria specified for LC-MSn as laid down in the Commission Decision 2002/657/EC august 2002 (2).

For glyburide measurement by LC-MS2, MS2-ions have to be monitored and the ratio between the two recorded fragment ions has to be calculated and compared with the ratio as obtained for either standards or fortified control samples.

7 Validation and Measurement uncertainty
The method described in this SOP was validated conform ARO/475. The method should be capable of detecting glyburide at 1 ng/g urine, this is also the level used for this method see annex 1 for an overview of the validation results. In table 2 a summary of the validation results is shown.

Table 4 Performance characteristics of the method, CCα, CCβ, reproducibility and uncertainty of measurement.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CCα</th>
<th>CCβ</th>
<th>Uncertainty of measurement at 1 ng g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyburide</td>
<td>0.07</td>
<td>0.12</td>
<td>0.31</td>
</tr>
</tbody>
</table>

10 Relating documents
- ResVal V2.0 Validation Report (SOP ARO/475, Method validation using ResVal)).
- Sterk SS, Blokland MH, Stephany RW. Incubation of glyburide with bovine liver microsomes and characterization of the metabolites by LC-ion trap-MS. Proceedings of the Euroresidue V Conference, Noordwijkerhout, the Netherlands 10-12 May 2004; 894-899.
Documentbeheer

Algemeen

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Wijzigingen ten opzichte van vorige versie:
Nieuwe procedure in verband met omzetting naar HTML (oud SOP ARO/483)

Beoordelaars

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Hyperlinks