2011 Joint SOFT-TIAFT Meeting
San Francisco, California, USA

Thermo Scientific Workshop
28 September 2011

Identification of New Amphetamine-Related Designer Drugs Using Exactive High Resolution Accurate Mass Spectrometry

Giampietro Frison

Laboratory of Environmental Hygiene and Forensic Toxicology
Department of Prevention
Azienda ULSS 12 Veneziana, Mestre (Venezia), Italy
Forensic toxicology at the LIATF

✓ **Seized drugs**
  • Determination of active ingredients, adulterants, diluents in seized material for Police and Court

✓ **Biological samples**
  • Determination of alcohol, psychoactive drugs, other drugs of toxicological interest in biofluids
  • Driving under the influence of alcohol and drugs
  • Post-mortem toxicology
  • Drug-facilitated crimes
  • Other forensic toxicology applications
The flood of new designer drugs

✓ Indane analogues of Ecstasy’s (MDAI, MDMAI)

✓ Tetraline analogues of Ecstasy’s (2-AT, MDAT)

✓ Tryptamines

✓ Cannabinomimetics (JWH-18, JWH-073, JWH-200, JWH-250,CP-47,497, ...)

✓ Beta-keto-amphetamines (designer cathinones)

✓ Piperazines
New A-R drugs encountered at LIAFT

Mephedrone
4-MEC
4-FA
MDPV
Methylone
Cl-Ph-Pip
Propylone
The flood of new designer drugs

The LIAFT experience with new designer drugs

Increasing number of police seizures and/or intoxication cases

Need to promptly obtain their structural characterization, in spite of poor availability of reference (p/m) standards
Analytical strategy to obtain the structural characterization of new A-R drugs

**GC-MS**
- Poor chromatography
- Non-specific EI MS

**GC-MS + der with 3ClEtCl for**
- Improved GC properties
- Distinctive MS behav.
- Highly inform. EI MS

**UHPLC-HRMS (Orbitrap)**
- Accurate mass measurements at 100,000 resolv. power
- Study of MH\(^+\) collision-induced product ions
- Comparison of exp. and calc. MH\(^+\) isotopic clusters
- Study of isotopic fine structure of M+1, M+2, M+3 clusters
GC/MS - Mephedrone

Rel. Ab.

6.31

Mephedrone
(4-Methyl-Meth-Cathinone)

Methanolic solution (b.p. 64.5 °C)

GC/MS

Agilent 7890 II - 5975
Full Scan EI (40-450 u)
Inj. 1 µl, 250C, split / splitless (1 min)
Carrier gas (He), 1 ml/min

Agilent HP-5MS Ultra Inert column
30 m x 0.25 mm x 0.25 µm
50C (0.5 min), 200C a 30C/min, 300C (5 min) a 10C/min
Interf. 280C, EMV + 300 V
GC/MS - Mephedrone

**GC/MS**

Agilent 7890 II - 5975
Full Scan EI (40-450 u)
Inj. 1 µl, 250C, split / splitless (1 min)
Carrier gas (He), 1 ml/min

Agilent HP-5MS Ultra Inert column
30 m x 0.25 mm x 0.25 µm
50C (0.5 min), 200C a 30C/min, 300C (5 min) a 10C/min
Interf. 280C, EMV + 300 V

MW 177

Rel. Ab. 58

m/z 56 65 91 119 162
Mephedrone deriv. with 3ClEtClfor

MW 177

+ Cl—CO—O—CH₂—CCl₃

50 µL 2,2,2 - 3ClEtClfor : Ethyl acetate 3 : 7

80 °C, 15 min, to dryness

Residue reconst. with 50 µL Ethyl acetate

MW 351

Forensic Toxicology Use Only
GC/MS of Mephedrone - 3ClEtClfor

**GC/MS (same instrument, same conditions)**

- **Agilent 7890 II - 5975**
- **Full Scan EI (40-450 u)**
- **Inj. 1 µl, 250C, splitless (1 min)**
- **Carrier gas (He), 1 ml/min**

- **Agilent HP-5MS Ultra Inert column**
- **30 m x 0.25 mm x 0.25 µm**
- **50C (0.5 min), 200C a 30C/min, 300C (5 min) a 10C/min**
- **Interf. 280C, EMV + 300 V**

Forensic Toxicology Use Only
GC/MS of Mephedrone - 3ClEtClfor
U-HPLC/HR-Orbitrap-MS and A-R drugs

Why choosing the Orbitrap technology

✓ Non-targeting approach (Full Scan) and “all ions” MS/MS

✓ Retrospective detection of analytes without new MS runs

✓ High resolving power: 100,000 at m/z 200

✓ High mass accuracy: < 5ppm (e.c.), < 2ppm (i.c.)

✓ High mass accur. for M+0 and M+1, M+2, ....ions: EC assign. with high confidence

✓ 100,000 RP: full separation of $^{15}$N from M+1 C isotope, and $^{34}$S from M+2 C isotope

$\omega = \sqrt{\frac{k}{m/z}}$
U-HPLC/HR-Orbitrap-MS and A-R drugs

Thermo Scientific

Exactive HCD MS

Analytical Conditions

**U-HPLC**

- **LC:** U-HPLC Thermo Scientific Accela 1250
- **Injection:** 10 µL
- **Column:** Thermo Scientific Hypersil Gold
  - 50 x 2.1 mm x 1.9 mcm
- **Phase A:** H₂O, 0.05% HCOOH, 5 mM HCOONH₄ pH 5
- **Phase B:** ACN, 0.05% HCOOH
- **Flow:** 400 µL/min
- **Gradient:** 2% B, to 30% B in 5 min, to 98% B in 5 min, maint. 2 min, to 2% B in 4 min, maint. 2 min.
- **Temp.:** Column 40°C, samples 15°C

**HR-Orbitrap-MS**

- **Mass detect.:** Thermo Scientific “Exactive” Orbitrap HRMS
- **Source:** HESI-II Ion Max
- **Spray volt.:** 2.5 KV
- **Sheath gas:** N₂ set to 45 a. u.
- **Aux. gas:** N₂ set to 5 a. u.
- **Cap.Temp.:** 290°C
- **HESI Temp.:** 260°C
- **Polarity** Pos
- **Mass range:** 50-800 u
- **Mass resol.:** 25.000 (HCD on, 25 eV) or 100.000 (HCD off), No Lock Mass
U-HPLC / HR-Orbitrap-MS analytical strategy

1. High chromatographic resolution of analytes

2. Accurate mass measurements of MH$^+$ ionic species in full scan conditions

3. Study of MH$^+$ collision-induced product ions obtained in MS/MS experiments

4. Comparison of experimental and calculated MH$^+$ isotopic clusters

5. Examination of the isotopic fine structure (IFS) of the (M+1), (M+2), (M+3) isotopic peaks relative to the monoisotopic (M+0) peaks
U-HPLC / HR-Orbitrap-MS analytical strategy

1. High chromatographic resolution of analytes

"Classic" amphetamines

- Amphetamine
- Methamphetamine
- MDA
- MDMA
- MDEA
- MBDB

New A-R drugs

- Mephedrone
- MDPV
- 4-MEC
- Propylone
- 4-FA
- Cl-Ph-Pip
- Methylenedioxymethamphetamine (MDMA)

**Mass Spectral Data:**

- Mass: 180.1265-128.1337 F: FTMS (1,1) + p ESI Full ms [100.00-800.00]
- MS: ICIS MIX
- amf-amfisimid-jwh-metolon-propilon

**MS Data:**

- m/z: 136.1110-136.1124
- F: FTMS (1,1) + p ESI Full ms [100.00-800.00]
- MS: ICIS MIX
- amf-amfisimid-jwh-metolon-propilon
## U-HPLC / HR-Orbitrap-MS analytical strategy

### 2. Accurate mass measurements of MH⁺ ionic species

<table>
<thead>
<tr>
<th>Substance</th>
<th>Elemental composition</th>
<th>Exact mass</th>
<th>MH⁺</th>
<th>MH⁺</th>
<th>Δm at 100K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exact mass</td>
<td>Accurate mass</td>
<td>ppm</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>C₉H₁₃N</td>
<td>135,1048</td>
<td>136,1121</td>
<td>136,1120</td>
<td>-0.73</td>
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<tr>
<td>Methamphetamine</td>
<td>C₁₀H₁₅N</td>
<td>149,1204</td>
<td>150,1277</td>
<td>150,1276</td>
<td>-0.67</td>
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<tr>
<td>MDA</td>
<td>C₁₀H₁₃NO₂</td>
<td>179,0946</td>
<td>180,1019</td>
<td>180,1017</td>
<td>-1.11</td>
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<tr>
<td>MDMA</td>
<td>C₁₁H₁₅NO₂</td>
<td>193,1103</td>
<td>194,1176</td>
<td>194,1174</td>
<td>-1.03</td>
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<tr>
<td>MDEA</td>
<td>C₁₂H₁₇NO₂</td>
<td>207,1259</td>
<td>208,1332</td>
<td>208,1329</td>
<td>-1.44</td>
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<tr>
<td>MBDB</td>
<td>C₁₂H₁₇NO₂</td>
<td>207,1259</td>
<td>208,1332</td>
<td>208,1329</td>
<td>-1.44</td>
</tr>
<tr>
<td>Methylone</td>
<td>C₁₁H₁₃NO₃</td>
<td>207,0895</td>
<td>208,0967</td>
<td>208,0968</td>
<td>-0.48</td>
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<tr>
<td>Mephedrone</td>
<td>C₁₁H₁₅NO</td>
<td>177,1154</td>
<td>178,1226</td>
<td>178,1225</td>
<td>-0.56</td>
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<tr>
<td>MDPV</td>
<td>C₁₆H₂₁NO₃</td>
<td>275,1521</td>
<td>276,1594</td>
<td>276,1589</td>
<td>-1.81</td>
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<tr>
<td>4-MEC</td>
<td>C₁₂H₁₇NO</td>
<td>191,1310</td>
<td>192,1383</td>
<td>192,1381</td>
<td>-1.04</td>
</tr>
<tr>
<td>Propylone</td>
<td>C₁₂H₁₇NO</td>
<td>191,1310</td>
<td>192,1383</td>
<td>192,1381</td>
<td>-1.04</td>
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<tr>
<td>4F-amphetamine</td>
<td>C₉H₁₂FN</td>
<td>153,0954</td>
<td>154,1027</td>
<td>154,1025</td>
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<tr>
<td>CI-Ph-piperazine</td>
<td>C₁₀H₁₃ClN₂</td>
<td>196,0767</td>
<td>197,0840</td>
<td>197,0838</td>
<td>-1.01</td>
</tr>
</tbody>
</table>

Δm = (Accurate mass – Exact mass) / Exact mass x 10⁶

100,000 resolving power

Mass accuracy < 2 ppm (no lock mass)
U-HPLC / HR-Orbitrap-MS analytical strategy

3. Study of MH+ product ions from MS/MS experiments

MDEA and MBDB: both C12H17NO2, MH+ exact mass 208,1332
U-HPLC / HR-Orbitrap-MS analytical strategy

3. Study of MH\(^+\) product ions from MS/MS experiments

4-MEC and Propylone: both C\(_{12}\)H\(_{17}\)NO, MH\(^+\) exact mass 192,1383
Elements, their isotopes, and EC calc.

- Data acquired even by ultrahigh mass accuracy and mass resolution can be insufficient for calculating unique elemental compositions without information about isotope ratios.

- Natural occurring elements can be monoisotopic (F, Na, P, I) or polyisotopic (H, C, N, O, S, Cl, Br).

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Name</th>
<th>Mass of atom</th>
<th>% Abundance</th>
</tr>
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<tbody>
<tr>
<td>^1H</td>
<td>Hydrogen</td>
<td>1.007825</td>
<td>99.9885</td>
</tr>
<tr>
<td>^2H</td>
<td>Deuterium</td>
<td>2.014102</td>
<td>0.115</td>
</tr>
<tr>
<td>^3H</td>
<td>Tritium</td>
<td>3.016049</td>
<td>*</td>
</tr>
<tr>
<td>^12C</td>
<td>Carbon</td>
<td>12.000000</td>
<td>98.93</td>
</tr>
<tr>
<td>^13C</td>
<td></td>
<td>13.003355</td>
<td>1.07</td>
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<tr>
<td>^14C</td>
<td></td>
<td>14.003242</td>
<td>*</td>
</tr>
<tr>
<td>^14N</td>
<td>Nitrogen</td>
<td>14.003074</td>
<td>99.632</td>
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<tr>
<td>^15N</td>
<td></td>
<td>15.000109</td>
<td>0.368</td>
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<tr>
<td>^16O</td>
<td>Oxygen</td>
<td>15.994915</td>
<td>99.757</td>
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<tr>
<td>^17O</td>
<td></td>
<td>16.999132</td>
<td>0.038</td>
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<tr>
<td>^18O</td>
<td></td>
<td>17.999160</td>
<td>0.205</td>
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<tr>
<td>^32S</td>
<td>Sulphur</td>
<td>31.972071</td>
<td>94.93</td>
</tr>
<tr>
<td>^33S</td>
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<td>0.76</td>
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<td>^34S</td>
<td></td>
<td>33.967867</td>
<td>4.29</td>
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<tr>
<td>^36S</td>
<td></td>
<td>35.967081</td>
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<tr>
<td>^35Cl</td>
<td>Chlorine</td>
<td>34.968853</td>
<td>75.78</td>
</tr>
<tr>
<td>^37Cl</td>
<td></td>
<td>36.965903</td>
<td>24.22</td>
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<tr>
<td>^79Br</td>
<td>Bromine</td>
<td>78.918338</td>
<td>50.69</td>
</tr>
<tr>
<td>^81Br</td>
<td></td>
<td>80.916291</td>
<td>49.31</td>
</tr>
</tbody>
</table>


Forensic Toxicology Use Only
U-HPLC / HR-Orbitrap-MS analytical strategy

4. Comparison of measured and calc. MH⁺ isotopic clusters

4-MEC

MH⁺ measured isotopic cluster

MH⁺ calculated isotopic cluster

MDPV

MH⁺ measured isotopic cluster

MH⁺ calculated isotopic cluster
U-HPLC / HR-Orbitrap-MS analytical strategy

4. Comparison of measured and calc. $MH^+$ isotopic clusters

Cl-PhPip

Mephedrone

MH$^+$ measured isotopic cluster

MH$^+$ calculated isotopic cluster

MH$^+$ measured isotopic cluster

MH$^+$ calculated isotopic cluster
U-HPLC / HR-Orbitrap-MS analytical strategy

4. Comparison of measured and calc. MH\textsuperscript{+} isotopic clusters

**Relative Isotopic Abundance (RIA)**

\[
\frac{M+1}{M+0} \quad (^{13}\text{C} / ^{12}\text{C})
\]

\[
\text{RIA error (\%)} = \frac{\text{RIA}_{\text{meas}} - \text{RIA}_{\text{calc}}}{\text{RIA}_{\text{calc}}} \times 100
\]

<table>
<thead>
<tr>
<th>Substance</th>
<th>RIA error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylone</td>
<td>- 1.76</td>
</tr>
<tr>
<td>Mephedrone</td>
<td>0.02</td>
</tr>
<tr>
<td>MDPV</td>
<td>7.17</td>
</tr>
<tr>
<td>4-MEC</td>
<td>- 3.38</td>
</tr>
<tr>
<td>Propylone</td>
<td>- 0.02</td>
</tr>
<tr>
<td>4F-amphetamine</td>
<td>- 0.86</td>
</tr>
<tr>
<td>Cl-Ph-piperazine</td>
<td>- 1.72</td>
</tr>
</tbody>
</table>
U-HPLC / HR-Orbitrap-MS analytical strategy

5. Isotopic Fine Structure (IFS) of ion clusters

\[ \text{MH}^+ \text{ measured isotopic cluster} \]

- **M+0 (\text{MH}^+)**: 197,0838
- **M+1**: 198,0872
- **M+2**: 199,0809
- **M+3**: 200,0842

Relative Abundance

Forensic Toxicology Use Only
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+0 of Mephedrone (monoisotopic)

Measured at 100.000 RP

Calculated
U-HPLC/HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+1 of Mephedrone isotopic cluster (12%M+0)

Measured at 100,000 RP

Calculated
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+2 of Mephedrone isotopic cluster (0.5% M+0)

Measured at 100,000 RP

Calculated
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+3 of Mephedrone isotopic cluster (0.01% M+0)

![Diagram showing relative abundance of isotopic peaks with m/z values and molecular formulas C_{10}^{13}CH_{16}N^{18}O and C_{8}^{13}C_{3}H_{16}NO.]

Measured at 100,000 RP

Calculated
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+0 of Methylone (monoisotopic)

C<sub>11</sub>H<sub>14</sub>NO<sub>3</sub>

Measured at 100.000 RP

Calculated
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+1 of Methylone isotopic cluster (12% M+0)

Measured at 100.000 RP

Calculated
U-HPLC/HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+2 of Methylone isotopic cluster (0.5%M+0)

Measured at 100.000 RP

Calculated
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+3 of Methylone isotopic cluster (0.01% M+0)

Measured at 100.000 RP

Calculated
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+0 of Cl-Phenyl-Piperazine (monoisotopic)

**Measured at 100.000 RP**

**Calculated**
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+1 of Cl-Phenyl-Piperazine isotopic cluster (11% M+0)

Measured at 100.000 RP

Calculated
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+2 of Cl-Phenyl-Piperazine isotopic cluster (33% M+0)

Measured at 100.000 RP

Calculated
U-HPLC / HR-Orbitrap-MS analytical strategy

5. IFS of (M+1), (M+2), (M+3) isotopic peaks vs. (M+0) peaks

M+3 of Cl-Phenyl-Piperazine isotopic cluster (3%M+0)

Measured at 100.000 RP

Calculated
Conclusions

1. Efficient chromatographic separation of A-R drugs

2. Accurate mass measurements of MH+ ionic species with a mass accuracy < 2 ppm for all A-R drugs

3. Characteristic collision-induced product ions of MH+ ions with same ECs

4. Fully superimposable experimental and calculated MH+ isotopic clusters (RIA error < 10% for all new A-R drugs)

5. Isotopic fine structure of the (M+1), (M+2), (M+3) isotopic peaks completely in accordance with theoretical clusters

Elucidation of elemental composition and structural characteristics of new amphetamine-related designer drugs