A New Look at Citrinin: Quantitative Analysis by HPLC and LCMS/MS Utilizing Immunoaffinity Purification
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Abstract
Citrinin, a naturally occurring toxin produced by Penicillium, Monascus, and Aspergillus species of fungus, is most commonly considered a nephrotoxic compound. It is a mycotoxin that typically occurs in the storage processes of grains such as corn, soybeans, wheat, barley, and rice (red yeast rice). Evaluations were conducted through an R-Biopharm Rhone immunoaffinity column. This method allowed a single-extraction validation of both HPLC and LCMS/MS with acceptable recoveries. This analysis uses the same purified extract to be injected onto the HPLC and/or LCMS/MS. Use of this method has shown limits of quantification at 10ppb for several different matrices giving a better look at detection of this toxin. EFSA (European Food and Safety Authority) recommends levels of daily Citrinin consumption in children to be below 53 µg/kg, and adults 100 µg/kg.

Procedure for Analyzing Citrinin by HPLC and LCMS/MS
Four Commodities (Feed, Pet Food, Red Yeast Rice, and Corn) were analyzed by both HPLC and LCMS/MS under the below conditions. The study was to prove that both Accuracy and Precision analysis would meet validation guidelines:
- Weigh out 25 g of sample into a 250 ml flask.
- Add 100 ml of 3/1 (MeOH/H2O). Shake for 1 hour. Filter sample after extraction.
- Dilute 2 ml extract with 18 ml PBS solution.
- Mix thoroughly.
- Filter extract through glass fiber filter. Take 10 ml of filtered sample through the column at a flow rate of 1-2 drops/second.
- Wash Columns with 10 ml of 10% Phosphoric Acid (pH 7.4).
- Elute columns with 1 ml of 100% Methanol, followed by 1 ml of DI Water for a 2 ml total.

LCMS/MS Conditions – API3000 Equipment or Equivalent LC Conditions
Shimadzu SIL-20AHT injector
LC-20AD Pumps
LCMS/MS Software
Column: Phenomenex Kinetex 2.6u C18 100A 100 x 3.0mm
Column temp: 40°C
Flow rate: 0.5ml/min
Mobile Phase A: LCMSMS grade water with 0.1% Formic Acid
Mobile Phase B: LCMSMS grade Methanol with 0.1% Formic Acid
Gradient:
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>2.0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>5.0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>10.49</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>10.50</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>
Total Run Time: 12.0 minutes Injection Volume: 50ul

MS Conditions
MS system: API3000
Ionization: ESI positive
Source Temp: 500°C
Curtain Gas: 15
Nebulizer Gas: 7
CAD Gas: 5
IS: +3000V
Acquisition: Analyst 1.6.2 (MRM with full scan)
* Special thanks to R-Biopharm for supplying columns for analysis purposes.
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Conclusions
Citrinin was analyzed, using the same extract, by both HPLC and LCMS/MS. This allows for confirmation of positive results to be determined by two different systems of analysis simultaneously.

For Feed Analysis:
HPLC
Accuracy percent recovery ranged from 70.3% - 74.2% Average = 72.1%
Precision spiked at 50 ppb ranged from 35.78 – 38.99, with a %RSD = 3.02

LCMSMS
Accuracy percent recovery ranged from 70.6% - 84.5% Average = 78.2%
Precision spiked at 50 ppb ranged from 38.45 – 41.79, with a %RSD = 2.99

For Red Yeast Rice Analysis:
HPLC
Accuracy percent recovery ranged from 71.4% - 87.3% Average = 80.3%
Precision spiked at 50 ppb ranged from 37.5 – 44.0, with a %RSD = 6.01

LCMSMS
Accuracy percent recovery ranged from 76.2% - 84.7% Average = 77.1%
Precision spiked at 50 ppb ranged from 38.8 – 42.6, with a %RSD = 3.36

For Corn Analysis:
HPLC
Accuracy percent recovery ranged from 80.9% - 84.1% Average = 82.5%
Precision spiked at 100 ppb ranged from 76.3 – 83.6, with a %RSD = 3.15

LCMSMS
Accuracy percent recovery ranged from 75.1% - 91.6% Average = 81.5%
Precision spiked at 25 ppb ranged from 20.7 – 25.0, with a %RSD = 10.9

Thus, both HPLC and LCMSMS analysis of Citrinin can be quantitatively reported at levels of 10 ppb.