Strengthening Results

Advanced Chromatography with Mass Detection
UV Detection – Why Not Use UV For Everything?

• **Identification**
  • Difficult selectivity in complex matrices

• **Sensitivity**
  • Need sufficient molar absorptivity

• **Method Development**
  • Peak assignments require confirmation runs

• **Quantitation**
  • Relatively high detection limits and narrow dynamic ranges

• **Definitive Confirmation**
  • May require confirmation analysis

• **Structural Information**
  • Limited information
What Are Some of the Benefits to Mass Detection?

UltiMate LC System
MSQ™ Plus Mass Detector
Benefits: Reduce Matrix Background Interference

- Mass detectors allow analyte-specific detection within matrices

- Column
  - Hypersil Gold PFP
  - 50 x 2.1, 1.9 um

- Gradient
  - H2O:ACN
  - 0.1% FA

- Flow Rate
  - 500 uL/min

- Injection
  - 2 uL

- Analyte
  - 10 ppm

- Detector
  - 200-400 nm

[Graph: Standard in Urine]
Benefits: Improve Limit of Quantitation

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  - Analyte
    - 10 ppm
  - Detector
    - ESI Positive
    - Capillary 4.5 kV
    - Probe 550 C

Filter contaminants, isolate analyte, and increase sensitivity
Minimize sample preparation, simplify workflow, increase throughput
Benefit: Improve Limit of Quantitation

- Mass detectors allow sensitive, robust, multi-component quantitation across large linear dynamic ranges

- Column
  - Hypersil Gold PFP
  - 100 x 2.1, 1.9 um

- Gradient (Ternary)
  - H2O:MeOH:ACN
  - 0.1% Acetic Acid

- Flow Rate
  - 1,000 uL/min

- Injection
  - 2 uL

- Analyte
  - Variable

- Detector
  - ESI Positive
  - Capillary 4.5 kV
  - Probe 450 C

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Caffeine</td>
</tr>
<tr>
<td>2</td>
<td>Ephedrine</td>
</tr>
<tr>
<td>3</td>
<td>Pseudoephedrine</td>
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<tr>
<td>4</td>
<td>Amphetamine</td>
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<tr>
<td>5</td>
<td>Hydrocodone</td>
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<td>Oxycodone</td>
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<td>3,4-MDMA</td>
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<td>9</td>
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<td>Noscapine</td>
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<td>13</td>
<td>THC</td>
</tr>
<tr>
<td>14</td>
<td>Cannabinol</td>
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</table>
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  - Variable

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  - Capillary 4.5 kV
  - Probe 450 C

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOQ (ng/mL)</th>
<th>LOD (ng/mL)</th>
<th>Linear Range (ng/mL)</th>
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<tr>
<td>ephedrine</td>
<td>1.21</td>
<td>0.36</td>
<td>1.3-2000</td>
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<tr>
<td>pseudoephedrine</td>
<td>1.25</td>
<td>0.38</td>
<td>1.3-1670</td>
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<td>amphetamine</td>
<td>1.78</td>
<td>0.53</td>
<td>1.3-1670</td>
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<td>methamphetamine</td>
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<td>1.3-1670</td>
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<td>3,4-MDMA</td>
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<td>oxycodone</td>
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<td>clonazepam</td>
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<td>THC</td>
<td>191</td>
<td>57.4</td>
<td>68.5-11100</td>
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</table>

*Improve limits of detection and quantitation for multi-component analyses*
Benefit: No Chromaphore required

- Mass detectors ionize and detect analytes that do not exhibit strong UV absorbencies
  - Column
    - Hypersil Gold PFP
    - 100 x 2.1, 1.9 um
  - Gradient
    - H2O:MeOH
    - Ammonium Formate
  - Flow Rate
    - 500 uL/min
  - Injection
    - 2 uL
  - Analyte
    - Triacetone triperoxide (TATP)
  - Detector
    - APCI Positive
    - Corona 30 uA
    - Probe 350 C

Overcome limitations associated with conventional LC detection
Benefit: Detect Inorganic Analytes

- Mass detectors allow inorganic analysis concurrent with organic analysis

- Column
  - Hypercarb
  - 100 x 2.1, 5 um

- Gradient
  - H2O:MeOH
  - 1% FA

- Flow Rate
  - 300 uL/min

- Injection
  - 5 uL

- Analyte
  - Gunpowder Extraction
  - ~ 50 ug/mL

- Detector
  - ESI Positive/Negative
  - Capillary 3.5 kV
  - Probe 550 C

Increase throughput by using an all-in-one detector
Benefit: Confirm Identification

- Mass detectors allows confident and efficient identification of analyte peaks
  - Column
    - Hypersil Gold PFP
    - 100 x 2.1, 1.9 um
  - Gradient
    - H2O:ACN
    - 0.1% Acetic Acid
  - Flow Rate
    - 1,000 uL/min
  - Injection
    - 2 uL
  - Analyte
    - 500 ppb
  - Detector
    - ESI Positive
    - Capillary 3.5 kV
    - Probe 450 C

Quickly identify peaks after changing columns, gradients, or flow rates
Benefit: Additional Information on Impurities

- Mass detectors strengthen purity analysis by generating impurity spectra
- Column
  - Hypersil Gold
  - 100 x 2.1, 1.9 um
- Isocratic
  - H2O:MeOH
- Flow Rate
  - 800 uL/min
- Injection
  - 5 uL
- Analyte
  - Simvastatin
- Detector (UV)
  - 254 nm
- Detector (Mass)
  - ESI Positive/Negative
  - Capillary 3.0 kV
  - Probe 550 C

Identify the mass, structure, and source of impurities
Benefit: Definitive Confirmation

- Mass detectors allow systematic and reproducible fragmentation of analyte for multiple orders of confirmation
  - Column
    - Hypersil Gold
    - 100 x 2.1, 1.9 um
  - Isocratic
    - H2O:MeOH
  - Flow Rate
    - 800 uL/min
  - Injection
    - 5 uL
  - Analyte
    - Illicit Drugs
  - Detector
    - ESI Positive
    - Capillary 3.5 kV
    - Probe 550 C

Effectuate compound-specific fragmentation and match to theoretical behavior
Benefit: Library Search

- Mass detectors allow matching of spectra against user-generated libraries
  - Column
    - Hypersil Gold PFP
    - 100 x 2.1, 1.9 um
  - Gradient
    - H2O:MeOH
    - Ammonium Formate
  - Flow Rate
    - 500 uL/min
  - Injection
    - 2 uL
  - Analyte
    - Soil Extraction
    - LOD 0.2 ug/kg
  - Detector
    - APCI Negative
    - Corona 30 uA
    - Probe 350 C

Confidently screen samples for target analytes using library matching
Mass Detection – Strengthen Chromatography

- **Easier Identification**
  - Ionize analyte with low molar absorptivity
  - Create positive and negative ions simultaneously

- **Greater Sensitivity**
  - Enhance selectivity
  - Filter contaminants

- **Faster Method Development**
  - Simplify peak assignment

- **Enhanced Quantitation**
  - Isolate analytes

- **More Definitive Confirmation**
  - Match against spectral libraries
  - Induce compound-specific fragmentation

- **Structural Information**
  - Identify impurities
  - Increase sensitivity
  - Improve quantitation

UltiMate LC System
MSQ™ Plus Mass Detector
How Does Mass Detection Work?

UltiMate LC System
MSQ™ Plus Mass Detector
What is a Mass Detection?

- Mass detection is the measurement of the mass-to-charge ratio of individual molecules that have been converted to gas phase ions.

- Mass Spectrum: A plot of mass to charge (m/z) vs. relative or absolute intensity.
MSQ Plus Mass Detector – Single Quadrupole

- Strengthen chromatography with enhanced mass detection
  - Mass confirmation
  - Robust quantitation
  - Structural elucidation
  - Positive identification

Diagram:
- Analyte from LC Column
- FastLoc Probe
- Entrance Cone
- Vacuum
- Cone Wash
- Transfer Lens
- Quadrupole Analyzer
- Exit Cone
- Ion Bright Detector
- Signal to Data System
MSQ Plus Mass Detector: Advanced LC Detection

- Expanded Application Versatility
  - Extended 17-2000 Da mass range
  - FastLoc™ ESI and APCI Probes
  - Maximum flow rate 2 mL/min for both ESI and APCI

- Minimized Operation Costs
  - Patented titanium entrance cone

- Increased Sensitivity
  - Scan speeds up to 12,000 amu/sec
  - Patented Cone Wash technology
MSQ Plus Mass Detector: Advanced LC Detection

- **Maximized Sample Throughput**
  - Patented M-path Source
  - Wipe clean surfaces for easy maintenance

- **Greater User Confidence**
  - Automatic tune and calibration

- **Improved Quantitative Ability**
  - Source CID (Collision Induced Dissociation) for ion fragmentation
  - Simultaneous quantitative and qualitative determinations by combining Full Scan and SIM.
  - Multiple scan modes with one injection (+/-, FS, SIM, overlapping segments)
Example Applications

UltiMate LC System
MSQ™ Plus Mass Detector
A Single Ternary Method for 14 Drugs

- Fourteen drugs baseline separated in twelve minutes by employing UHPLC/MS with a ternary solvent gradient.

Identified

Quantified

Hypersil GOLD RFP 1.9μm, 100 x 2.1 mm; ternary gradient of water, acetonitrile, and methanol with acetic acid at 1 mL/min; 1 μL injection; ESI Positive
A Single Ternary Method for 14 Drugs

Ternary Gradient Profile

- Water
- Methanol
- Acetonitrile

Percent Composition vs. Time (min)

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Time (min)
Hallucinogenic Mushrooms in Chocolate

- Easily separate psilocybin and psilocin using UHPLC/MS, avoiding detection difficulties such as low concentration, thermal lability, and matrix interference that are typical with other techniques

- **Hypersil GOLD PFP 1.9um, 100 x 2.1 mm; ternary gradient of water, acetonitrile, and methanol with acetic acid at 1 mL/min; 2 uL injection; ESI Positive**
Confirmation of Psilocin

- Definitively confirm the presence of illicit drugs using UHPLC/MS, by creating and searching spectral libraries for fragmentation patterns produced through in-source collision induced dissociation.

**Psilocin**

- **(low cone voltage)**
  - m/z 205.0
  - Identified

- **(medium cone voltage)**
  - m/z 160.2
  - Confirmed

- **(high cone voltage)**
  - m/z 115.1
  - Confirmed

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Rodriguez Cruz, Sandra E., "Analysis and Characterization of Psilocybin and Psilocin Using Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC-ESI MS) with Collision-Induced-Dissociation (CID) and Source-Induced-Dissociation (SID)", Microgram Journal, Volume 3 Numbers 3-4 (2005)
LSD in Candy Hearts

- Positively identify LSD within a complex matrix using UHPLC/MS, requiring no downtime for instrument bake-out and clean-up

Hypersil GOLD PFP 1.9um, 100 x 2.1 mm; ternary gradient of water, acetonitrile, and methanol with acetic acid at 1 mL/min; 2 uL injection, ESI Positive
Marijuana in Brownies

- Efficiently separate and detect THC, cannabinol, and cannabidiol using UHPLC/MS, consuming only milligram amounts of evidence

Hypersil GOLD PFP 1.9um, 100 x 2.1 mm; ternary gradient of water, acetonitrile, and methanol with acetic acid at 1 mL/min; 2 uL injection; ESI Positive
Pseudoephedrine in Tablets

- Quantify ephedrine, pseudoephedrine, methamphetamine, amphetamine, and 3,4-MDMA using UHPLC/MS; and, elucidate the current manufacturing pathways of clandestine chemistry.

Hypersil GOLD PFP 1.9um, 100 x 2.1 mm; binary gradient of water and acetonitrile with acetic acid at 1 mL/min; 1 uL injection; ESI Positive

Identified

Quantified

Hypersil GOLD PFP 1.9um, 100 x 2.1 mm; binary gradient of water and acetonitrile with acetic acid at 1 mL/min; 1 uL injection; ESI Positive
Pseudoephedrine in Tablets

- Definitively confirm the presence of illicit drug product using UHPLC/MS, by creating and searching spectral libraries for fragmentation patterns produced through in-source CID.

![Diagram of molecular ion and in-source CID for Pseudoephedrine](image)

- Molecular Ion (low cone voltage): 148.1
- In-Source CID (high cone voltage): 148.1
- Pseudoephedrine (RT: 2.60min): 166.0

Hypersil GOLD PFP 1.9um, 100 x 2.1 mm; binary gradient of water and acetonitrile with acetic acid at 1 mL/min; 1 uL injection; ESI Positive
Salt Forms of Product

- Characterize illicit drugs and their precursors by distinguishing unique salt forms with low mass, polarity switching UHPLC/MS

Hypercarb 3um, 50 x 2.1 mm; binary gradient of water and methanol with formic acid at 300 uL/min; 2 uL injection; ESI Positive and Negative
Gunpowder in Particles

- Determine the make and manufacturer of gunpowder found in particles, pellets, and residue by characterizing their unique salt compositions through low mass, polarity switching UHPLC/MS

![Graphs showing the normalized abundance of various elements for Hodgdon Triple Seven and Hodgdon Pyrodex gunpowders.](image)

**Hodgdon Triple Seven**
- Sodium
- Potassium
- Nitrate
- Gluconate
- Perchlorate
- Dicyandiamide
- Benzoate

**Hodgdon Pyrodex**
- Sodium
- Potassium
- Nitrate
- Gluconate
- Perchlorate
- Dicyandiamide
- Benzoate

- Hypercarb 5μm, 100 x 2.1 mm; binary gradient of water and methanol with formic acid at 300 uL/min; 5 uL injection; ESI Positive and Negative
Explosive Compounds in Soil

- Simultaneously analyze nitroamines, nitroaromatics, nitrate esters, and peroxide explosives by UHPLC/MS in under 15 minutes. Confirm the identifications by spectral library search.

<table>
<thead>
<tr>
<th>Peak Compound</th>
<th>Match scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 HMTD</td>
<td></td>
</tr>
<tr>
<td>2 EGDN</td>
<td></td>
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<tr>
<td>3 TNB</td>
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<tr>
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<td>17 PETN</td>
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</tr>
</tbody>
</table>

Library spectrum

Target spectrum

Match scores

Relative Abundance

Time (min)
MSQ Plus Mass Detector: Advanced LC Detection

For cost conscious chemists who require increased productivity through dependable simplicity, the Thermo Scientific MSQ is a turnkey UHPLC/MS solution providing superior chromatographic efficiency and enhanced mass detection with unsurpassed ease of use, performance, and ruggedness.
Increase Productivity

Ultra-Fast Speed, Resolution and Mass Detection

Thermo Scientific Dionex
UltiMate 3000 System

Thermo Scientific
MSQ Plus Mass Detector
Thank you!