Identification of Artificial Dyes in Food Using LC-HR-MS/MS Non-Target Screening

André Schreiber
SCIEX, Concord, Ontario (Canada)
Overview – Artificial Dyes

• People associate certain colors with certain flavors
• Use of artificial colors and dyes to make food visually more appealing and “flavorful”
• Some dyes are banned because of known toxicity and Carcinogenicity, such as Sudan dyes and other azo-dyes
• Other dyes are approved for use in foods and are regulated by Codex Alimentarius, the US-FDA, EFSA etc.
• Nature derived color additives (pigments derived from vegetables, minerals or animals) are exempt from certification
• Public concern about the use of artificial dyes (i.e. recent research shows link between artificial colors and behavioral problems)
• Analytical methods include LC-UV and LC-MS/MS
• New approach: LC-HR-MS/MS using the SCIEX X500R QTOF system to identify and quantify artificial colors and dyes in food samples
Experimental

- **ExionLC™ AD system**
  - Phenomenex Luna Omega 1.6 µm Polar C18 (50 x 2.1 mm)
  - Gradient of water/methanol + 5 mM ammonium formate
  - Flow 0.5 mL/min
  - Injection of 5 µL

- **SCIEX X500R QTOF system**
  - Turbo V™ source with ESI probe, TEM = 450°C
  - Mass calibration using the integrated calibrant delivery system (CDS) with the TwinSprayer probe (dual ESI needle).
  - Information dependent acquisition (IDA) in negative polarity
    - TOF-MS 100-1000 Da (200 msec) DP = -80 V
    - 10x MS/MS 50-1000 Da (50 msec) CE = -35 V and CES = 15 V
    - Dynamic background subtraction on with intensity threshold of 10 cps
  - Looped TOF-MS experiment in negative polarity
    - TOF-MS 50-1000 (200 msec) CE = -50 V and CES = 20 V (to find molecular ions with fragment ion of 79.95735 SO₃⁻)

- **SCIEX OS Software** for data acquisition and processing
SCIEX X500R QTOF System powered by SCIEX OS Software

SCIEX X500R QTOF system

SCIEX ExionLC™ system

SCIEX OS software
Sample-Control Comparison in SCIEX OS Software

Begin by creating a results table or opening an existing one.

1. Select batch samples to process
   - Available samples
   - Selected samples

2. Select a processing method
   - GUS Dyes method

3. Select a comparison sample for Non-targeted workflow
   - Sugar (dyes neg 02_sugar 1.wf2.2)

Select samples
Select control
Non-Target Screening in SCIEX OS Software

Setup:
- Peak finding
- Library searching
- Traffic lights
- Formula finding

Peak detection sensitivity (fast to exhaustive)
Table is Filtered by Formula Finding Results and Sorted by Area

Sample was diluted with water/sugar solution (10,000x) to simulate dissolved cake icing.
Review of Formula Finding Results in SCIEX OS Software

Evaluation of TOF-MS and MS/MS Error and ChemSpider Hit Count

<table>
<thead>
<tr>
<th>Index</th>
<th>Sample Name</th>
<th>Component Name</th>
<th>Area (Da)</th>
<th>Retention Time</th>
<th>Found At Mass</th>
<th>Used</th>
<th>Library Finder</th>
<th>Library</th>
<th>Library Hit</th>
<th>Error</th>
<th>Error MS/MS (ppm)</th>
<th>Hit Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>R 10000x</td>
<td></td>
<td>834.6484</td>
<td>5.03</td>
<td>834.6481</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>94.470</td>
</tr>
<tr>
<td>384</td>
<td>R 10000x</td>
<td></td>
<td>858.8542</td>
<td>4.87</td>
<td>858.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.774</td>
</tr>
<tr>
<td>86</td>
<td>R 10000x</td>
<td></td>
<td>878.8542</td>
<td>4.87</td>
<td>878.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.441</td>
</tr>
<tr>
<td>37</td>
<td>R 10000x</td>
<td></td>
<td>900.8542</td>
<td>4.87</td>
<td>900.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.774</td>
</tr>
<tr>
<td>402</td>
<td>R 10000x</td>
<td></td>
<td>920.8542</td>
<td>4.87</td>
<td>920.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.441</td>
</tr>
<tr>
<td>211</td>
<td>R 10000x</td>
<td></td>
<td>940.8542</td>
<td>4.87</td>
<td>940.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.774</td>
</tr>
<tr>
<td>313</td>
<td>R 10000x</td>
<td></td>
<td>960.8542</td>
<td>4.87</td>
<td>960.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.441</td>
</tr>
<tr>
<td>373</td>
<td>R 10000x</td>
<td></td>
<td>980.8542</td>
<td>4.87</td>
<td>980.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.774</td>
</tr>
<tr>
<td>73</td>
<td>R 10000x</td>
<td></td>
<td>1000.8542</td>
<td>4.87</td>
<td>1000.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.441</td>
</tr>
<tr>
<td>401</td>
<td>R 10000x</td>
<td></td>
<td>1020.8542</td>
<td>4.87</td>
<td>1020.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.774</td>
</tr>
<tr>
<td>403</td>
<td>R 10000x</td>
<td></td>
<td>1040.8542</td>
<td>4.87</td>
<td>1040.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.441</td>
</tr>
<tr>
<td>85</td>
<td>R 10000x</td>
<td></td>
<td>1060.8542</td>
<td>4.87</td>
<td>1060.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.774</td>
</tr>
<tr>
<td>10</td>
<td>R 10000x</td>
<td></td>
<td>1080.8542</td>
<td>4.87</td>
<td>1080.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.441</td>
</tr>
<tr>
<td>245</td>
<td>R 10000x</td>
<td></td>
<td>1100.8542</td>
<td>4.87</td>
<td>1100.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.774</td>
</tr>
<tr>
<td>81</td>
<td>R 10000x</td>
<td></td>
<td>1120.8542</td>
<td>4.87</td>
<td>1120.8542</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>0.0</td>
<td>N/A</td>
<td>93.441</td>
</tr>
</tbody>
</table>

**Formula with 5 Hits**

**Link to ChemSpider**

**Zoom**
Tentative identification of Erythrosine in RED food color
Tentative identification of Sunset Yellow in **RED** food color
Semi-Quantitative Analysis of Dyes

99.4% of Peak Area in Dye Sample (Not Present in Control Sample)

- 834.6 @ 5.0 min  Erythrosine  88.6%
- 708.7 @ 4.9 min  Erythrosine – I  6.1%
- 407.0, 200.0, 171.0 @ 2.8 min  Sunset Yellow  3.0%
- 890.7 @ 5.5 min  Erythrosine + C₃H₄O  1.0%
- 582.8 @ 4.9 min  Erythrosine – I₂  0.4%
Review of ChemSpider Results in SCIEX OS Software

Automatic In-silico Fragmentation of Structure Found in ChemSpider

Tentative identification of Tartrazine in **YELLOW** food color
Review of ChemSpider Results in SCIEX OS Software

Automatic In-silico Fragmentation of Structure Found in ChemSpider

Tentative identification of Brilliant Blue in **BLUE** food color
Tentative identification of Erythrosine, Allura Red and Sunset Yellow in **BROWN** food color
Summary

• Identification and quantitation of artificial dyes using LC-HR-MS/MS non-target screening

• Use SCIEX X500R QTOF system with SCIEX OS software
  – High resolution TOF-MS and MS/MS data acquired in IDA mode
  – Non-target peak finding
  – Sample-control comparison
  – Automatic formula finding, ChemSpider searching and structural elucidation of MS/MS spectra to identify compounds

• Several certified colors were identified in diluted icing sugars, including Tartrazine (E102), Sunset Yellow (E110), Erythrosine (E127), Allura Red (E129), and Brilliant Blue (E133)
AB Sciex is doing business as SCIEX.

For Research Use Only. Not for use in diagnostic procedures.

© 2016 AB Sciex. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.
Thank you for your attention!