Analysis of trimethoprim, lincomycin and sulfadoxin using Laser Diode Thermal Desorption Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry in manure

Overview

Purpose
- Develop an ultra-fast analysis method for the determination of antibiotics in manure.
- Develop a simple analysis method
- Avoid time consuming steps like derivatization, liquid chromatography and solid phase extraction prior to analysis.

Method
- Use of Laser Diode Thermal Desorption Atmospheric Pressure Chemical Ionization as an ionization source.
- LDTD parameters to be optimized: Laser power, laser pattern, flow gas volume in each well.
- MS parameters

Results
- Good recovery values for solid-liquid extraction between 77-86%
- Intra and inter day precision were evaluated with RSDs < 15%
- Calibration curves show good linearity with an R² > 0.985
- Limits of detection ranged from 8.3 to 15.1 µg/L
- Calibration curves in spiked manure showed linearity in a complex matrix with an R² > 0.985

Introduction
- In the last several decades, antibiotics have been widely used in swine, cattle and poultry breeding. They have been administered routinely in therapeutic doses to prevent disease, improve feed and that can limit bacterial growth. A novel ultra-fast analysis method for the determination of antibiotics in manure.

Method

Instrumentation
- LDTD model T-960 controlled by LexSoft 4.0 software, Phyrionic Technologies.
- TSQ Quantum Ultra AM controlled by Xcalibur 2.0 software, Thermo Fisher Scientific.

Compounds
Lincomycin
- M.W. 406.54 g/mol
- M.W. 292.32 g/mol
- M.W. 310.33 g/mol

Trimethoprim
- (m/z) 407.2
- 311.3
- 156.1*

Sulfadoxin
- (m/z) 311.3
- 255.2
- 90

Internal standards
- Trimethoprim-13C as internal standard

MS Parameters
- APPI (+)
- Scan time: 0.005 s
- Q1 width: 0.75 amu
- Q3 width: 0.75 amu
- Corona discharge: 4 µA
- Corona voltage: 5500 V

Method validation parameters

Table 1: SIM parameters of the LDTD-APCI-MS/MS analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor Ion (m/z)</th>
<th>Product Ion (m/z)</th>
<th>Reaction Energy (eV)</th>
<th>Tube-Lens (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lincomycin</td>
<td>407.2</td>
<td>126.1</td>
<td>25</td>
<td>86</td>
</tr>
<tr>
<td>Sulfadoxin</td>
<td>311.3</td>
<td>156.1</td>
<td>17</td>
<td>80</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>291.1</td>
<td>255.2</td>
<td>23</td>
<td>77</td>
</tr>
</tbody>
</table>

Figure 3: Laser pattern

Figure 4: Laser power

Figure 2: LDTD-AFCI-MS/MS setup and Laser plate

Figure 5: LDTD peak shape of thermal desorption

Conclusion
- LDTD-APCI source coupled to MS/MS is a high-throughput method for the analysis of antibiotics in a complex matrix.
- Simple, less expensive, faster method (15s per sample).
- Solid-liquid extraction step show good recovery values between 77% and 86% for all three antibiotics in manure.
- The intra- and inter day precision with RSDs < 15%.
- Calibration curves in spiked manure showed linearity in a complex matrix with an R² > 0.985 and LOD (µg/L): Trimethoprim-13C: 20, Sulfadoxin: 10, Lincomycin: 2.
- The limits of detection (LOD) ranged from 8.3 to 15.1 µg/L.

Future research direction:
- Test the method in a non-manure sample.
- Test the method with other veterinary antibiotics.

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References
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