Fluensulfone on Potatoes and Sugar Beets

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Target Compounds

Fluensulfone

Metabolites

Thiazole Sulfonic Acid

Butene Sulfonic Acid
Reference Method

Fluensulfone
1. Blend (5 minutes) then shake (5 minutes) in 50:50 acetonitrile : water
2. Decant and centrifuge
3. Filter aliquot
4. Analyze for fluensulfone by LC/MS/MS in positive mode with an acetonitrile/water gradient

Metabolites
1. Remove acetonitrile by evaporation from an aliquot equivalent to 1 g of matrix
2. Elute aqueous fraction through C18 spe cartridge, wash cartridge with water and combine fractions
3. Analyze for metabolites by LC/MS/MS in negative mode with an acetonitrile/water gradient
Initial Changes

To improve sensitivity, changed mobile phase to methanol/water from acetonitrile/water.

To improve peak shape, changed calibration standard solution organic/water composition.

Metabolites - Reference method worked relatively well
Fluensulfone - Recoveries were low likely due to matrix interference.

Modification: Used the C18 cartridge to clean-up the fluensulfone extract by adding a wash step after metabolite elution. Fluensulfone was eluted with acetonitrile.
Metabolite 1.6 ng/mL calibration standard

- **TIC**
- **BSA**
- **TSA**
Metabolite - potato flakes

Untreated Control

0.01ppm fortification
Untreated Control

0.01 ppm fortification
Interfering co-extractives were not effectively retained by C18.

- Metabolite - flakes and chips - substituted a 1 g polymeric hydrophilic-lipophilic balanced spe sorbent for C18
- Fluensulfone - flakes - substituted a 1 g C18 spe cartridge for 0.5 g cartridge
- Fluensulfone - chips - substituted a 1 g polymeric hydrophilic-lipophilic balanced spe sorbent for C18
Butene Sulfonic Acid after Modification

BSA

chips

flakes
Fluensulfone on Sugar Beet

Beet Roots
Beet Tops
Sugar
Molasses
Dried Pulp

Beet (Sugar)
PR#10908 (2013)
Sugar Beet – Roots and Tops

Roots

• Fluensulfone recoveries often <70%
• BSA recoveries were acceptable
• TSA recoveries near or below 70% at higher fortification levels

Tops

• Matrix interferences tended to increase recoveries at lower fortification levels
Modifications - Roots and Tops

To improve recoveries

- Additional shake
- Additional solvent
- Increased shaking time to 30 minutes
To improve extract clean-up

- Weak anion exchange sorbents were used for metabolite extract clean-up.
  - Tops - polymeric weak anion exchange sorbent
  - Roots – NH₂ silica based sorbent
- Change in retention mechanism allowed the use of wash solvents containing organic solvents
## Recoveries for Roots and Tops

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Level (ppm)</th>
<th>Number of Obs.</th>
<th>Fluensulfone</th>
<th>TSA</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td>0.01</td>
<td>9</td>
<td>74 ± 7</td>
<td>90 ± 4</td>
<td>84 ± 4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>13</td>
<td>76 ± 6</td>
<td>80 ± 5</td>
<td>86 ± 4</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7</td>
<td>---</td>
<td>70 ± 2</td>
<td>85 ± 4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5</td>
<td>---</td>
<td>70 ± 5</td>
<td>88 ± 10</td>
</tr>
<tr>
<td>Tops</td>
<td>0.01</td>
<td>12 (8)</td>
<td>75 ± 4</td>
<td>(109 ± 7)</td>
<td>86 ± 6</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>11</td>
<td>76 ± 8</td>
<td>86 ± 7</td>
<td>87 ± 6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>---</td>
<td>75 ± 7</td>
<td>91 ± 5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>8</td>
<td>---</td>
<td>72 ± 4</td>
<td>94 ± 6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5</td>
<td>---</td>
<td>66 ± 3</td>
<td>91 ± 4</td>
</tr>
</tbody>
</table>
• Beet sugar was successfully extracted and analyzed using the working method for beet tops with slight modifications.

• One extract shaking step of 5 minutes

• Extract solution required stirring while aliquots were taken as phases began to separate after standing for a few minutes.
Processed Fraction - Molasses

- Fluensulfone – The beet sugar method was used for extraction and analysis.
- Metabolites – The beet sugar method was used for extraction and clean-up, but the LC mobile phase was changed to deal with chromatographic interferences.
Molasses – untreated - methanol/water
Molasses – untreated - acetonitrile/water
# Recoveries for Molasses

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Level (ppm)</th>
<th>Number of Obs.</th>
<th>Fluensulfone</th>
<th>TSA</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol &amp; water</td>
<td>0.01</td>
<td>3</td>
<td>92 ± 10</td>
<td>115 ± 4</td>
<td>132 ± 6</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3</td>
<td>92 ± 15</td>
<td>87 ± 2</td>
<td>101 ± 1</td>
</tr>
<tr>
<td>Acetonitrile &amp; water</td>
<td>0.01</td>
<td>3</td>
<td>---</td>
<td>92 ± 5</td>
<td>105 ± 6</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3</td>
<td>----</td>
<td>86 ± 3</td>
<td>100 ± 4</td>
</tr>
</tbody>
</table>
Sugar Beet - Dried Pulp

5 g sugar beet pulp

5 g sugar beet pulp + water and enzymes
Sugar Beet - Dried Pulp

- Dried pulp rapidly expands with the addition of water. Pulp absorbs extraction solvent after rehydration.
- Cellulase and pectinase enzymes with water rehydrates matrix and less extraction solvent is absorbed.
- 5 g sample size
- Blend for 5 minutes and shake once for 30 minutes.
- Fluensulfone – clean-up with 0.5 g polymeric hydrophilic-lipophilic balanced spe sorbent
- Metabolites – clean-up with polymeric weak anion exchanger
### Recoveries – Dried Pulp

<table>
<thead>
<tr>
<th>Level (ppm)</th>
<th>Number of Obs.</th>
<th>Fluensulfone</th>
<th>TSA</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>3</td>
<td>70 ± 2</td>
<td>126 ± 1</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>0.1</td>
<td>3</td>
<td>75 ± 2</td>
<td>93 ± 16</td>
<td>85 ± 3</td>
</tr>
</tbody>
</table>