FT-MRR: Reinventing Rotational Spectroscopy as an Analytical Tool

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Overview

FT-MRR Technique: High Resolution Broadband Spectroscopy

Room Temperature Analysis (Small molecules, <125 amu)

Molecular Beam Analysis (Molecules 100-500+ amu)

Pharmaceutical Impurities

Environmental Analysis (Air/Water)

Chiral Analysis – Diastereomers, Enantiomers, Absolute Configuration

Industrial Gas Impurities

If PAC – February 28, 2017

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The FT-MRR Technique: Fundamentals

- **Molecular Rotational Spectroscopy**
  - Fingerprint spectra based on 3-dimensional moments of inertia – isomers, conformers, and isotopologues are fully resolved
  - Spectra are repeating patterns in microwave to millimeter-wave
  - Used for basic research: gas phase structural determination, interstellar chemistry, identifying transient compounds, and more

- **Requirements for FT-MRR Suitability:**
  - Molecules must have a permanent dipole moment
  - Analytes must be in low-pressure, gas-phase environment
  - At room temperature: molecular weight <125 amu
  - In cold beams: higher mol wts accessible
The FT-MRR Technique: Fundamentals

Limited past viability as commercial products
- Not sensitive enough for trace analysis
- Slow: hours to measure full spectrum

What has changed?
- **Better, cheaper components** (from radar, electronic communications, imaging) improve sensitivity and performance
- **High speed digital electronics** bring measurement times down to seconds-to-minutes
- **Fourier Transform technique** gives massive performance improvements (analogous to FT-NMR)
BrightSpec

• Founded in 2012 to commercialize innovative technology developed at UVa

• Chiral analysis capabilities (Harvard) added in 2014

• 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, Virginia
Identifications are done by multi-line matches between mixture spectra and pure reference spectra.

Overlaps are rare, and most molecules have many lines so no chemometrics are necessary (even for mixtures, most channels are empty).

Analysis returns partial pressures for each identified species – which are then related back to concentration in the original sample.
FT-MRR fills in gaps where chromatography is challenging:

- FT-MRR has excellent performance on small, polar analytes that often are hard to separate (and often require derivatization)
  - Including very small compounds: NH$_3$, H$_2$O, H$_2$CO, PH$_3$, HCN, H$_2$S, OCS...

- FT-MRR distinguishes molecules with similar structures that are hard to separate by chromatography, including enantiomers

Simpler method development, is faster, and requires fewer consumables than chromatography

Unlike most spectroscopy techniques, FT-MRR is naturally suited to complex matrices because molecular features are highly resolved. If the matrix has an FT-MRR signature, it is resolved from that of the analyte.
Trace gas analysis of small molecules

Molecular Rotational Resonance Spectroscopy

February 28, 2017

n 18 ppb
3 minutes

Introduction

Molecular Rotational Resonance Spectroscopy (MRRS) is a technique for analyzing trace gases in the atmosphere. MRRS is based on the principle that molecules absorb microwave radiation at specific frequencies, which are characteristic of their rotational transitions. This technique is particularly useful for analyzing small molecules with low concentrations.

468 ppb PH₃ in N₂
Direct Sampling
3 minute measurement
LDL = 80 ppb

468 ppb PH₃ in N₂
Preconcentrated—1 L of gas
20 minute collection +
measurement
LDL = 100 ppt

Molecule | LDL
--- | ---
Water | 5 ppb
Formaldehyde | 10 ppb
Carbonyl Sulfide | 18 ppb
Ammonia | 35 ppb
Ethylene Oxide | 75 ppb
Phosphine | 200 ppb
Hydrogen Sulfide | 300 ppb

Flow rate:
Frequencies:

572498.6 MHz – Ammonia

Formaldehyde

468 ppb PH₃ in N₂

Direct Sampling

3 minute measurement

LDL = 80 ppb

Preconcentrated—1 L of gas

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November 2014

MRRS (Molecular Rotational Resonance Spectroscopy)

Molecular Rotational Resonance Spectroscopy (MRRS) is a technique for analyzing trace gases in the atmosphere. MRRS is based on the principle that molecules absorb microwave radiation at specific frequencies, which are characteristic of their rotational transitions. This technique is particularly useful for analyzing small molecules with low concentrations.
Very fast eluting volatiles pose a challenge:

- co-elution with solvent
- requires special columns and low temperatures to resolve
- difficult to capture with purge & trap

FT-MRR Headspace Method

• Follows many of the same principles as headspace GC
  FT-MRR runs without carrier gas – we evacuate the headspace vial before injection, and vacuum pulls the analytes into transfer loop, and then into measurement chamber
  We observe faster equilibration times (60-90 sec) than standard GC

• Solvents: same as for GC – water, DMSO, DMF, DMAC all practical

• Can integrate autosampling technology for automated batch runs

Headspace module - heats samples, transfers headspace into vacuum chamber
Highly volatile species can be detected with excellent sensitivity in a simple static headspace measurement.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LDL – 260-290 GHz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broadband (10 min total)</td>
</tr>
<tr>
<td></td>
<td>Targeted (40 s/analyte)</td>
</tr>
<tr>
<td>Chloromethane</td>
<td>5 µg/L</td>
</tr>
<tr>
<td></td>
<td>0.1 µg/L</td>
</tr>
<tr>
<td>Bromomethane</td>
<td>22 µg/L</td>
</tr>
<tr>
<td></td>
<td>0.8 µg/L</td>
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<tr>
<td>Chloroethane</td>
<td>26 µg/L</td>
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<tr>
<td></td>
<td>0.5 µg/L</td>
</tr>
<tr>
<td>Vinyl Chloride</td>
<td>42 µg/L</td>
</tr>
<tr>
<td></td>
<td>0.5 µg/L</td>
</tr>
<tr>
<td>Dichlorodifluoromethane</td>
<td>100 mg/L</td>
</tr>
<tr>
<td></td>
<td>1 mg/L</td>
</tr>
<tr>
<td>Trifluorochloromethane</td>
<td>100 mg/L</td>
</tr>
<tr>
<td></td>
<td>1 mg/L</td>
</tr>
</tbody>
</table>

Targeted method setup when monitoring known analytes:

- Measure any number of compounds, in any order
- Allocate measurement time based on necessary DLs
- Best sensitivity when target compounds are known – directs measurement time on strongest resonances
Solid Headspace: Example

Sample: Diphenhydramine Hydrochloride (commercially obtained secondary standard)
-Heat sample to 195°C (until fully melted), hold 5 minutes
-Cool to ~100°C (allows API and semi-volatile species to condense)
-Transfer sample to measurement cell

<table>
<thead>
<tr>
<th>Run #</th>
<th>Solid wt (mg)</th>
<th>Signal (μV)</th>
<th>Part. Press. (μTorr)</th>
<th>Amount in sample (ppmw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>2.8</td>
<td>21</td>
<td>55</td>
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<tr>
<td>2</td>
<td>48</td>
<td>3.4</td>
<td>25</td>
<td>69</td>
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<td>3</td>
<td>54</td>
<td>4.6</td>
<td>34</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Average</strong> 70.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>r.s. dev</strong> 20%</td>
</tr>
</tbody>
</table>

Solution Headspace (dissolved in water) – 79.8 ppm (10% RSD, n=3)
MDL: Solution Headspace 20 ppm, Solid Headspace <0.1 ppm

Upcoming work: Temperature ramp automation to improve reproducibility
Chiral Analysis by FT-MRR

Technique Capabilities:

1) Unambiguous resolution with small structural changes – diastereomer analysis
   *First application: Diastereomer purity in a continuous manufacturing process*

2) Absolute Chiral Configuration of Enantiomers

3) Rapid Measurements of Enantiomeric Excess

Image Credit:
http://doktori.bme.hu/bme_palyazat/2013/honlap/Bagi_Peter_en.htm
Dihydroartemisinic Acid
(Real time monitoring of diastereomer content during continuous manufacturing, in conjunction with Prof. Frank Gupton, Virginia Commonwealth University)

DHAA produced by catalytic hydrogenation of artemisinic acid – opening possibility of creating an inversion at 1 chiral center

2 ways to determine relative stereochemistry:
1) Isotopic substitution (changes in moment of inertia with natural abundance $^{12}$C -> $^{13}$C substitution)

Requires 100+ mg sample, 12+ hours instrument time, but gets a full gas-phase 3-D structure of carbon heavy atom backbone

2) Use derived parameters (moments of inertia, permanent dipole moment direction, sometimes fine structure) and compare to electronic structure theory.

Requires <10 mg sample (development target <1 mg), <1 hour instrument time

(data: Brooks Pate, University of Virginia)
Cavity Instrument for Rapid Monitoring

Based on published instrument designs – can do single line measurement in seconds-to-minutes

1000x faster than broadband instrument to reach equal sensitivity on one transition
Two methods for absolute configuration and enantiomeric excess by FT-MRR:

**Three-Wave Mixing**  
2013, Harvard University

- Uses a triply resonant microwave field + phase-sensitive detection
- Absolute configuration made through phase referencing to dipole moment absolute sign from theory

**Chiral Tagging**  
2016, University of Virginia

- Uses chiral complexation to resolve enantiomers
- Absolute configuration made through referencing complex geometry to theory


Absolute Configuration of Solketal

Step 1: Measure and Assign Spectrum using Racemic Tag Sample

Even for weakly bound complexes, errors in structural parameters are low (1-2% on rotational constants, 5° dipole moment direction) – and computations can be done on a desktop PC.

Step 2: Measure with Known Enantiopure Tag to Identify Spectrum that Persists

The homochiral spectrum is observed. The propylene oxide sample is S. The solketal sample must be (S)-solketal.

Measurement Time: 10 min
Sample Consumption: 3 μL

Thank you

Financial Support

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Overview

**Pharmaceutical Impurities**

**Room Temperature Analysis**
(Small molecules, <125 amu)

**FT-MRR Technique:**
High Resolution Broadband Spectroscopy

**Molecular Beam Analysis**
(Molecules 100-500+ amu)

**Chiral Analysis – Diastereomers, Enantiomers, Absolute Configuration**

**Industrial Gas Impurities**

**Environmental Analysis**
(Air/Water)

+ more...

**Artemisinin**

Artemisinic Acid
Dihydroartemisinic Acid

H₂/cat. → O₂/hv

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Products: Room Temp, Small Molecules

Both Products

BrightSpec Discovery
- Modular form factor
- Tool for basic research

BrightSpec One
- Integrated form factor
- Tool for analytical lab
- Can add autosampler for QA/QC
Linearity and Quantitation

Data is overlaid from measurements across 3 different days, with 3 different operators.

Fully automated spectral measurement and analysis
- broadband analysis: <10 minutes (including measurement and analysis)
- targeted analysis: ~10 seconds per component

No calibration required other than automated electronic instrument normalization.
The FT-MRR Technique: Fundamentals

**Fourier Transform MRR**
- Development began in the 1970s (W.H. Flygare, University of Illinois)
- Time domain excitation; a range of pulse sequences are accessible
- Free induction decay (FID) signal contains spectral information, and is recorded on a fast digitizer background free

**Broadband (Chirped Pulse) Spectroscopy**
Brooks Pate group, University of Virginia
- Can characterize many data channels (up to $10^6$) simultaneously
- Efficiently uses modern microwave & millimeter wave materials and digital processing components

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http://faculty.virginia.edu/bpate-lab/research.html
Measurement is under ideal gas conditions: spectra are independent of matrix

Total sample in chamber about 1 μmol
Typical analyte detection limits ~1 pmol
(varies depending on analyte molecular weight and dipole moment)

-Detection limits:
dontrth direct gas: ppm-level
static headspace: ppm (volatile) to ppb (very volatile)
solid headspace: ppb
from sorbent (solution or air): ppb or better

Measurements take seconds (analyte-specific) to minutes (broadband investigations)
How does FT-MRR distinguish very similar structures?

Dihydroartemisinic acid is synthesized by catalyzed hydrogenation of artemisinic acid.

DHAA: 2 conformers, both populated in jet

Side products if hydrogenation of AA produces the wrong chiral center

(data: Brooks Pate, University of Virginia)
Dihydroartemisinic Acid

**Theory** is very accurate, even for molecules of this size – and desktop PC can do the calculations. Small changes in structure give noticeable effects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1</th>
<th>Δ R Diastereomer Conf. 1</th>
<th>Δ R Diastereomer Conf. 2</th>
<th>Δ S Diastereomer Conf. 1</th>
<th>Δ S Diastereomer Conf. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (MHz)</td>
<td>717.521006</td>
<td>-1.3%</td>
<td>-1.6%</td>
<td>-2.5%</td>
<td>-3.7%</td>
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<tr>
<td>B (MHz)</td>
<td>311.494215</td>
<td>+0.1%</td>
<td>+0.6%</td>
<td>-0.8%</td>
<td>-0.4%</td>
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<tr>
<td>C (MHz)</td>
<td>254.837601</td>
<td>-1.0%</td>
<td>+0.3%</td>
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<td>+2.0%</td>
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<tr>
<td>θ</td>
<td>49.5°</td>
<td>+4.3°</td>
<td>-5.6°</td>
<td>+14.5°</td>
<td>-20.5°</td>
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<tr>
<td>φ</td>
<td>23.2°</td>
<td>+2.2°</td>
<td>+50°</td>
<td>-19.2°</td>
<td>+45.9°</td>
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<tr>
<td>ID?</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
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</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 2</th>
<th>R Diastereomer Conf. 1</th>
<th>R Diastereomer Conf. 2</th>
<th>S Diastereomer Conf. 1</th>
<th>S Diastereomer Conf. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (MHz)</td>
<td>715.1335</td>
<td>-0.9%</td>
<td>-1.2%</td>
<td>-2.2%</td>
<td>-3.4%</td>
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<td>B (MHz)</td>
<td>312.860449</td>
<td>-0.3%</td>
<td>+0.1%</td>
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<td>-0.8%</td>
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<td>C (MHz)</td>
<td>258.630249</td>
<td>-2.5%</td>
<td>-1.1%</td>
<td>-3.4%</td>
<td>-0.5%</td>
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<tr>
<td>θ</td>
<td>38.7</td>
<td>+15.1°</td>
<td>+5.2°</td>
<td>+25.2°</td>
<td>-9.7°</td>
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<tr>
<td>φ</td>
<td>72.2</td>
<td>-46.8°</td>
<td>+1.0°</td>
<td>-68.2°</td>
<td>-3.1°</td>
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<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>
Quantitative Enantiomeric Excess Ratios

The strongest transitions from the homochiral and heterochiral (S)-verbenone / 3-butyn-2-ol complexes were used in the analysis (~50 transitions from each spectrum).