Caffeine in Beverages

Detection and Quantification Options

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Review of the Levels Targeted

- Caffeine occurs naturally in a number of beverages / foods and can be added to foods
  - Coffee: 80-190 per cup

- Levels of Caffeine found in some beverages

<table>
<thead>
<tr>
<th></th>
<th>Regular Cola</th>
<th>Diet Cola</th>
<th>Tea</th>
<th>Coffee</th>
<th>Energy Drinks</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/250ml</td>
<td>20-25</td>
<td>35</td>
<td>40</td>
<td>80-190</td>
<td>50-125</td>
</tr>
</tbody>
</table>
| mg/355ml             | 30-35        | 50        | 57  | 110-270| 70-180
Testing is supportive of certain aspects of:

- Quality Assurance / Compliance
- Enforcement initiatives
- Data Collection by regulators for Risk Assessment
Even Though Testing is not the Most Effective Approach ...

Testing may be Required to Ascertain Levels of Caffeine in Various Products – in particular Beverages.

Analytical Methods have to meet a “fit for purpose” requirement

- Ability to meet the detection and quantification requirements (ppm levels)
- Ability to meet the precision requirements:
  - collection of data versus compliance,
  - allowance for discrepancy in quantification by regulatory provisions
  - screening approach (semi-quantitative), versus confirmatory approach (quantitative)

Matrix requirements:
- Sample preparation requirements
- Matrix effects / interferences
- Pre-concentration / clean-up needed?
Choosing an analytical method can be a matter of trade-offs / compromise

- **Rapid Response / Screening techniques:**
  - May lack in precision, selectivity and lead to higher detection levels

- **Precise and low detection levels require:**
  - Sample Preparation and removal of interferences
  - Separation techniques may be needed
    - Chromatography (TLC / LC / GC)
  - Require selective approaches
    - Mass Spectrometry detection offers better selectivity / specificity than spectroscopic detection (UV Vis / Fluorescence)
  - Can be time-consuming / resource intensive
Caffeine May Co-occur with Other Relevant Chemicals

Caffeine has structurally analogous substances that act differently:

- Subjected to different regulatory requirements
  - Caffeine: 1,3,7-trimethylxanthine
  - Theobromine: 3,7-dimethylxanthine
  - Theophylline: 1,3-dimethylxanthine

- Structurally related compounds may act as interferences and may co-occur in food matrices
Overview of Caffeine Determination Methods

Spectroscopic methods: No separation – Direct application to the detection device after sample preparation

<table>
<thead>
<tr>
<th>Methods</th>
<th>Extraction</th>
<th>LOD and LOQ</th>
<th>Food commodities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrometry-UV Vis</td>
<td>Yes (Filtration + dichloromethane)</td>
<td>Not published</td>
<td>Coffee beans, tea leaves</td>
<td>(Belay et al., 2008; Atomssa &amp; Gholap, 2011)</td>
</tr>
<tr>
<td>Spectrometry-NIR (Need an RP-HPLC calibration)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Green tea leaves</td>
<td>(Luypaert et al., 2003; Lee et al., 2014)</td>
</tr>
<tr>
<td>Spectrometry-UV Vis</td>
<td>Dilution and filtration</td>
<td>LOD: 2 ppm</td>
<td>Soluble coffee, tea and beverages</td>
<td>(Aldpogan et al, 2002)</td>
</tr>
</tbody>
</table>

- A lot of overlap with others components:
  - Phenolic components, other methylxanthines, catechins
- Either manual purification before the quantification or treatment of the results after the experiment.
Overview of Caffeine Determination Methods (2)

Spectroscopic methods: With separation – HPLC as a standard reference. First method established in 1976

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</thead>
<tbody>
<tr>
<td>HPLC-photodiode array</td>
<td>Degassing + Centrifugation</td>
<td>LOD= 0,023 ppm LOQ=0,077 ppm</td>
<td>Energy drinks</td>
<td>(Gliszczynska-Świgło &amp; Rybicka, 2015)</td>
</tr>
<tr>
<td>HPLC-UV</td>
<td>Liquid-liquid extraction + filtration</td>
<td>LOD: 2,1 ppm LOQ: 4,2 ppm</td>
<td>Coffee</td>
<td>(Bizzetto et al., 2013)</td>
</tr>
<tr>
<td>HPLC-UV</td>
<td>Degassing + Filtration</td>
<td>LOD=0,023 ppm LOQ=0,07 ppm</td>
<td>Beverages</td>
<td>(Ali et al., 2012)</td>
</tr>
<tr>
<td>HPLC-UV</td>
<td>Clean up procedure before injection</td>
<td>LOD=0,07 ppm LOQ=0,2 ppm</td>
<td>Tea, coffee powder, cocoa product</td>
<td>(Srdjenovic et al., 2008)</td>
</tr>
<tr>
<td>HPLC-UV with Capillary monolithic column</td>
<td>Water infusion</td>
<td>LOD: 0,05 ppm LOQ: 0,16 ppm</td>
<td>Tea, coffee and cocoa products</td>
<td>(Al-Othman et al., 2012)</td>
</tr>
<tr>
<td>UPLC-UV</td>
<td>Water infusion</td>
<td>LOD: 0,12 ppm LOQ: 0,40 ppm</td>
<td>Black tea</td>
<td>(Pan et al., 2017)</td>
</tr>
</tbody>
</table>

- Use for multiple detection in one time: catechins, phenolic compounds, etc.
- Reduction of selectivity issue
### Overview of Caffeine Determination Methods (3)

#### Alternative Methods

<table>
<thead>
<tr>
<th>Methods</th>
<th>Extraction</th>
<th>LOD and LOQ</th>
<th>Advantages</th>
<th>Food commodities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid Phase - Fourier transform-Raman spectroscopy</td>
<td>Solid Phase extraction</td>
<td>LOQ: 0,05 ppm</td>
<td>Faster than HPLC and reduction of reagent consumption</td>
<td>Energy Drinks</td>
<td>(Armenta et al, 2005)</td>
</tr>
<tr>
<td>FT-IR spectroscopy</td>
<td>Liquid/liquid extraction</td>
<td>LOQ: 0,035 ppm</td>
<td>Faster than HPLC</td>
<td>Tea leaves</td>
<td>(Najafi et al, 2003)</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Drop-to-drop solvent microextraction</td>
<td>LOQ: 0,004 ppm</td>
<td>Reduction of solvent consumption</td>
<td>Various</td>
<td>(Shrivas &amp; Wu, 2007)</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>Liquid/liquid extraction + filtration</td>
<td>LOD: 8,7 ppm</td>
<td>Faster than HPLC but less sensitive</td>
<td>Decaffeinated coffee</td>
<td>(Meinhart et al, 2010)</td>
</tr>
<tr>
<td>Thin layer chromatography and HP-TLC</td>
<td>Filtration + liquid/liquid extraction</td>
<td>LOQ: 0,1ppm</td>
<td>Cheap but long</td>
<td>Not really (Urine and saliva)</td>
<td>(Fenske, 2007)</td>
</tr>
</tbody>
</table>
### Mass Spectrometry Detection

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>LC-MS</td>
<td>Infusion + filtration</td>
<td>Quantification by RP-HPLC–UV</td>
<td>Tea</td>
<td>(Wu et al., 2012)</td>
</tr>
<tr>
<td>UPLC-MS/MS (ESI)</td>
<td>Infusion</td>
<td></td>
<td>Wine, Juice and Tea</td>
<td>(Gruz et al., 2008)</td>
</tr>
</tbody>
</table>

- Very high specificity, faster than HPLC, more sensitive but in a smaller range
Official AOAC methods

- Method 967.11 for non-alcoholic beverages
- Introduced in 1967
- UV spectrometry
- Based on complex and time consuming treatment of the sample with acid and basic Celite columns

Historical Approach

Performance Issues:
Highly manual, time and solvent consuming, reproducibility, etc.
Official AOAC Methods (2)

- 979.08 for carbonated beverages
- Introduced in 1979 and updated in 1984
- RP-HPLC with UV detector
- Mobile phase is Acetic acid (20% v/v), pH 3
- One standard solution (0.05 ppm of caffeine) – 2 injections
- No sample Preparation other than degassing (and possible filtration for particle containing samples)
- Injection of prepared samples in duplicates

Possible Issues with Precision:
Overestimation or underestimation of caffeine content
Standard HPLC method – Closest to Method in FSSAI Manual

- RP-HPLC with UV detector
- Mobile phase is very often Ethanol/ Water : Ammonium Phosphate or acetonitrile and water
- Development of a calibration curve (from 300 ppm to 1 ppm)
- Limited Sample Preparation: Degassing/Sonication – Filtration
- Calibration curve with a $r^2 > 0.98$
- Injection of prepared samples in duplicates

Performance Characteristics:
- Sensitivity, linearity range, reproducibility, repeatability, analytical recovery and robustness

Possible Issues:
- Time and solvent consuming
Mass-Spectrometry Detection: HPLC or UPLC-MS/MS

- RP-HPLC with MS detector
- Tuning of the molecule to know what fragments can be obtained

Fragments will depend on the cone voltage (U) and energy collision (V)
New method: HPLC or UPLC-MS/MS

- Use of internal standard:
  - To be added to the sample after homogenization **but prior to** the sample preparation step.

- Internal Standard set at the same level for all samples

![Comparison between UV and MS](image)

*Linearity is not always possible for MS-based detection systems*
New method: HPLC or UPLC-MS/MS

- Calibration Curve carried out in the targeted matrix:
  - Which may represent challenges as some of the matrices contain inevitably added levels of Caffeine
- Quantification is made based on the Internal Standard
- Limited sample preparation: Filtration is required
- Calculation can account for the matrix effect:
  - Matrix effect: \((1 - \text{signal of the extracted samples/signal of pure standards}) \times 100\%\)
- Relative Error and Relative Standard Deviation < 15%
- LOQ close to 0.01 ppm

Main Benefits:
- Rapid
- Precise

Issues:
- Training required
- Higher investment and maintenance cost
Some References for LC Methods

Several Options for the Detection:

- HPLC + ultraviolet detection (UV)
  - Hadad et al., 2012

- HPLC + electrochemical detection (ECD)
  - Novak et al., 2010

- HPLC + mass spectrometry (MS)
  - Pelegrini et al. 2007
  - Fraser et al. 2012
  - Wu et al., 2012

- HPLC + particle beam/electron ionization mass spectrometry (PB/EIMS)
  - Castro et al., 2010