Ultra-Fast Separation and Quantification of isobaric Barbiturates in Serum using LDTD-MS/MS combined with differential mobility spectrometry

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Barbiturates used to be prescribed for anxiety and insomnia, but they have been replaced by safer medications (Benzodiazepines). Because barbiturates are not as freely prescribed, they are not as easy to acquire as they were years ago, and therefore adult abuse has gone down. Teens however, have not followed this pattern. In the last 10 years, Barbiturate Abuse has increased among teens. According to high school surveys, about 8% of teens abuse barbiturates.

Why do teens abuse barbiturates?

1. Teens abuse barbiturates or “downers” to counteract other drugs.
2. Teens are too young to remember the repercussions (OVERDOSE) of barbiturate abuse from the 1950's to the 1970's.
3. Barbiturates are often used in suicide attempts.
And how do we analyze Barbiturates in 2015? Really!

Based on the Recommended Methods for the Detection Assay of Barbiturates and Benzodiazepines in Biological Specimens by the United Nations International Drug Control Program, Barbiturates are normally detected in plasma and urine by immunoassay, GC or HPLC.

Analysis of Isobaric molecules with similar structures requires sufficient time in GC-MS or LC-MS/MS to get an adequate separation. Adequate separation in this case means **20 to 30 minutes**.

Outcome: Assay throughput reduction and/or a limited number of samples that can be tested for abuse of Barbiturates.
Can we do it differently in 2015? Yep!

Chromatographic separation is a time consuming bottleneck in a LC or GC–MS analysis workflow. Can we do it without chromatographic separation?

1- LDTD ion source is used to desorb and ionize the Barbiturates.

2- Differential mobility spectrometer (DMS) allows separation of Isobaric molecules at sub second speed.

3- Qtrap Mass Spectrometer separates all the components based on their fragmentation pattern.
As an example, we used Amobarbital and Pentobarbital which differ only by the position of a methyl group.

Both Barbiturates have the same MS/MS fragmentation pattern. Therefore, we optimized the DMS to provide an additional separation as means to distinguish them.
Show it to me! The Instruments
**Instrumentation – LDTD Overview**

- High-Throughput Ion source
- Minimal gas phase ionic suppression
- 4-10 seconds sample to sample analysis time
- Sensitivity, accuracy, linearity and reproducibility equivalent or superior to LC-ESI and APCI
- Can be combined with Ion Mobility for separation of Isobaric compounds.
- Used for small molecule analysis (<1500 amu)
Instrumentation – DMS Overview

DMS Technologies

SelexION™ Curtain Plate
Updated version of the traditional curtain plate to accommodate the differential ion mobility cell. Maintains the same level of robustness and stability associated with the original design.

Differential Mobility Cell
Compact and simple design allows the cell to be installed without the use of any tools and in less than two minutes.

Diagram showing the components of the DMS setup with labels indicating the various parts and their functions.
Use of Modifiers in DMS

- Modifier gas makes clusters under low electric field and de-clusters during the high field cycle.
- Clustering action depends on the molecular functional group.
- Addition of modifiers changes the mobility which may give more specificity for some compounds.
- The most frequently used Modifiers are solvents.
- Modifiers tested for barbiturates in this assay: Methanol, Acetonitrile, Isopropanol and Water.
- Despite the stronger action of the compensation voltage, Methanol, Acetonitrile and Isopropanol strongly suppress ion signal.
- Associated suppression is too high making these modifiers unsuitable.
- Water used as a modifier gives a better separation than pure nitrogen without noticeable ion suppression.
DMS final parameters

• Separation voltage set to the maximum stable value which does not cause arcing: 4300 Volts.
• Temperature set at Low for maximum separation. No liquid: no need for desolvation.
• Modifier is Water and set at Low. (Better separation with less suppression – No Solvent used in the LDTD ion Source.)
• DMS resolution (DR) is set at High for adequate separation of Amobarbital and Pentobarbital.
• COV is tuned individually for each molecule.
COV optimization

Instrumentation – DMS Overview
Extraction Procedure

• 25 µL patient serum sample (or STD or Cal).

• 50 µL internal standard (5 µg/mL phenobarbital-d5 in MeOH/HCl (0.1 N) 25:75).

• 100 µL Ethyl Acetate.

• Vortex 30 seconds.

• Transfer 2 µL of the upper layer in LazWell™ plate.

• Analyze after complete solvent evaporation.
Serum Sample Analysis

Instrumentation
- LDTD model: S-960
- MS: Sciex 5500 QTrap® SelexION™

MS Parameters
- APCI (-)
- Separation Voltage: 4300 V
- DMS modifier: Water
- DP: -80 V
- Dwell: 25 msec
- CE: -46 V

LDTD Parameters
- Laser power pattern:
  - Increase laser power to 45 % in 6.0 sec
  - Decrease laser power to 0 %
- Carrier gas flow: 3 L/min (Air)

MRM mode:
- Amobarbital: 225 → 42 (COV: 6.8V)
- Pentobarbital: 225 → 42 (COV: 5.8V)
- Phenobarbital-d5: 236 → 42 (COV: 5.2V)
Serum Sample Analysis

Linearity:
- Standard calibration curve ranging from 125 to 25 000 ng/mL prepared in blank plasma samples.

Pentobarbital calibration curve with LDTD-DMS MS/MS. $R = 0.99760$ with a weighting factor $1/x$.

Amobarbital calibration curve with LDTD-DMS MS/MS. $R = 0.99732$ with a weighting factor $1/x$. 
# Serum Sample Analysis

## Intra-run Accuracy and Precision

<table>
<thead>
<tr>
<th></th>
<th>LLOQ</th>
<th>QC-Low</th>
<th>QC-Med</th>
<th>QC-High</th>
<th>ULOQ</th>
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</thead>
<tbody>
<tr>
<td>Conc. (ng/mL)</td>
<td>125</td>
<td>250</td>
<td>2500</td>
<td>12500</td>
<td>25000</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
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<tr>
<td>Mean (ng/mL)</td>
<td>107.8</td>
<td>253.3</td>
<td>2827.6</td>
<td>13109.8</td>
<td>23880.8</td>
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<tr>
<td>%RSD</td>
<td>15.0</td>
<td>13.0</td>
<td>5.1</td>
<td>6.7</td>
<td>3.6</td>
</tr>
<tr>
<td>%Nom</td>
<td>86.2</td>
<td>101.3</td>
<td>113.1</td>
<td>104.9</td>
<td>95.5</td>
</tr>
</tbody>
</table>

**Pentobarbital:** Accuracy is between 86.2 and 113.1% while precision is between 3.6 and 15.0%.

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<tr>
<td>N</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mean (ng/mL)</td>
<td>122.9</td>
<td>222.6</td>
<td>2742.0</td>
<td>12597.2</td>
<td>24163.6</td>
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<tr>
<td>%RSD</td>
<td>5.9</td>
<td>6.1</td>
<td>9.6</td>
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<td>8.6</td>
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<tr>
<td>%Nom</td>
<td>98.3</td>
<td>89.0</td>
<td>109.7</td>
<td>100.8</td>
<td>96.7</td>
</tr>
</tbody>
</table>

**Amobarbital:** Accuracy is between 89.0 and 109.7% while precision is between 3.3 and 9.6%.
Serum Sample Analysis

Amobarbital specificity:
• ULOQ level QC of Amobarbital (25000 ng/mL) is desorbed while monitoring Amobarbital, Pentobarbital and IS to quantify Isobaric Contribution.

<table>
<thead>
<tr>
<th></th>
<th>QC-Amobarbital</th>
<th>QC-Pentobarbital</th>
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</thead>
<tbody>
<tr>
<td>Conc. (ng/mL)</td>
<td>25000</td>
<td>0</td>
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<tr>
<td>N</td>
<td>4</td>
<td>4</td>
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<td>Mean (ng/mL)</td>
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<tr>
<td>%RSD</td>
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<td>21.4</td>
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<tr>
<td>%Nom</td>
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<tr>
<td>%Interference</td>
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</table>

QC at 25000 ng/mL of Amobarbital desorbed: 1% Isobaric Contribution to Pentobarbital.
**Serum Sample Analysis**

**Pentobarbital specificity:**
- ULOQ level QC of Pentobarbital (25000 ng/mL) is desorbed while monitoring Amobarbital, Pentobarbital and IS to quantify Isobaric Contribution.

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<tr>
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<td>0</td>
<td>25000</td>
</tr>
<tr>
<td>N</td>
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<tr>
<td>Mean (ng/mL)</td>
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<tr>
<td>%RSD</td>
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<td>3.3</td>
</tr>
<tr>
<td>%Nom</td>
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<td>117.5</td>
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<tr>
<td>%Interference</td>
<td>No interference</td>
<td></td>
</tr>
</tbody>
</table>

QC at 25000 ng/mL of Pentobarbital desorbed: **No Isobaric Contribution to Amobarbital.**
Conclusion

• Quantification of Isobaric Barbiturates in serum can be performed in 9 seconds using LDTD-DMS-MS/MS.
• SelexION™ technology allows separation of two isobaric molecules with similar structure by varying the COV parameters.
• Intra-run precision and accuracy comparable to LC-MS and GC-MS methods.