SULFONAMIDE ANTIBACTERIALS IN MILK

SPE Procedure

Oasis® MCX 3 cc/60 mg

CONDITION:
A. 2 mL methanol
B. 2 mL water

LOAD:
5 mL whole homogenized milk

WASH 1:
2 mL 5% methanol in water

WASH 2:
1 mL 0.5 M aqueous hydrochloric acid

WASH 3:
2 mL 20% methanol in water

WASH 4:
1 mL methanol

ELUTE:
2.5 mL 90:10 methanol/water (200 mM ammonium bicarbonate pH 8.5)

Reconstitute in 0.5 mL mobile phase

LC Conditions

Instrument: Waters ACQUITY UPLC® System
Column: Waters ACQUITY UPLC BEH C18, 2.1 x 100 mm, 1.7 µm
Flow rate: 0.2 mL/min
Mobile phase: A. 0.05% formic acid/water
B. 0.05% formic acid/methanol
Gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>3.25</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>3.26</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Detector: ACQUITY UPLC PDA Detector

MS Conditions

Instrument: Waters Quattro Premier™ XE
Ionization mode: Positive electrospray (ESI+)
Multiple reaction monitoring

Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfapyridine</td>
<td>249.80 → 91.90</td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>249.80 → 155.80</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>253.70 → 91.90</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>253.70 → 155.80</td>
</tr>
</tbody>
</table>

MRM method parameters.

Results

Whole milk (5 ng/mL) typical LC/MS/MS analysis (MRM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Sulfadiazine</td>
<td>79</td>
<td>9.2</td>
</tr>
<tr>
<td>2-Sulfathiazole</td>
<td>75</td>
<td>6.5</td>
</tr>
<tr>
<td>3-Sulfapyridine</td>
<td>68</td>
<td>10.2</td>
</tr>
<tr>
<td>4-Sulfamerazine</td>
<td>77</td>
<td>9.1</td>
</tr>
<tr>
<td>5-Sulfamethizole</td>
<td>73</td>
<td>10.1</td>
</tr>
<tr>
<td>6-Sulfamethazine</td>
<td>67</td>
<td>7.0</td>
</tr>
<tr>
<td>7-Sulfamethoxypyridazine</td>
<td>79</td>
<td>10.0</td>
</tr>
<tr>
<td>8-Sulfachloropyridazine</td>
<td>60</td>
<td>7.0</td>
</tr>
<tr>
<td>9-Sulfamethoxazole</td>
<td>71</td>
<td>9.1</td>
</tr>
<tr>
<td>10-Sulfadimethoxine</td>
<td>80</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Ordering Information

<table>
<thead>
<tr>
<th>Description</th>
<th>Part Number</th>
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</thead>
<tbody>
<tr>
<td>Oasis MCX, 3 cc/ 60 mg, 100/box</td>
<td>186000254</td>
</tr>
<tr>
<td>LCMS Certified Vials</td>
<td>600000751CV</td>
</tr>
<tr>
<td>ACQUITY UPLC BEH C18, 2.1 x 100 mm, 1.7 µm</td>
<td>186002352</td>
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