DETERMINATION OF NITROIMIDAZOLES AND THEIR METABOLITES IN MILK USING GC/MS-NCI

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According to the directive 96/23/EC, the use of Nitroimidazoles in animals is prohibited. Consequently, analytical strategies are needed for monitoring their use through analysis of samples of animal origin.

Milk, as a product of animal origin, is of prime concern due its consumption by a vulnerable part of the population, namely children. A method was therefore developed and validated for the determination of Nitroimidazoles in milk. An internal standard for each nitroimidazole was used.
NITROIMIDAZOLES

- Metronidazole (MTZ)
- Ipronidazole (IPZ)
- Ronidazole (RNZ)
- Dimetridazole (DMTZ)
- Hydroxy Ipronidazole (IPZ–OH)
- Hydroxy Metronidazole (MTZ–OH)
- HMMNI
The estimated value of $CC_\alpha$ has been found to be between 0.14—0.74 µg/l which is lower than the proposed MRPL (Minimum Requirement Performance Limit) (3 µg/l), so the method has been proved to be appropriate to use for the analysis of Nitroimidazoles.

The method provides a tool to Competent Food Authorities/National Reference Laboratories to monitor the abuse of this class of veterinary drugs and thus attaining one more step towards the further improvement of public health protection.
Thorough mixing/adjusted to pH 2 using 2M HCl

Buffer solution added (pH 3) to vol. of 8 ml, 1 ml of protease added

Hydrolyze phase for at least 2 hr or overnight at 37°C

pH adjusted to 3

pH 6 adjusted with 5M NaOH, filled up to 8 ml

Hexane phases discarded

Hexane for defatting (10 ml), centrifugation

The above step is repeated (using 5 ml)

XTR cartridges used (20 min waiting time), 9 ml of ethyl acetate/tBME (1:1) added, elution with 2×9 ml of ethyl acetate/tBME (1:1)

Concentration to dryness, glass vial rinsed twice with 0.5 ml of ethyl acetate/tBME (1:1)

Combined extracts evaporated to dryness

BSA/n-heptane 1:1 (v:v) added

Derivatisation for 1 hr at 50°C, 1 µl injection into GC-MS

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**Instrumentation**

- Agilent GC 6890N
- MS-NCI detector 5973-inert

**Parameters of the GC-MS/NCI:**
- Analytical Column HP-5MS (30 m×0.25 mm×0.25 µm)
- Precolumn: Fused Silica Intermediate Polarity (1 m×0.32 mm)
- Reagent Gas: Methane
- In. Temp.: 85°C
- Carrier Gas: Helium
- In. Time: 1.50 min

**Validation**

The method was validated according to the European Union Decision 657/2002/EC.²

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Results and Discussion

Due to matrix interferences the standard curve of spiked samples was used for quantification. Four levels of spiking were used: 1, 2, 3, 4 µg/l in order to evaluate:

- Decision limit ($CC_\alpha$)
- Detection capability ($CC_\beta$)
- Repeatability
- Reproducibility
- Recovery

ISO/IEC 17025

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## Validation Data

<table>
<thead>
<tr>
<th></th>
<th>CC&lt;sub&gt;α&lt;/sub&gt; (µg/l)</th>
<th>CC&lt;sub&gt;β&lt;/sub&gt; (µg/l)</th>
<th>Rep/ty (%RSD, n=24) at MRPL</th>
<th>Reprod/ty (%RSD, n=24) at MRPL</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNZ</td>
<td>0.56</td>
<td>0.85</td>
<td>4.6</td>
<td>9.9</td>
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<tr>
<td>MTZ</td>
<td>0.16</td>
<td>0.36</td>
<td>6.0</td>
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<td>DMZ</td>
<td>0.29</td>
<td>0.49</td>
<td>7.3</td>
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<tr>
<td>IPZ</td>
<td>0.14</td>
<td>0.34</td>
<td>10.5</td>
<td>15.9</td>
<td>100</td>
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<tr>
<td>MTZ-OH</td>
<td>0.74</td>
<td>1.29</td>
<td>15.8</td>
<td>28.5</td>
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<td>IPZ-OH</td>
<td>0.54</td>
<td>0.81</td>
<td>7.3</td>
<td>13.9</td>
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</tr>
</tbody>
</table>

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Chromatographic Results from Spiked Samples
Conclusions

Quantities of nitroimidazoles are not affected by discarding the fat layer.

The method is a multiresidue method and can be applied in routine laboratory processes.

The determined $CC_\alpha$, $CC_\beta$ are more than satisfactory.

Precision and recoveries were well resolved.

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Thanks for Listening