Applications of FT-MRR Spectroscopy for Impurity Identification and Quantification

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**FT-MRR Basics**

**Fourier Transform – Molecular Rotational Resonance**

- Molecules have fingerprint rotational spectra based on 3-dimensional moments of inertia – isomers, conformers, and isotopologues resolved (Additional capabilities for enantiomers)

- Extremely selective – no false positives

- Spectra measured using FT technique (analogous to FT-NMR) that dramatically enhances sensitivity

- Analyte targets are:
  - *Volatile* – measurement is in low pressure gas phase
  - *Polar* – interaction is through permanent dipole moment
  - *Low weight* – <125 amu for room-temperature analysis, up to 500 amu using molecular beams

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**Simple relationship between ab initio electronic structure and spectra**

**Site-specific isotope resolution**
BrightSpec

• Founded in 2012 to commercialize innovative technology from the University of Virginia. IP portfolio of 7 patents (UVa, Harvard, and BrightSpec-filed)

• Team of 10 full-time employees
  - 4 experts in FT-MRR technique
  - Complementary expertise in engineering, applications design, instrument design, software
  - Experienced management team

• Based in Charlottesville, VA

All products CE certified
21 CFR Part 11 compatible

BrightSpec FT-MRR One
BrightSpec Discovery Series
BrightSpec Chiral Broadband
I will present three examples of client-driven analyses where FT-MRR is being applied:

1) Quantification of residual solvent impurities in nutritional IV solutions, raw materials, and drug products

2) ID & quantification of a trace level mutagenic impurity in a drug product

3) Chiral purity monitoring of a continuous pharmaceutical synthetic process

Collaboration with B. Frank Gupton, Virginia Commonwealth University
Residual Solvent Analysis by FT-MRR

Residual solvent analysis (USP <467>):
- Pharmaceutical manufacturers must verify that residual impurities used in synthesis are at safe levels in their products – typically ppm sensitivity required (Class 2)

- Gas chromatography is standard, but simpler and faster methods are desirable if they can demonstrate equivalent performance

Client sent samples of a IV-administered nutritive solution with impurities of methanol, ethanol, and isopropanol they needed to quantify. Samples are also thermally unstable - a problem for GC.

FT-MRR Requirements:
- Unambiguously resolve analytes without chromatography
- Reach detection limits of 1 mg/L with <10% measurement accuracy
- Simpler, faster, and easier analysis than existing methods
Analytical Methods

**Static Headspace FT-MRR**

- Similar advantages as SHS-GC (concentration of volatiles; matrix simplification)
- Only consumable is nitrogen for cleaning
- Interfaces with autosampler

- Reference library used to find lines of each analyte without overlaps
- Method validity established by measuring stock solutions in concentration range 1-100 mg/L
- Regular blanks to confirm no carryover
- Accuracy maintained in customer’s sample matrix – demonstrated through spiked recovery analysis

**FT-MRR Measurement Cycle**

<table>
<thead>
<tr>
<th>Description</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evacuate vial</td>
<td>2 min</td>
</tr>
<tr>
<td>Inject solution and equilibrate</td>
<td>3 min</td>
</tr>
<tr>
<td>Transfer sample to measurement chamber</td>
<td>45 sec</td>
</tr>
<tr>
<td>Measure analytes</td>
<td>15 sec</td>
</tr>
<tr>
<td>Clean system</td>
<td>3 min</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>9 min</strong></td>
</tr>
</tbody>
</table>

**Case Study 1:** Routine analysis of residual solvent impurities

**Case Study 2:** Genotoxic impurities in a final drug product

**Case Study 3:** Chiral analysis during continuous manufacturing process
Method Results

Case Study 1: Routine analysis of residual solvent impurities

Case Study 2: Genotoxic impurities in a final drug product

Case Study 3: Chiral analysis during continuous manufacturing process

MDL:
- Methanol 0.2 mg/L
- Isopropanol 1.0 mg/L
- Ethanol 0.3 mg/L

Repeatability typ 10%

Linearity $R^2 > 0.99$
Mutagenic Impurity Analysis

- Policies outlined in ICH-M7 for the assessment and control of impurities that are potentially DNA reactive

- Can arise from raw material impurities, side reactions, or degradation – and may be described as a set of related structures, so structure characterization may be needed as well as quantification

- Low detection limits (<1 ppm) needed, with good quantification – challenge for method development

Client (Top 25 global pharma) provided formulated drug capsules with a known chloroethane impurity. Goal is to quantify chloroethane, as well as to learn other information about the samples using FT-MRR.

**FT-MRR Requirements:**
- Structure ID and quantification capabilities in one measurement
- Low detection limits
- Good reproducibility and accuracy
BrightSpec Thermal Evolution Method

- Heat dry powder in an evacuated headspace vial, followed by vacuum-driven transfer of headspace vapor into FT-MRR chamber for characterization

- Can perform broadband investigative analysis (including unknowns) or targeted analysis of known compounds

- At higher temperatures, degradation products can be generated as well as impurities (though oxygen-free environment prevents combustion)

- Very sensitive for dry powders because volatile impurities can be separated efficiently from matrix

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Profile Results

Case Study 1: Routine analysis of residual solvent impurities

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Chloroethane resolved in complex mix
Quantitative Results

BrightSpec software directly determines analyte partial pressures in measurement chamber, which are converted to impurity mass in original sample.

<table>
<thead>
<tr>
<th></th>
<th>Chloroethane mass (w/w, ppm)</th>
<th>RSD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot A</td>
<td>8.8</td>
<td>11%</td>
</tr>
<tr>
<td>Lot B</td>
<td>8.4</td>
<td>12%</td>
</tr>
<tr>
<td>Lot C</td>
<td>14.0</td>
<td>3%</td>
</tr>
<tr>
<td>MDL</td>
<td>&lt;0.1</td>
<td></td>
</tr>
</tbody>
</table>

Case Study 1: Routine analysis of residual solvent impurities
Case Study 2: Genotoxic impurities in a final drug product
Case Study 3: Chiral analysis during continuous manufacturing process
Chiral Analysis in Continuous Manufacturing

- Interest in continuous manufacturing of pharmaceuticals is growing rapidly

- In API synthesis, chirality is a critical attribute that is very challenging to measure – with no good method for automated, on-line analysis

- FT-MRR is unique in that even very subtle structural changes cause very clear differences in the spectrum

- In drugs with multiple chiral centers, unwanted side products may be diastereomers of the main product – giving different fingerprints in FT-MRR

- In the last few years, two methods have been developed to resolve enantiomers by FT-MRR:
  1) *3-wave mixing* (D. Patterson and J. Doyle, Harvard) – enantiomers produce radiation opposite in phase under particular conditions
  2) *Chiral tagging* (B.H. Pate, Univ. of Va.) – sample is complexed with a chiral resolving agent, producing structurally distinct species
Artemisinin

- Well known antimalarial (discovered by Tu Youyou, 2015 Nobel Prize in Medicine)
  Natural product originally isolated from a form of wormwood native to Asia, but now chemically synthesized
  Artemisinin combination therapy recommended for treatment of *P. falciparum* malaria

First target: Hydrogenation step creates a 5\textsuperscript{th} chiral center and HPLC+NMR are currently used off-line to assess the reaction specificity after the fact. An on-line solution is desired.
Analysis Methods

1) Characterization of FT-MRR signatures of target analytes using broadband spectrometer

Confirmed identity of DHAA by measuring frequency shifts upon $^{13}$C isotopic substitution – detected at each position in natural abundance. (12 hour measurement time, ~100 mg sample)

(When less sample is available, comparisons between experimental and theoretical parameters are sufficient for ID.)

Data: Brooks Pate, Univ. of Va.

\[ \textbf{DHAA computed 3-D structure with experimental carbon positions (small circles)} \]

\[ \textbf{Line FWHM 70 kHz} \]
Analysis Methods

2) Cavity-enhanced spectrometer coupled to sampling manifold for rapid analysis

Current cycle time: 18 minutes limited by speed of thermal cycling, evacuation of reservoir between samples

*First test case: conformer ratio in pure DHAA (set by beam dynamics)*

Prototype design, based on spectrometer design of R.D. Suenram et al.

Connects to continuous manufacturing sample line directly – due to sample handling requirements does not take in sample continuously

Currently can detect down to 5% impurity, with work in progress to extend down to <1%

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Summary

FT-MRR is being applied to a range of problems in impurity characterization – both volatile residual impurities and chiral structural analysis (different instruments, same technique)

Advantages over other techniques include simpler method development and operation, straightforward resolution of complex mixtures - including isomers, and simple quantitation

Speed and ease of measurement, linearity of response, and quantitative accuracy are all on track to meet customer expectations

*We are seeking collaborations on developing applications of FT-MRR in process R&D environments!*
Thank You

Financial Support

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