Analytical Technologies and Compound Identification

Daniel L. Norwood, MSPH, PhD
SCĪO Analytical Consulting, LLC.
Characterization is the discovery, identification, and quantitation of each individual organic and inorganic chemical entity present in an extract above a specified level or threshold. Such thresholds can be based on patient safety considerations, materials considerations, the capabilities of analytical technology, etc.

Scouting is the process of acquiring general chemical information that provides insight into the nature and magnitude of extractables.

Discovery is the process of searching for, and ultimately finding, individual organic and inorganic chemical entities present in an extract.

Identification is the process of assigning a molecular structure to an organic extractable, or assigning constituent elements in the case of an inorganic extractable.

Quantitation is the process of measuring the level, or concentration, of an individual organic or inorganic chemical entity contained in an extract.
Identification – Sub-categories

• Qualitative Analysis – A type of chemical analysis in which the analyte or analytes in a sample are identified (YES/NO result).

• Structural Analysis - A type of chemical analysis in which the molecular structure(s) of an analyte(s) is elucidated.

• Quantitative Analysis - A type of chemical analysis in which the amount of each analyte in a sample is determined (numerical result).
Compound Specific Detectors
(currently available for Trace Organic Analysis)

- Single-crystal X-ray Spectrometer
- FTIR (Fourier Transform Infrared Spectrophotometer)
- NMR (Nuclear Magnetic Resonance Spectrometer)
- Mass Spectrometer
  - GC/MS
  - LC/MS
Why a mass spectrometry centered approach?

• **Sensitivity** – mass spectrometers can generate structural information from high pg to low ng of analyte.
• **Selectivity** – mass spectrometers can distinguish between vast numbers of different analytes.
• **Duty Cycle** – the duty cycle of a mass spectrometer is amenable to the duty cycles of GC and HPLC.
• **Sample Quality** – mass spectrometers can accommodate complex mixtures of analytes and (in many cases) relatively “dirty” samples.
Mass Spectrometry Nomenclature

Nominal Mass
  Integer mass of the most abundant stable isotopes

Theoretical Accurate Mass
  Calculated mass of a particular molecular formula

Measured Accurate Mass
  Experimentally determined mass of a particular molecular formula

Protonated Molecular Ion [M+H]^+
  An ion formed by addition of a proton (either in solution or in the gas phase) to an intact molecule

Deprotonated Molecular Anion [M-H]^-
  An ion formed by abstraction of a proton (either in solution or in the gas phase) from an intact molecule
MS/MS
A technique by which a selected precursor ion is fragmented (usually through collisions with neutral gas molecules) to produce structural information

Resolving Power/Resolution
A measure of the ability of a mass spectrometer to separate ions of two different masses
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td>electron ionization</td>
</tr>
<tr>
<td>CI</td>
<td>chemical ionization</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
<tr>
<td>LSIMS</td>
<td>liquid secondary ion</td>
</tr>
<tr>
<td>TSP</td>
<td>thermospray</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray</td>
</tr>
<tr>
<td>APCI</td>
<td>atmospheric pressure CI</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix assisted laser desorption</td>
</tr>
<tr>
<td>LMIS</td>
<td>liquid metal ion source</td>
</tr>
</tbody>
</table>
Hyphenated Analytical Techniques

- LC/MS  LC/NMR  GC/MS  GC/FTIR
- What distinguishes a “Hyphenated Analytical Technique” from any other analytical technique? What is the “Line of Demarcation”?
  - e.g. Why is LC/UV not considered to be a hyphenated analytical technique?
- Norwood’s Definition –
  - “A hyphenated analytical technique is one in which the detector has significantly greater scientific impact than the sample introduction system.”
“Modern” LC/MS Systems

LTQFT Ultra
“FTMS”

QTOF
(SYNAPT G2)

API 5000
(Triple Quadrupole)

“A good field to get out of.”

Bill Haddon, 1982 ASMS Meeting
Mass Spectrometry Data Elements

a. Mass spectrometric fragmentation behavior/expert mass spectrum interpretation
b. Confirmation of molecular weight
c. Confirmation of elemental composition
d. Mass spectrum matches automated library or literature spectrum
e. Mass spectrum and chromatographic retention index match authentic reference compound
f. Supporting spectral information from an orthogonal method (e.g., NMR)
## LC/MS Instrument Summary

<table>
<thead>
<tr>
<th>Instrument</th>
<th>MW</th>
<th>In-source Frag.</th>
<th>MS/MS</th>
<th>MS^n</th>
<th>Accurate Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Quad.</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Triple Quad.</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Ion Trap</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>TOF</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>FTMS</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>(*</td>
<td>*</td>
</tr>
<tr>
<td>QTOF</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Trap-FTMS</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Quad-FTMS</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>(*</td>
<td>*</td>
</tr>
</tbody>
</table>
Identification Categories

• A **Tentative** identification means that data have been obtained that are consistent with a class of molecule only. This is typically the case when only information such as a or d is available.

• A **Confident** identification means that the tentative identification has been bolstered by additional and sufficient confirmatory information to preclude all but the most closely related structures. This would be the case, for example, if the tentative information (a and/or d) were augmented by b, c, or f. The more confirmatory information obtained, the greater the level of confidence.

• A **Confirmed** identification means that the preponderance of evidence confirms that the entity in question can only be the identification that is provided. *Although it is possible that a highly confident identification may meet the standard implied by the preponderance of evidence (for example, having a, b, c, e, and f), the only means of providing a confirmed identification is via mass spectral and retention time match with an authentic reference compound (item e).*
Scan 5446 (21.468 min): 07110301.D

#180831: Zn

m/z--> Abundance

Abundance
Total Ion Chromatogram of Three Representative Extractables/Leachables

[Graph showing chromatogram with peaks labeled: Irganox 1076, DEHP, Tetramethylthiourea]
Electron Ionization Mass Spectrum of Tetramethylthiourea (130 pg on-column)

Average of 5.742 to 5.801 min.: 071019010.D\data.ms (-)

#21710: Thiourea, tetramethyl- (CAS) $$ N,N,N',N'-Tetramethylthiourea
Electron Ionization Mass Spectrum of Bis-2-ethylhexylphthalate (304 pg on-column)

Abundance

Average of 11.248 to 11.308 min.: 071019010.D\data.ms (-)

#230979: 1,2-Benzene dicarboxylic acid, bis(2-ethylhexyl) ester (CAS:

[Chemical Structure Image]
Electron Ionization Mass Spectrum of Irganox 1076 (500 pg on-column)
Irganox 1076 APCI Negative Ion FT-ICR Mass Spectrum
(1.0 ng on-column, 2.4 ppm mass error)

SP071016021 #264  RT: 5.07  AV: 1  SB: 24 5.22-5.41 , 4.70-4.89  NL: 5.43E5
T: FTMS - p APCI corona Full ms [515.00-545.00]

[M-H]−
ESI+ TOF Mass Spectrum of TMTMS

![TOF Mass Spectrum of TMTMS](image)

- $[M+H]^+$
- $[M+Na]^+$
- $[2M+Na]^+$
Expanded ESI+ TOF Mass Spectrum of TMTMS

[Diagram showing mass spectrum with peaks at m/z 231.0006 and 233.0006 labeled as [M+Na]+]

Max 1.3e6 counts
Elemental Composition Report for TMTMS from ESI+ TOF-MS

Single Mass Analysis - displaying only valid results
Tolerance = 10.0 PPM / DBE: min = -0.5, max = 20.0

Monoisotopic Mass, Odd and Even Electron Ions
43463 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>231.0052</td>
<td>231.0052</td>
<td>0.0</td>
<td>0.2</td>
<td>8.0</td>
<td>12C4 1H2 14N9 23Na 32S</td>
</tr>
<tr>
<td>231.0051</td>
<td></td>
<td>0.1</td>
<td>0.6</td>
<td>9.5</td>
<td>12C11 1H7 14N2 32S2</td>
</tr>
<tr>
<td>231.0060</td>
<td></td>
<td>-0.8</td>
<td>-3.6</td>
<td>1.5</td>
<td>12C6 1H12 14N2 23Na 32S3</td>
</tr>
</tbody>
</table>
MS/MS Spectrum of Tetramethylthiuram Disulfide (ESI+; products of m/z 241)

Daughters of 241ES+

[M+H]^+
Structure and Fragmentation of Tetramethylthiuram Disulfide in Positive Ion APCI (MS/MS)

\[
\text{N} \quad \text{S} \quad \text{S} \quad \text{N} + \text{H}^+ \\
\text{m/z 241}
\]

\[
\text{N} \quad \text{S} - \text{S} - \text{S} + \\
\text{m/z 196}
\]

\[
\text{N} \quad \text{S} - \text{S} + \\
\text{m/z 88}
\]

\[
\text{N} + \\
\text{m/z 44}
\]

\[
\text{N} \quad \text{S} + \\
\text{m/z 120}
\]
LC/MS Analysis of Cyclic Oligomer Co-polymer (COC) Extract

Negative Ion APCI
Total Ion Chromatogram

UV = 280 nm

- 916 mw (A)
- 1120 mw (B)
- 1208 mw (C)
- Irganox 1010
Negative Ion APCI Mass Spectrum of Irganox 1010 in the COC Extract

-TOF MS: 13.105 to 13.395 min from KM20110926002.wiff Agilent, subtracted (13.539 to 13.937 min)

Max. 1.0e5 counts.

\([M-H]^-\)
Negative Ion APCI Mass Spectrum of Irganox 1010 Degradation Product A
Negative Ion APCI Mass Spectrum of Irganox 1010 Degradation Product B
Negative Ion APCI Mass Spectrum of Irganox 1010 Degradation Product C
Positive Ion HESI Mass Spectrum of Irganox 1010 Degradation Product C

SP120712006 #869  RT: 11.54  AV: 1  NL: 4.80E5
T: FTMS + c ESI Full ms [150.00-1500.00]
Positive Ion HESI Mass Spectrum of Irganox 1010 Degradation Product C

SP120712026 #869  RT: 11.54  AV: 1  NL: 4.80E5
T: FTMS + c ESI Full ms [150.00-1500.00]

(expanded view of molecular ion region)

\[ [M+\text{NH}_4]^+ \]

\[ [M+\text{Na}]^+ \]

\[ [M+\text{K}]^+ \]
Negative Ion HESI Mass Spectrum of Irganox 1010 Degradation Product C

$[M-H]^-$

$[M+TFA]^-$
Summary of Mass Spectrometry Data from Irganox 1010 and Related Structures

<table>
<thead>
<tr>
<th></th>
<th>[M+H]^+</th>
<th>^1Adduct Ions (+)</th>
<th>[M-H]^−</th>
<th>^3Adduct Ions (−)</th>
<th>^4Formula</th>
</tr>
</thead>
</table>

^1From positive ion ESI spectra (ACN: acetonitrile; EA: ethylamine).
^2From negative ion ESI and APCI spectra.
^3From negative ion ESI spectra (TFA: trifluoroacetic acid; HAc: acetic acid).
^4From [M+NH₄]^+ in positive ion ESI spectra (ppm deviation of experimental mass from calculated mass at 50,000 resolving power).
SPE LC/NMR (in-line) UV chromatogram of Cyclic Olefin Copolymer Extract

- 916 mw (A)
- 1120 mw (B)
- 1208 mw (C)

Irganox 1010
Flow-Probe $^1$H-NMR Spectrum of Irganox 1010
(2 collections)
Flow-Probe $^1$H-NMR Spectrum of C
(10 collections)

* = minor component
Structure of Irganox 1010 Degradation
Product C

m/z 915
High-Field Cryoprobe HMBC Spectrum of C
Concluding Thoughts

• There is no such thing as an *unknown*.
• There is only the limit of our need and motivation to identify.
• Mass spectrometry techniques that were once considered advanced are now becoming routine.
• Please use the standard identification criteria and categories.
Thank You!