"New Drugs 2014"

May 14-15, 2014, Rome

Capabilities of High Resolution/High Accuracy Mass Spectrometry in Structural Characterization of New Psychoactive Substances with Amphetamine-like Properties

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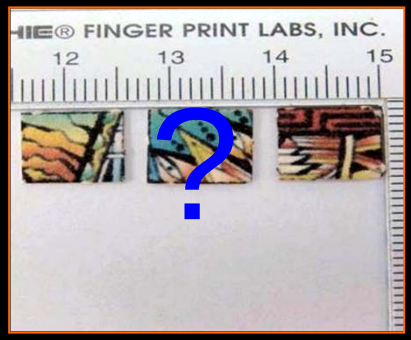
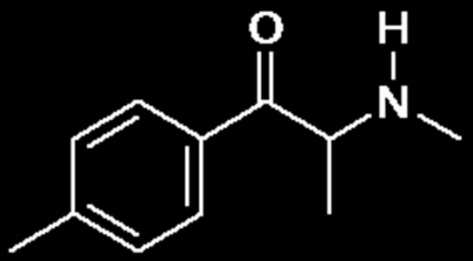
The LIATF experience with NPS



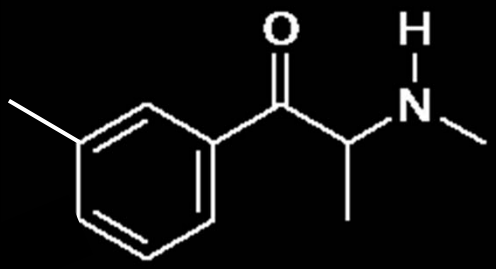
- Large number of police seizures and some intoxication cases
- Need to promptly obtain NPS structural characterization, in spite of poor availability of reference (p/m) standards



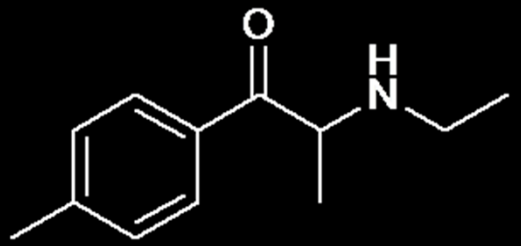

25 "A-R drugs" identified at LIAFT



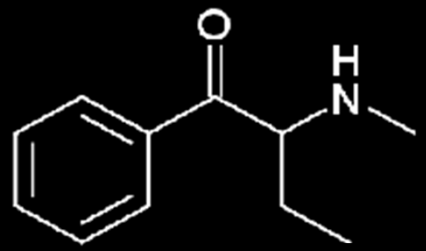
Mephedrone (4-MMC)



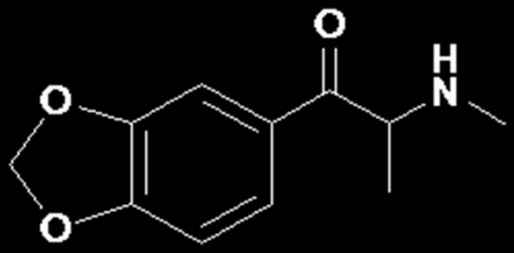
3-MMC



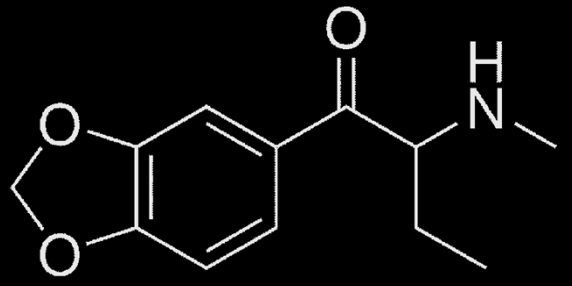
4-MEC



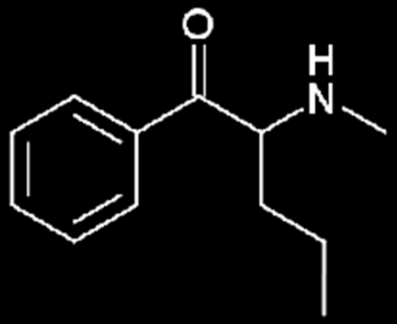
Buphedrone



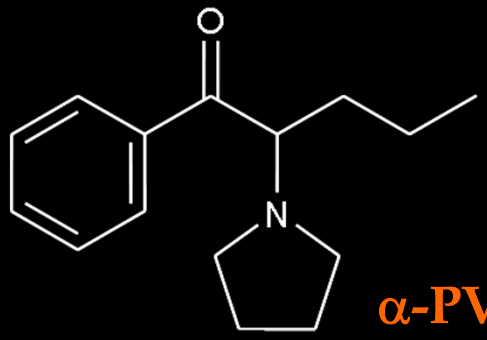
Methylone



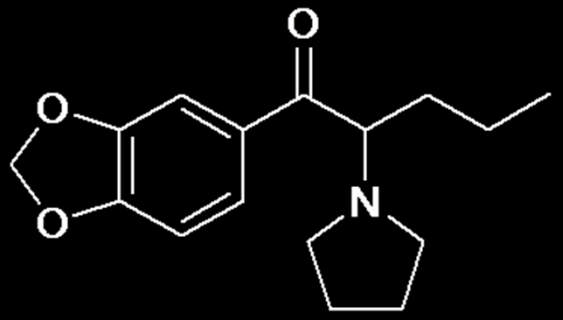
Butylone



Pentedrone



α-PVP



MDPV

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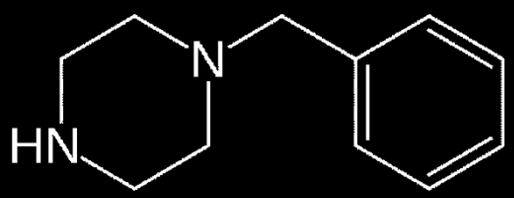
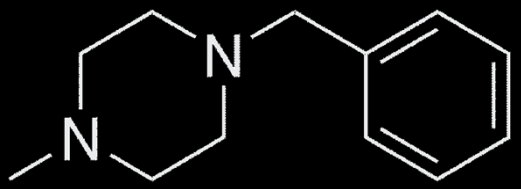
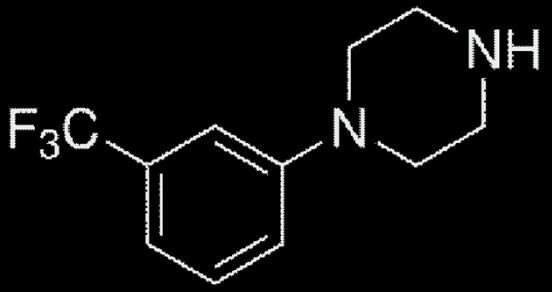


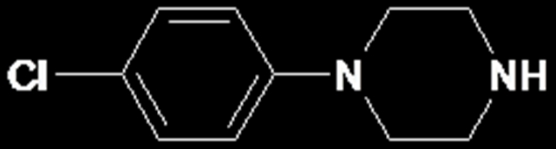
Benzyl-Piperazine



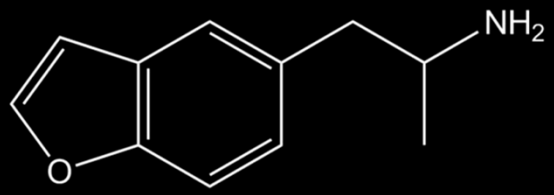
Benzyl-Methyl-Piperazine



TriF-Methyl-Phenyl-Piperazine

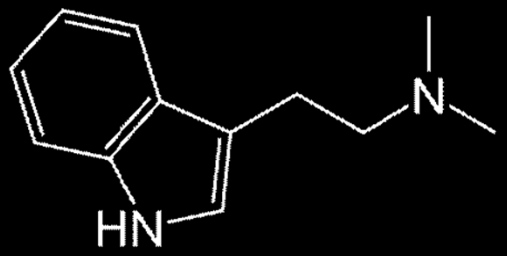


Cl-Phenyl-Piperazine



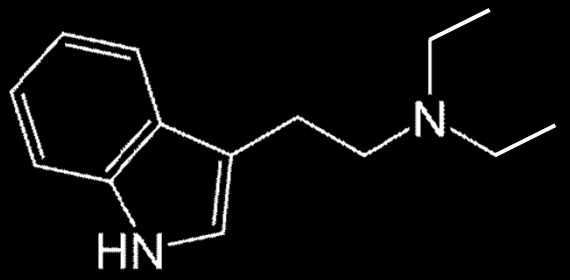
5-APB

Methoxethamine



DMT

4-AcO-DMT



DET

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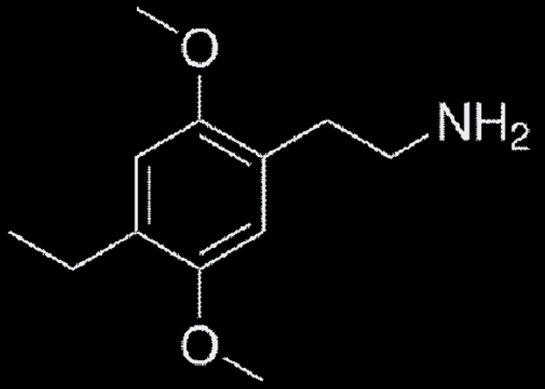
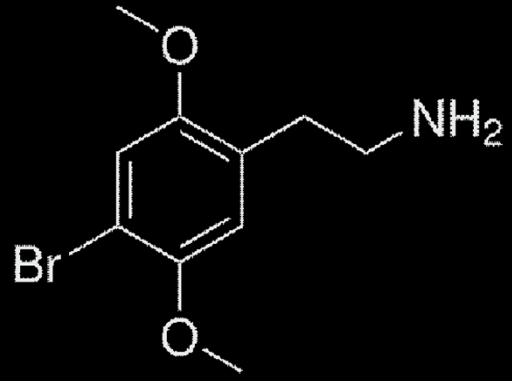


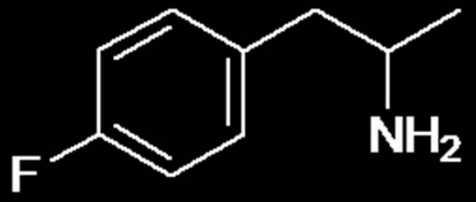
2C-E



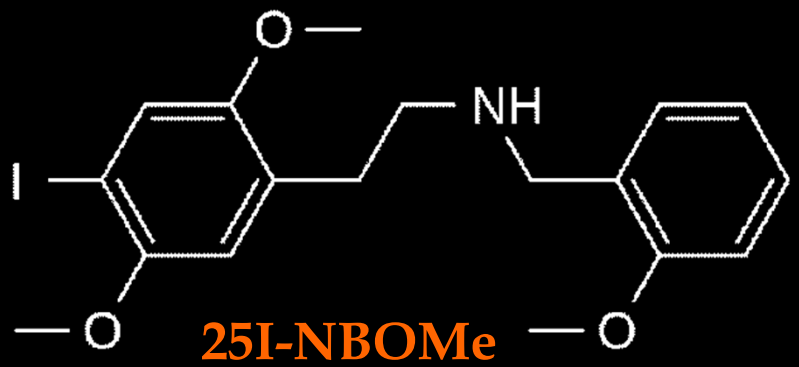
2C-B

bk-2C-B

2,4,5-TMA



4-FA



25I-NBOMe

25B-NBOMe

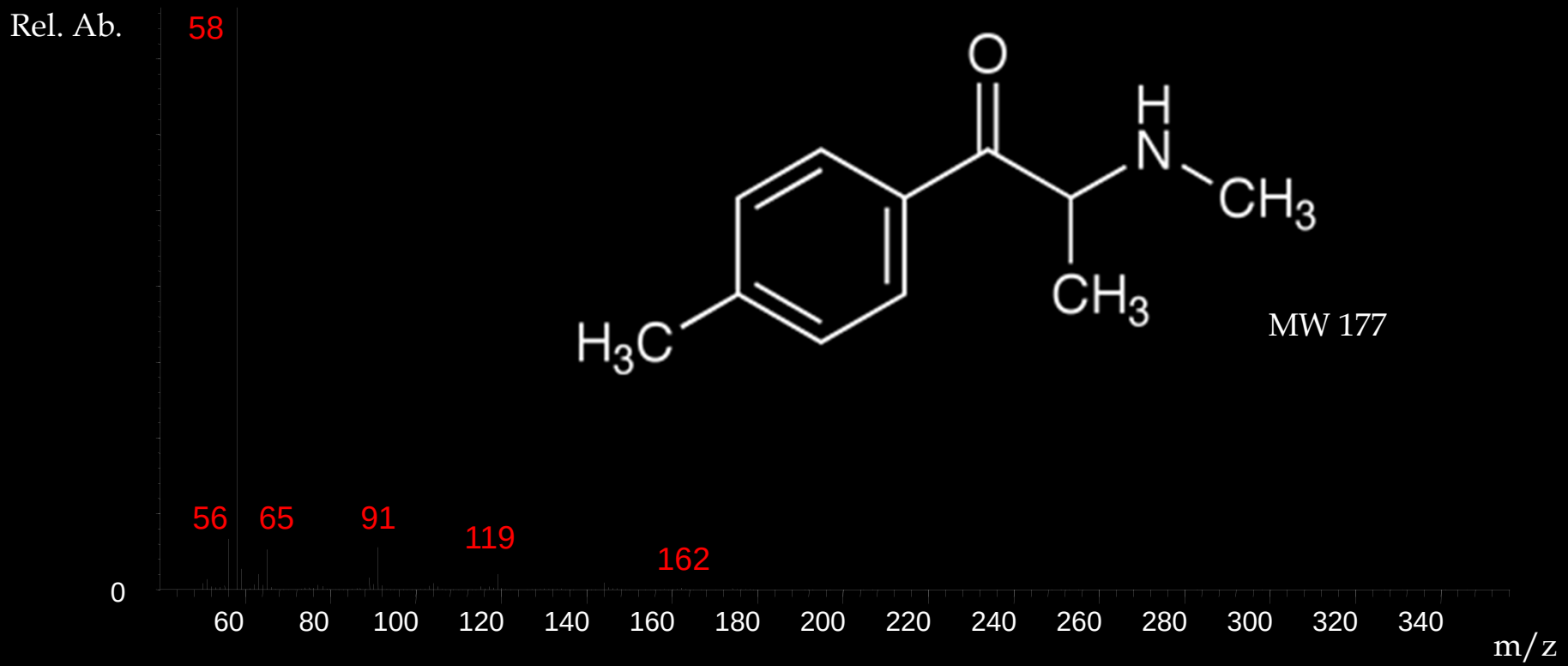
Analytical strategy to obtain the structural characterization of new A-R drugs

GC-MS
of underivatized drugs

GC-MS
after derivatization with 3Cl-ethyl-chloroformate

UHPLC-HRMS
(High resolution/high accuracy MS)

GC/MS - Mephedrone

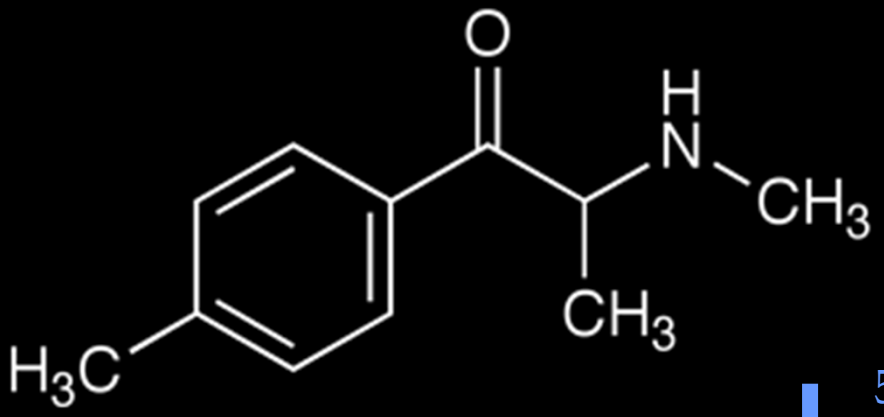


GC/MS

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

Mephedrone deriv. with 3ClEtClfor



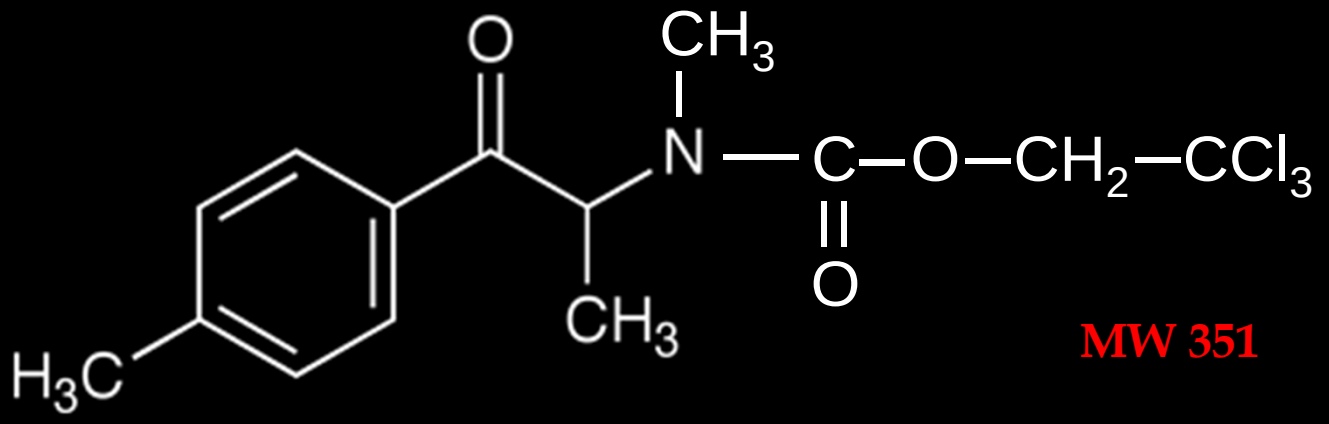
MW 177

+ Cl—CO—O—CH_2—CCl_3

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

80 ° C, 15 min, to dryness

Residue reconst. with 50 µL Ethyl acetate

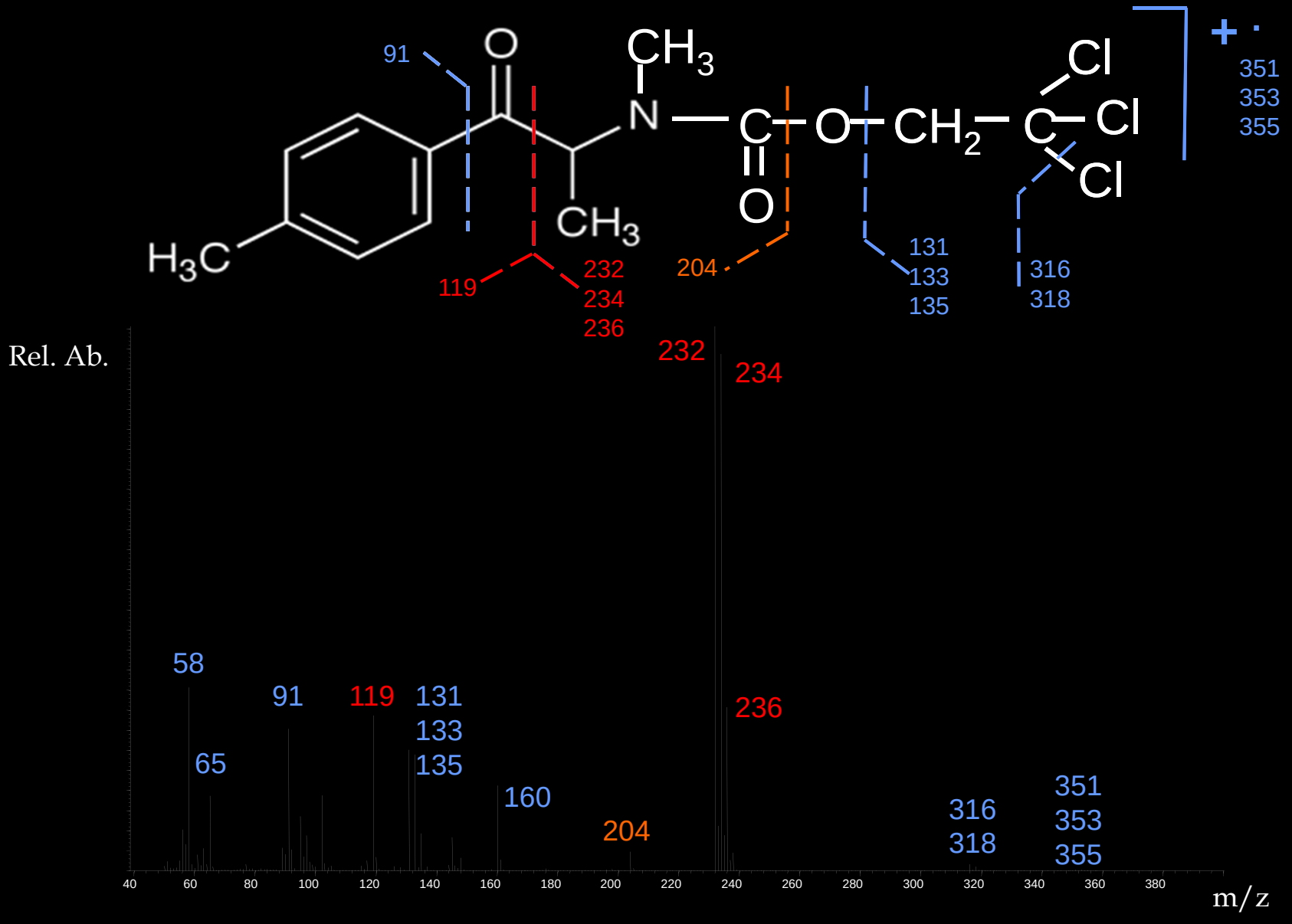


MW 351

1) G. Frison et al. *Rapid Commun. Mass Spectrom.* 2005, **19**, 919–927

2) G. Frison et al. *Rapid Commun. Mass Spectrom.* 2011, **25**, 387–390

GC/MS of Mephedrone - 3ClEtClfor

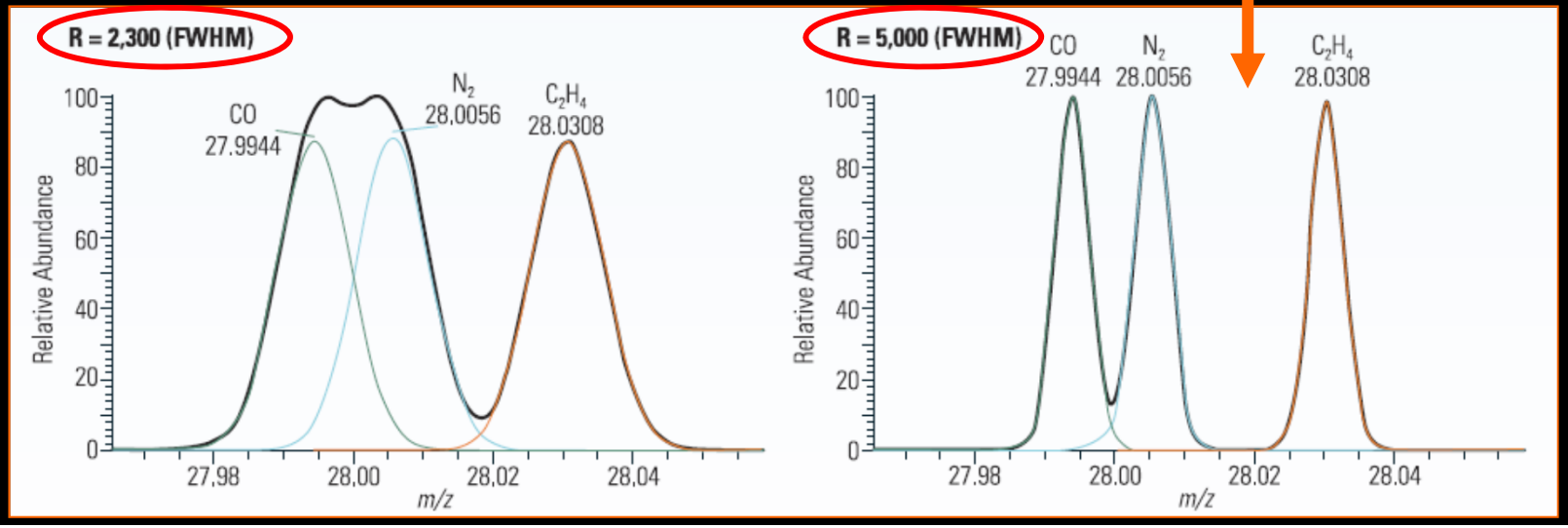


High resolution/High accuracy MS

Mass resolution = $m_1 / m_1 - m_2$

Low resol. MS	High resol. MS
$^{1}H = 1$	$^{1}H = 1.007825$
$^{12}C = 12$	$^{12}C = 12.000000$
$^{16}O = 16$	$^{16}O = 15.994915$
$^{14}N = 14$	$^{14}N = 14.003074$

Ions at m/z 28	Exact mass
CO^+	27.994915
N_2^+	28.006158
CH_2N^+	28.018723
$C_2H_4^+$	28.031299



High resolution/High accuracy MS

Mass accuracy

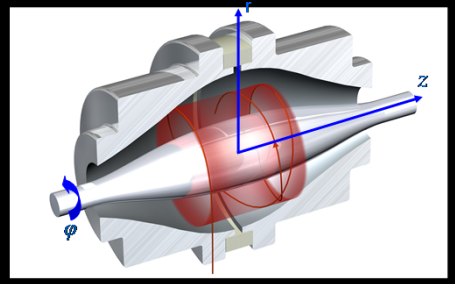
The ability to "measure the exact mass"

(accurate mass) of a monoisotopic ion

$$\Delta m = \frac{\text{(Accurate mass – Exact mass)}}{\text{Exact mass}} \cdot 10^6$$

High resolution
High accuracy MS → Elemental Composition (EC)!

U-HPLC/HR-Orbitrap-MS



$$\omega = \sqrt{\frac{k}{m/z}}$$

- High mass resolution: 100.000 at *m/z* 200
- High mass accuracy: < 5 ppm (ext. cal.)
  < 2 ppm (int.cal.)

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:	H_2O, 0,05% HCOOH, 5 mM $HCOONH_4$,pH 5
Phase B:	ACN, 0,05% HCOOH
Flow:	400 µL/min
Gradient:	2% B, to 50% B in 8 min, to 98% B in 5 min, maint. 2 min, to 2% B in 2 min, maint. 2 min.
Temp.:	Column 40° C, samples 15° C

HR-Orbitrap-MS

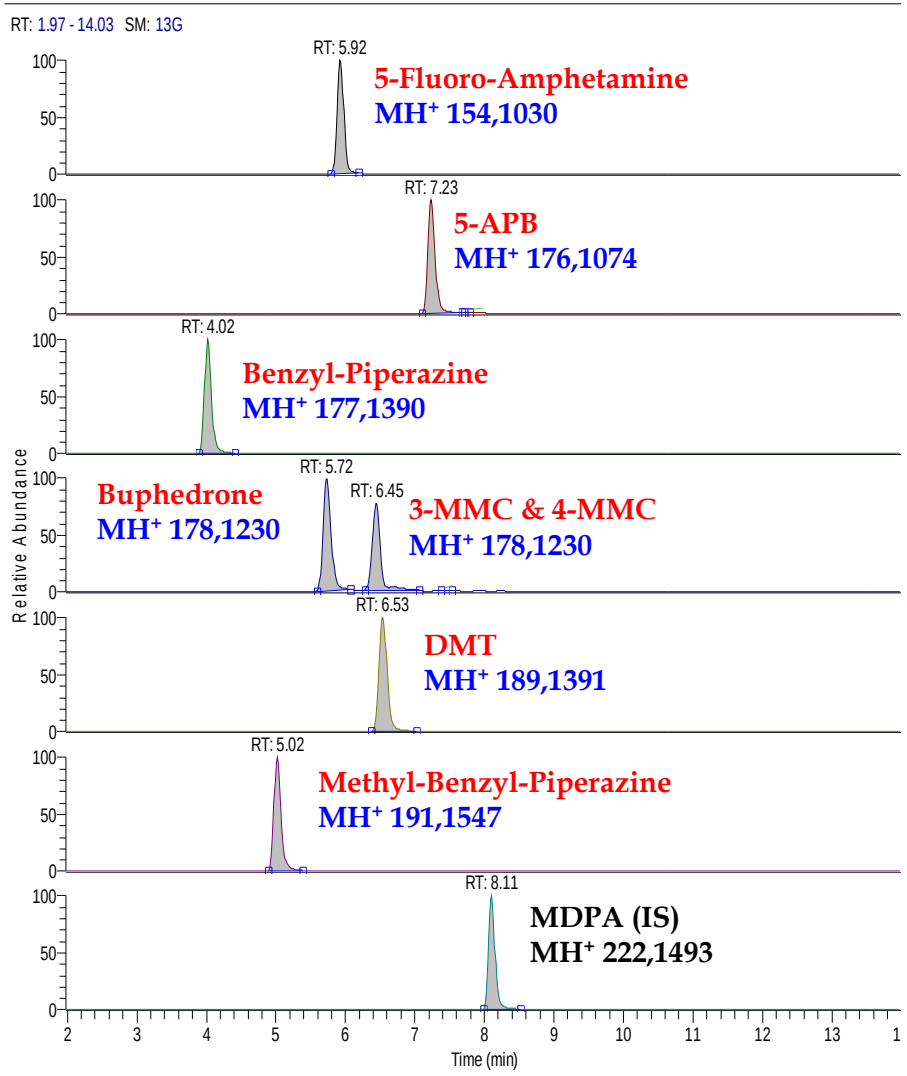
Mass detect.:	Thermo Scientific "Exactive" Orbitrap HRMS
Source:	HESI-II Ion Max
Spray volt.:	2,5 KV
Sheath gas:	N_2 set to 45 a. u.
Aux. gas:	N_2 set to 5 a. u.
Cap.Temp.:	290 ° C
HESI Temp.:	260° C
Polarity	Pos
Mass range:	50-800 u
Mass resol.:	25.000 (HCD on, 25 eV) or 100.000 (HCD off), No Lock Mass

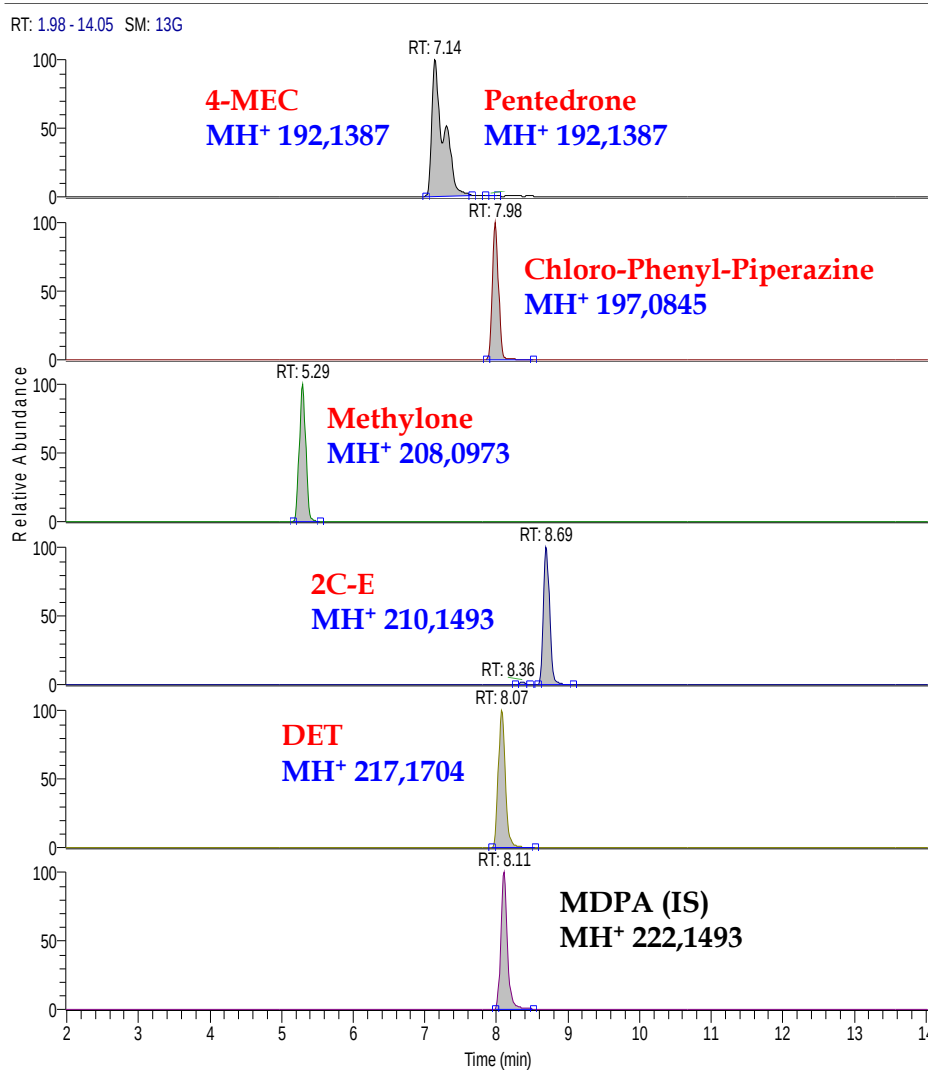
U-HPLC / HR-Orbitrap-MS analytical strategy

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 patterns
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

1. High chromatographic resolution of analytes

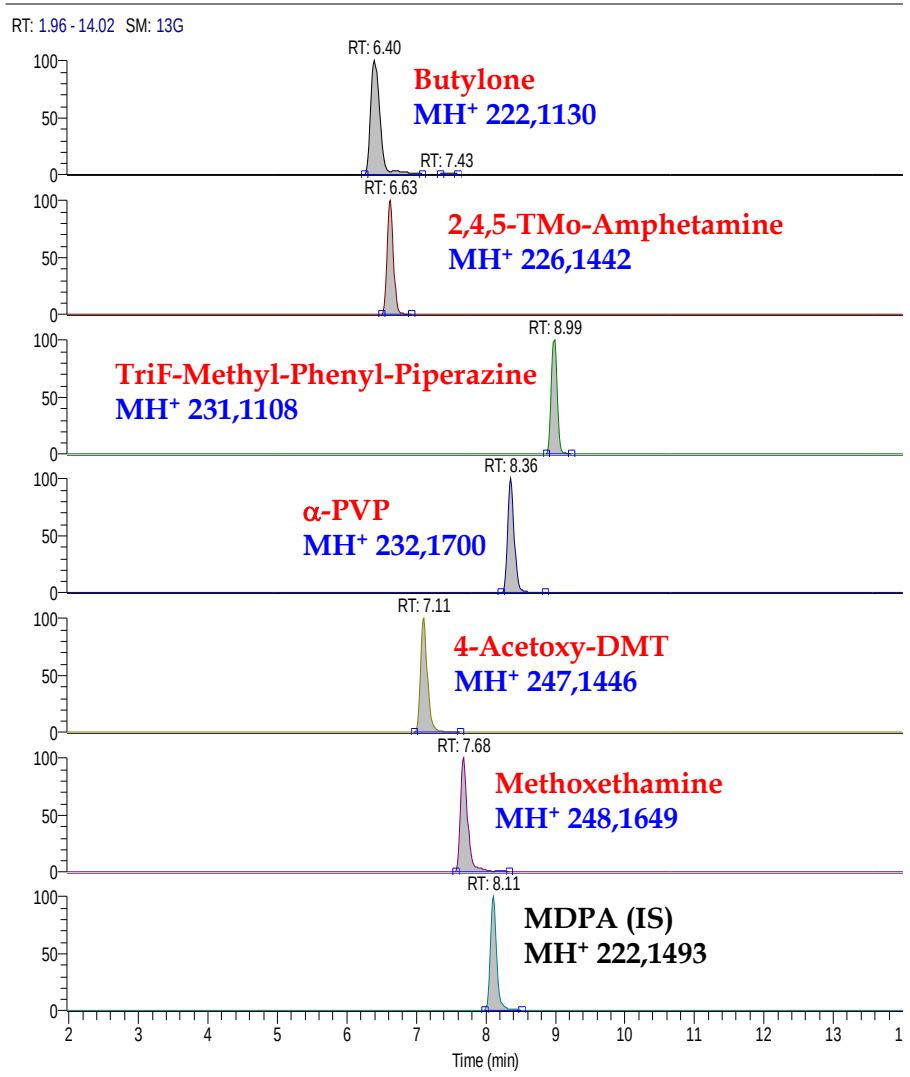
HR ion chromatograms of 25 AR-drugs

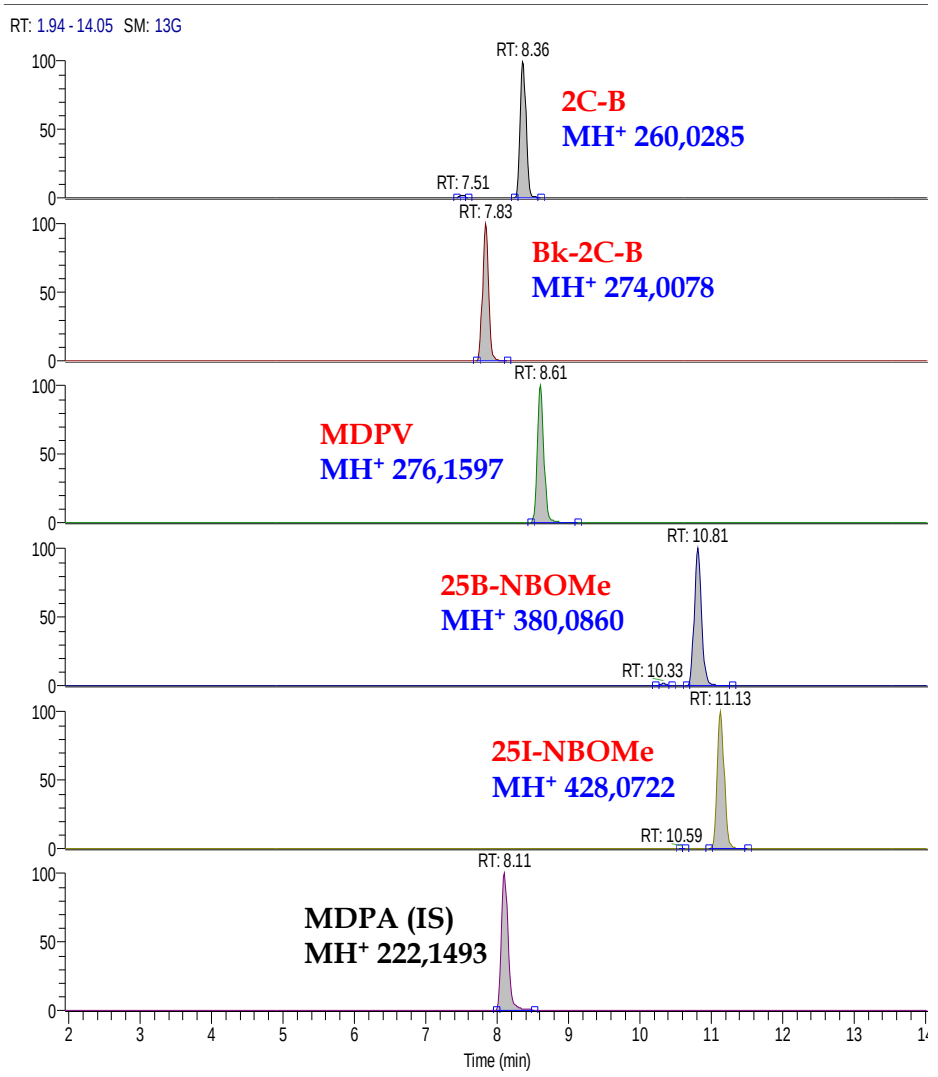




1. High chromatographic resolution of analytes

HR ion chromatograms of 25 AR-drugs





1. High chromatographic resolution of analytes

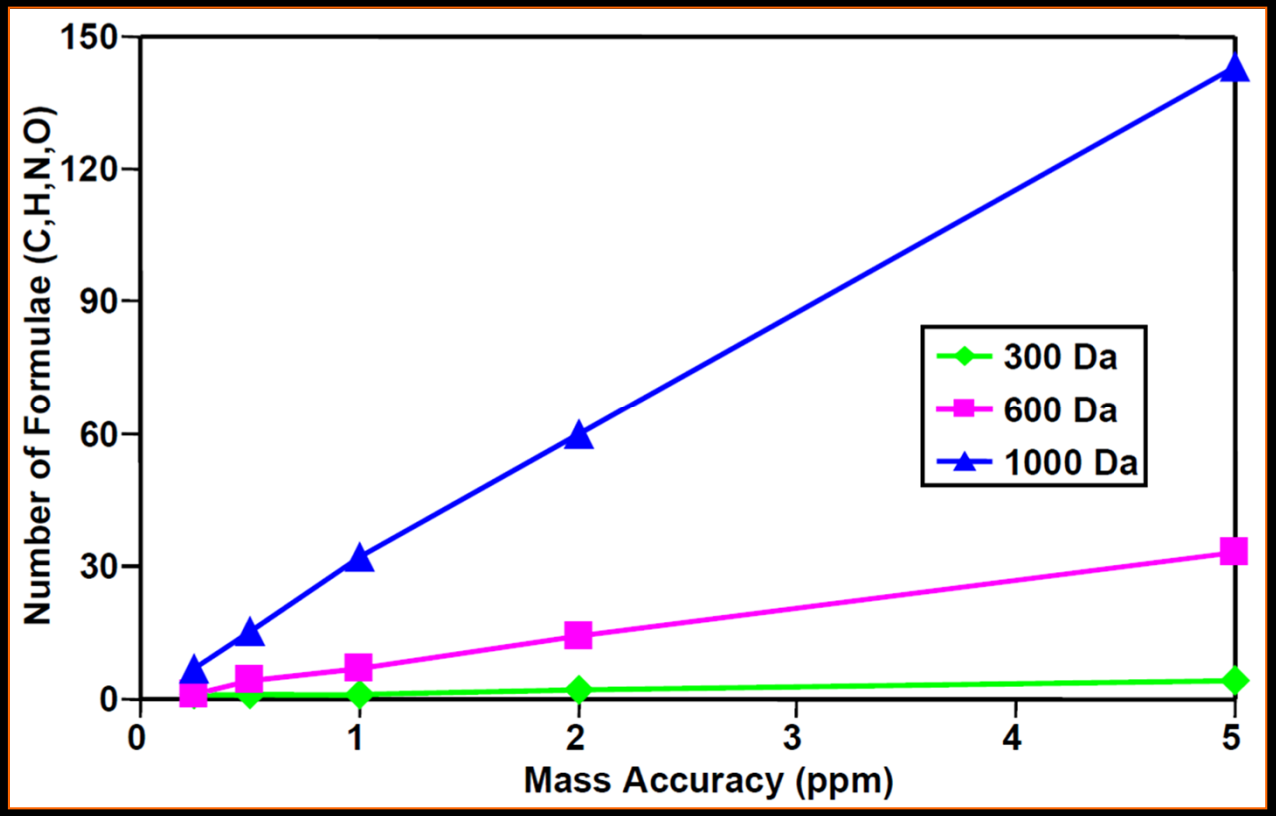
High chromatographic resolution and on-going optimization of analytical conditions help to minimize co-elution and ion suppression

" … Co-elution and ion suppression significantly hinder the ability to make an accurate mass mesurement and, in some cases, become more important than the instrument mass resolution and mass measurement accuracy …"

Croley TR et al. The chromatographic role in high resolution mass spectrometry for non-targeted analysis. *J Am. Soc. Mass Spectrom*, 2012, **23**, 1569-1578.

High resolution/High accuracy MS

$$\Delta m = \frac{(\text{Accurate mass} - \text{Exact mass})}{\text{Exact mass}} \cdot 10^6$$



The better is the measure of exact mass the lower is the number of EC formulae!

2. MH^+ accurate mass measurements

Acc. mass meas. of MH^+ ionic species for 25 AR-drugs at 100.000 RP (no lock mass)

Substance	Elemental composition	Exact mass	MH^+ Exact mass	MH^+ Accurate mass	Δm at 100K (ppm)
4-Fluoro-Amphetamine	$C_9H_{12}FN$	153,0954	154,1027	154,1030	1,94
5-APB	$C_{11}H_{13}NO$	175,0997	176,1070	1761074	2,27
BenzylPiperazine	$C_{11}H_{16}N_2$	176,1313	177,1386	177,1390	2,25
Mephedrone (4-MMC)	$C_{11}H_{15}NO$	177,1153	178,1226	178,1230	2,24
3-MMC	$C_{11}H_{15}NO$	177,1153	178,1226	178,1230	2,24
Buphedrone	$C_{11}H_{15}NO$	177,1153	178,1226	178,1230	2,24
Dimethyltryptamine (DMT)	$C_{12}H_{16}N_2$	188,1313	189,1386	189,1391	2,64
Methyl-Benzyl-Piperazine	$C_{12}H_{18}N_2$	190,1470	191,1543	191,1547	2,09
4-MEC	$C_{12}H_{17}NO$	191,1310	192,1383	192,1387	2,08
Pentedrone	$C_{12}H_{17}NO$	191,1310	192,1383	192,1387	2,08
Chloro-Phenyl-Piperazine	$C_{10}H_{13}ClN_2$	196,0767	197,0840	197,0845	2,53
Methylone	$C_{11}H_{13}NO_3$	207,0895	208,0968	208,0973	2,40
2C-E	$C_{12}H_{19}NO_2$	209,1415	210,1489	210,1493	1,90

Δm = (Accurate mass – Exact mass) / Exact mass x 10^6

2. MH^+ accurate mass measurements

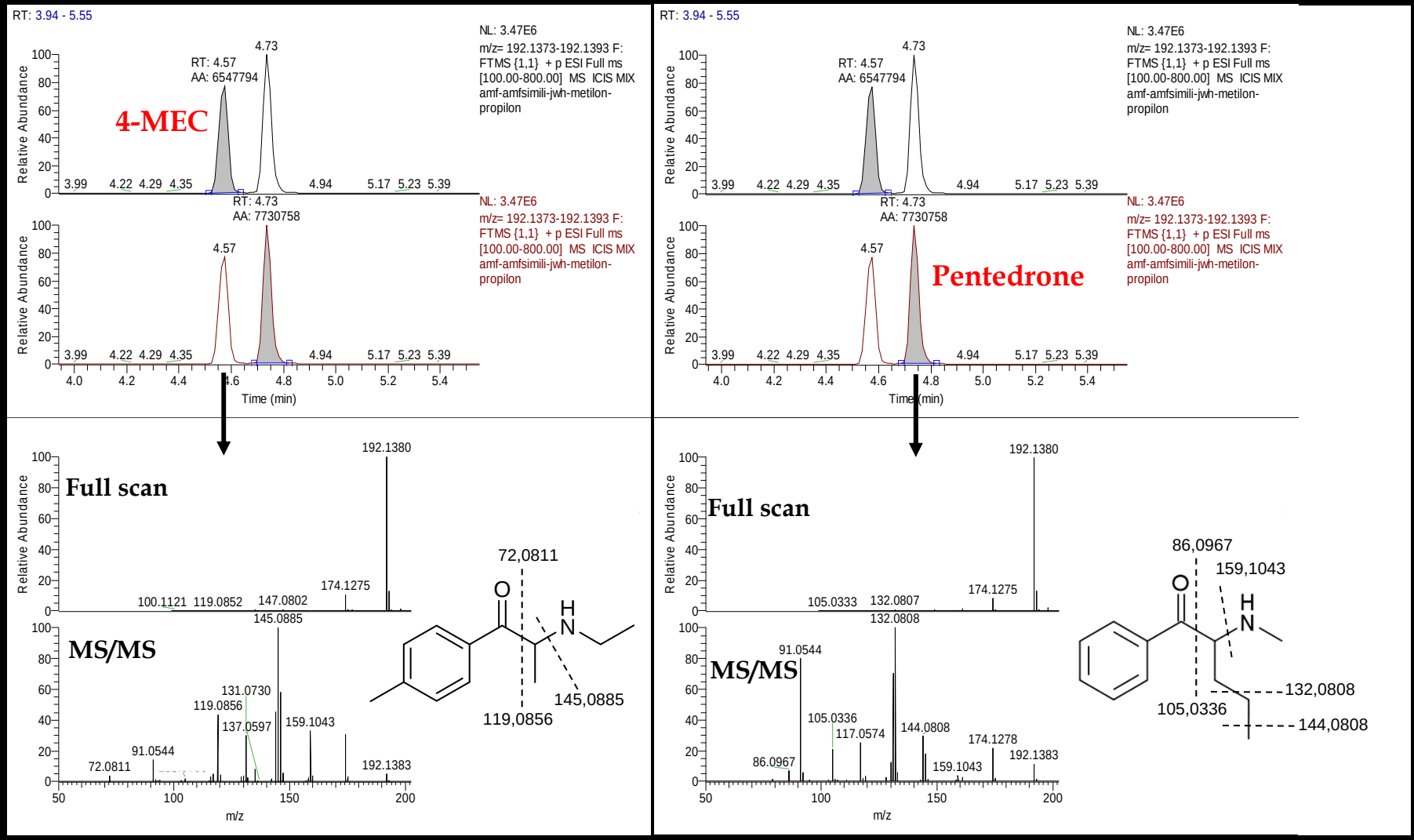
Acc. mass meas. of MH^+ ionic species for 25 AR-drugs at 100.000 RP (no lock mass)

Substance	Elemental composition	Exact mass	MH^+ Exact mass	MH^+ Accurate mass	Δm at 100K (ppm)
Diethyltryptamine (DET)	$C_{14}H_{20}N_2$	216,1626	217,1699	217,1704	2,30
Butylone	$C_{12}H_{15}NO_3$	221,1052	222,1125	222,1130	2,25
2,4,5-Trimethoxyamphetamine	$C_{12}H_{19}NO_3$	225,1365	226,1438	226,1442	1,76
Trifluoromethylphenylpiperazine	$C_{11}H_{13}F_3N_2$	230,1031	231,1104	231,1108	1,73
α-PVP	$C_{15}H_{21}NO$	231,1623	232,1696	232,1700	1,72
4-AcO-DMT	$C_{14}H_{18}N_2O_2$	246,1368	247,1441	247,1446	2,02
Methoxethamine	$C_{15}H_{21}NO_2$	247,1572	248,1645	248,1649	1,61
2C-B	$C_{10}H_{14}BrNO_2$	259,0208	260,0281	260,0285	1,54
Bk-2C-B	$C_{10}H_{12}BrNO_3$	273,0001	274,0074	274,0078	1,82
MDPV	$C_{16}H_{21}NO_3$	275,1521	276,1594	276,1597	1,08
25B-NBOMe	$C_{18}H_{22}BrNO_3$	379,0783	380,0856	380,0860	1,05
25I-NBOMe	$C_{18}H_{22}INO_3$	427,0646	428,0719	428,0722	1,17

Δm = (Accurate mass – Exact mass) / Exact mass x 10^6

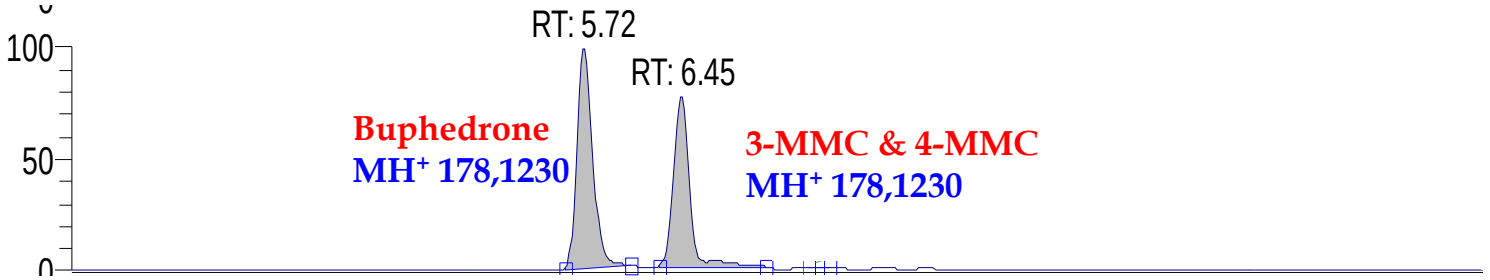
3. Study of MH+ product ions (MS/MS)

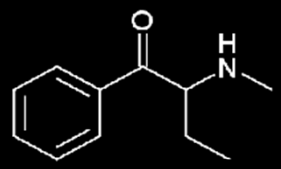
4-MEC and Pentedrone: both $C_{12}H_{17}NO$, MH^+ exact mass 192,1383

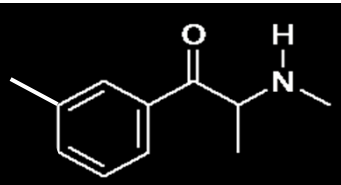


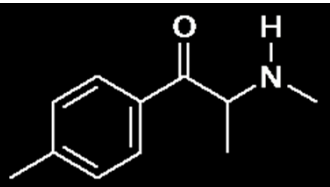
3. Study of MH+ product ions (MS/MS)

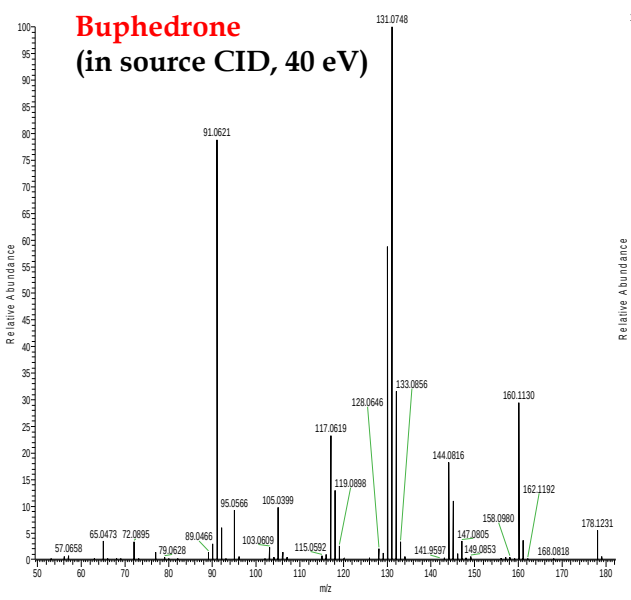
Buphedrone, 3-MMC, 4-MMC: all $C_{11}H_{15}NO$, MH^+ exact mass 178,1226

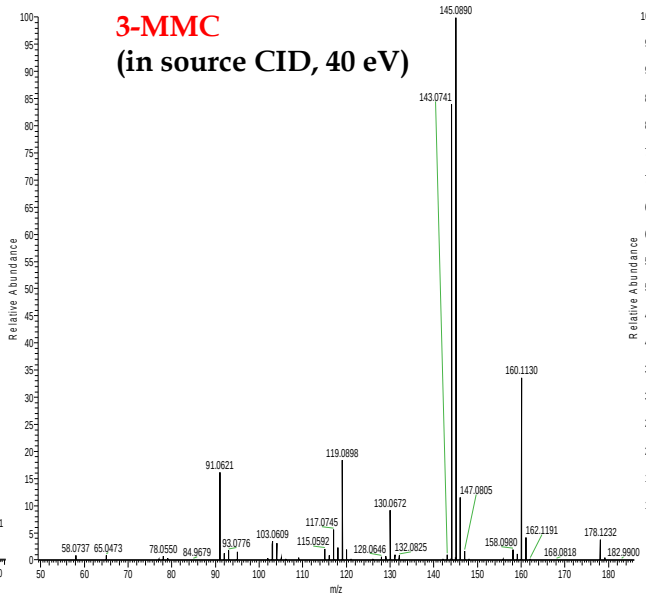


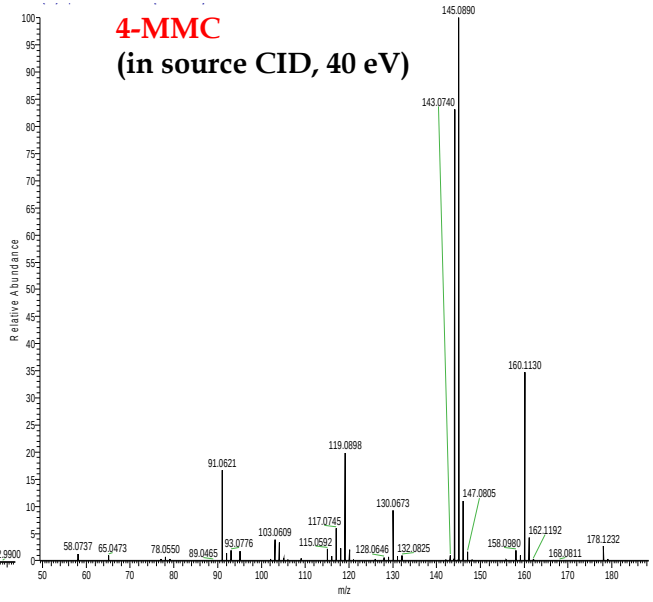






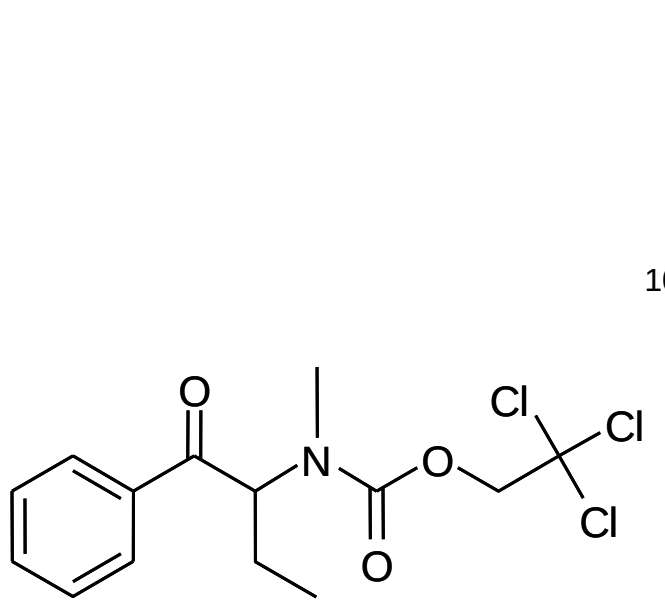




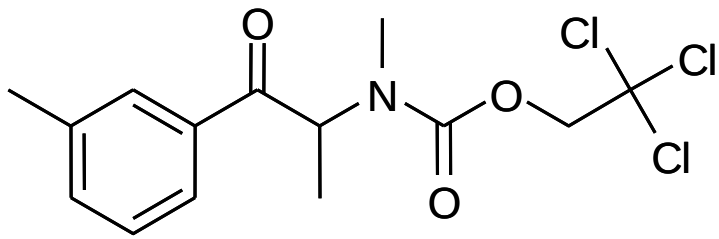


LC/HR-MS & GC/MS (after 3ClEtClfor deriv.)

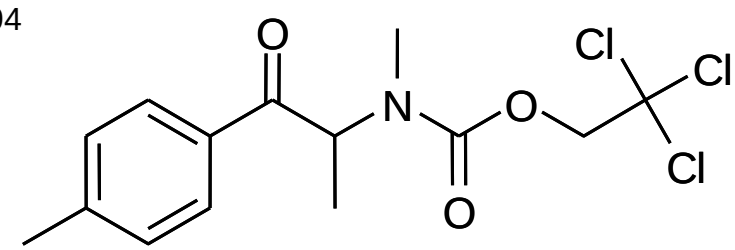
Buphedrone, 3-MMC, 4-MMC deriv. with 2,2,2-trichloroethyl chloroformate



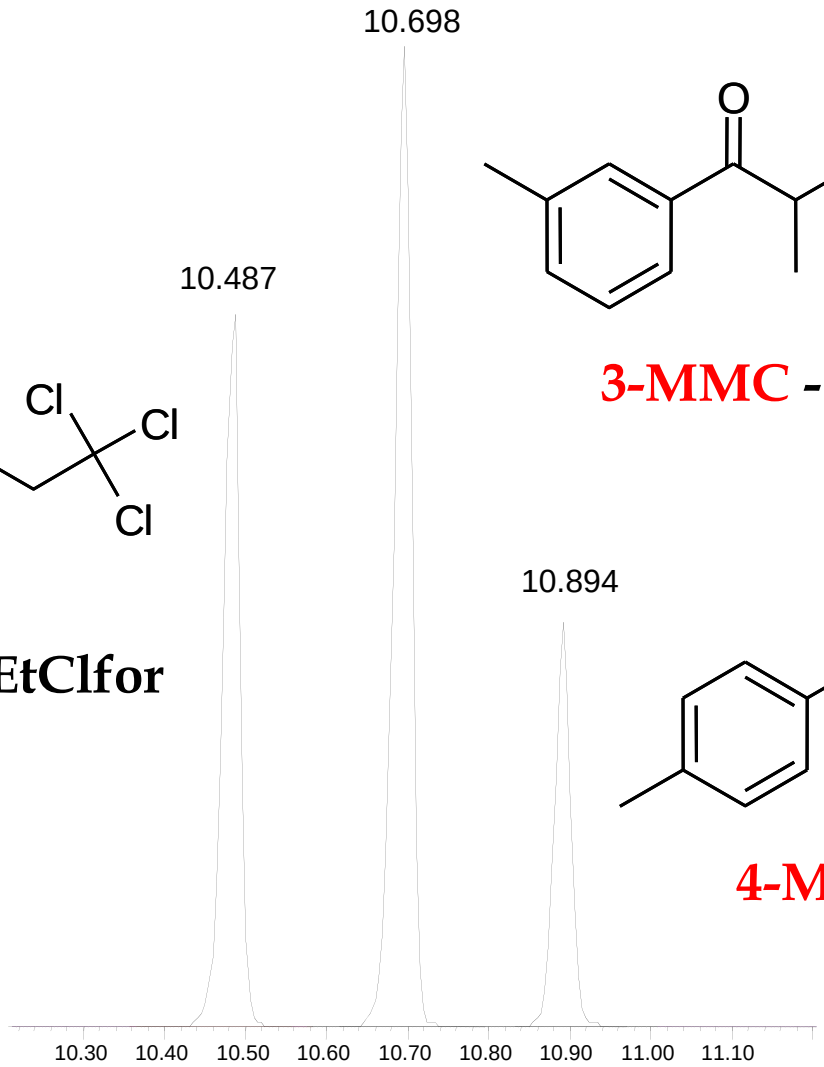
Buphedrone - 3ClEtClfor



3-MMC - 3ClEtClfor



4-MMC - 3ClEtClfor



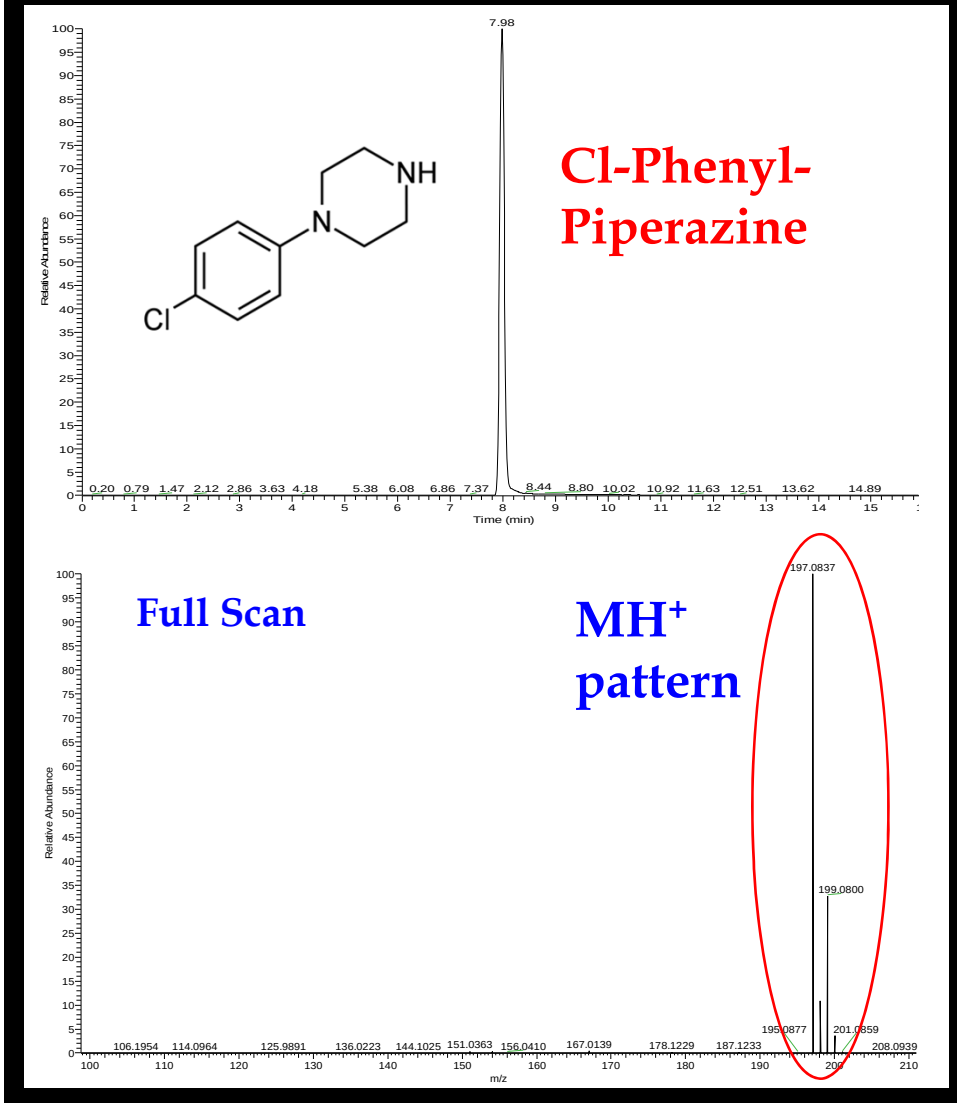
Elements, their isotopes, and EC calc.

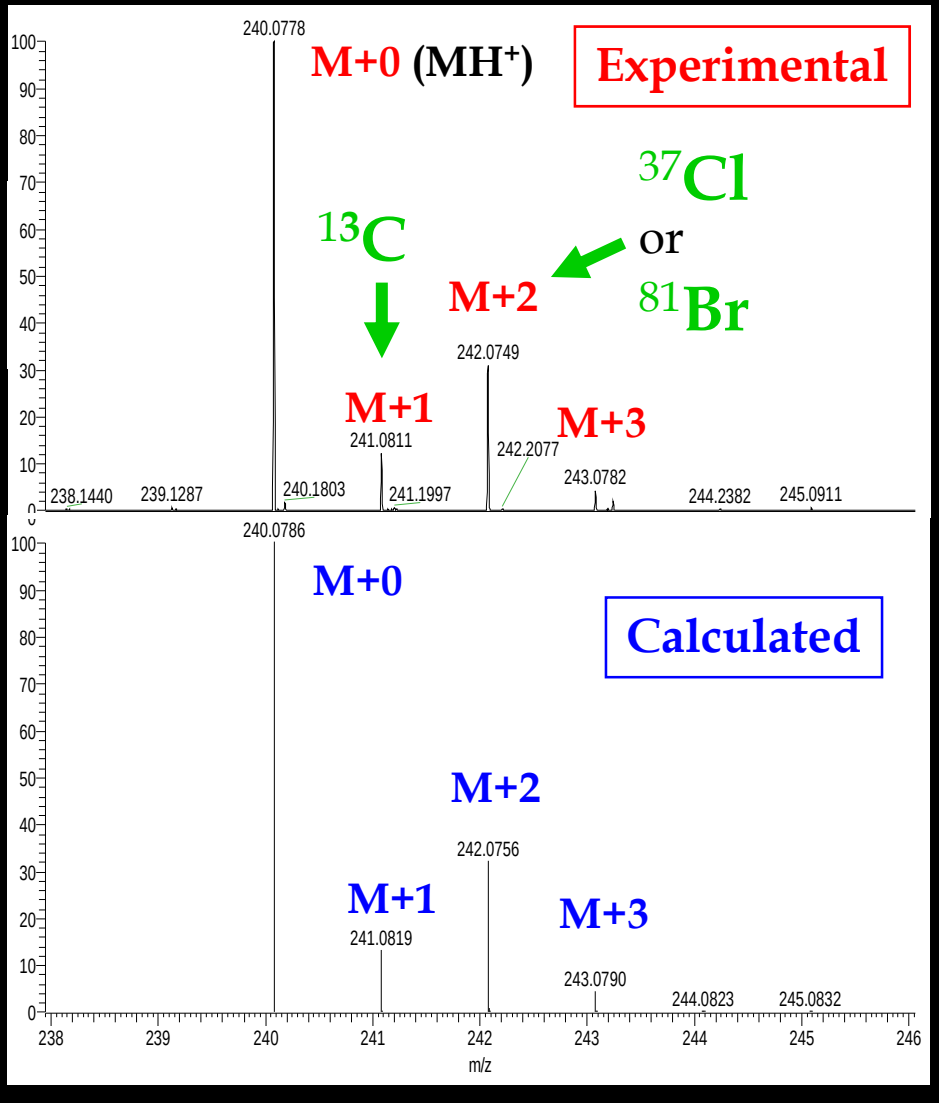
- ✓ Data acquired even by ultrahigh mass accuracy and mass resolution can be insufficient for calculating unique elemental compositions without information about isotope ratios
- ✓ Natural occurring elements can be monoisotopic (F, Na, P, I) or polyisotopic (H, C, N, O, S, Cl, Br)

Symbol	Name	Mass of atom	% Abundance
^{1}H	Hydrogen	1.007825	99.9885
^{2}H	Deuterium	2.014102	0.115
^{3}H	Tritium	3.016049	*
^{12}C	Carbon	12.000000	98.93
^{13}C		13.003355	1.07
^{14}C		14.003242	*
^{14}N	Nitrogen	14.003074	99.632
^{15}N		15.000109	0.368
^{16}O	Oxygen	15.994915	99.757
^{17}O		16.999132	0.038
^{18}O		17.999160	0.205
^{32}S	Sulphur	31.972071	94.93
^{33}S		32.971458	0.76
^{34}S		33.967867	4.29
^{36}S		35.967081	0.02
^{35}Cl	Chlorine	34.968853	75.78
^{37}Cl		36.965903	24.22
^{79}Br	Bromine	78.918338	50.69
^{81}Br		80.916291	49.31

De Laeter JR, Bohlke JK, De Bievre P, Hidaka H, Peiser HS, Rosman KJR, Taylor PDP: **Atomic weights of the elements: Review 2000 - (IUPAC technical report).** *Pure and Applied Chemistry* 2003, **75(6):**683-800.

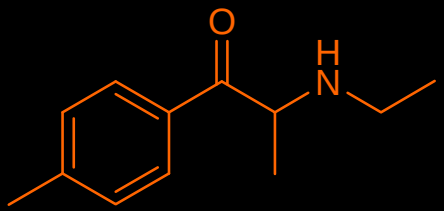
4. MH^+ isotopic patterns





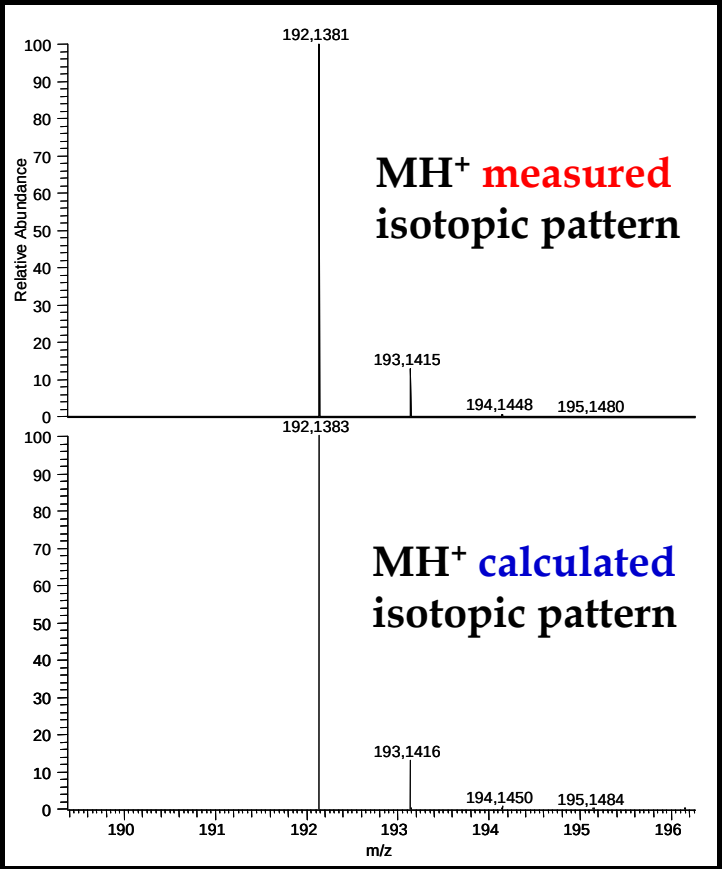
4. MH^+ isotopic patterns

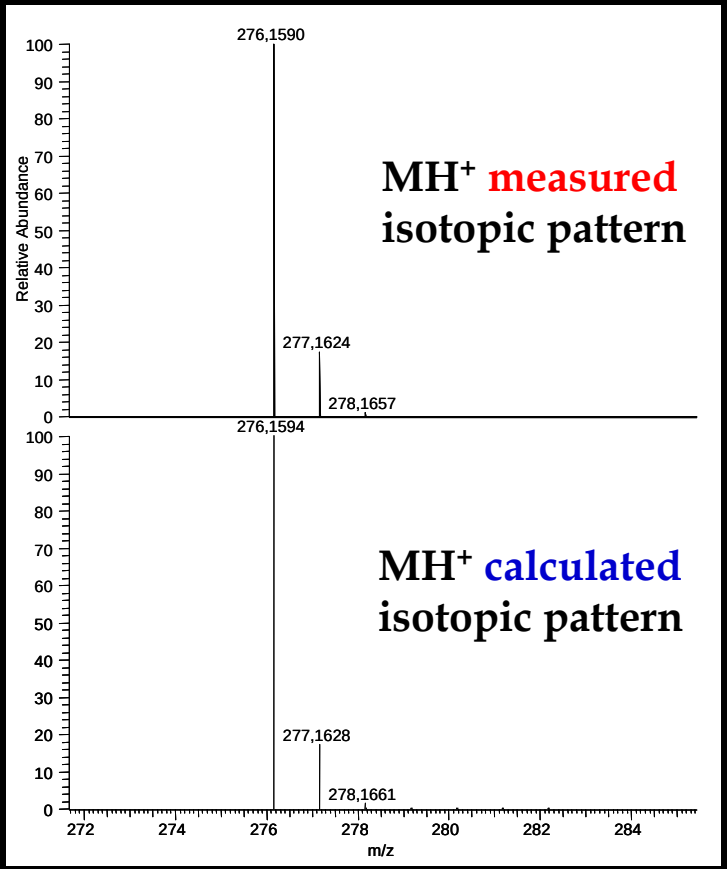
Comparison of measured and calculated MH^+ isotopic patterns



4-MEC

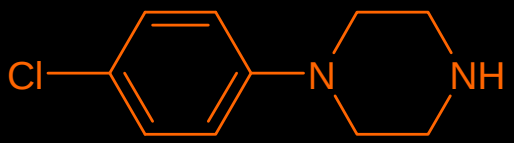
MDPV



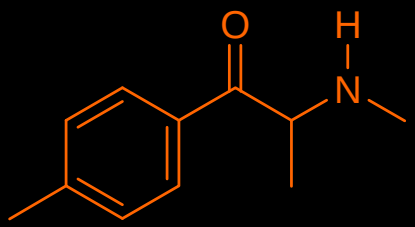


4. MH^+ isotopic patterns

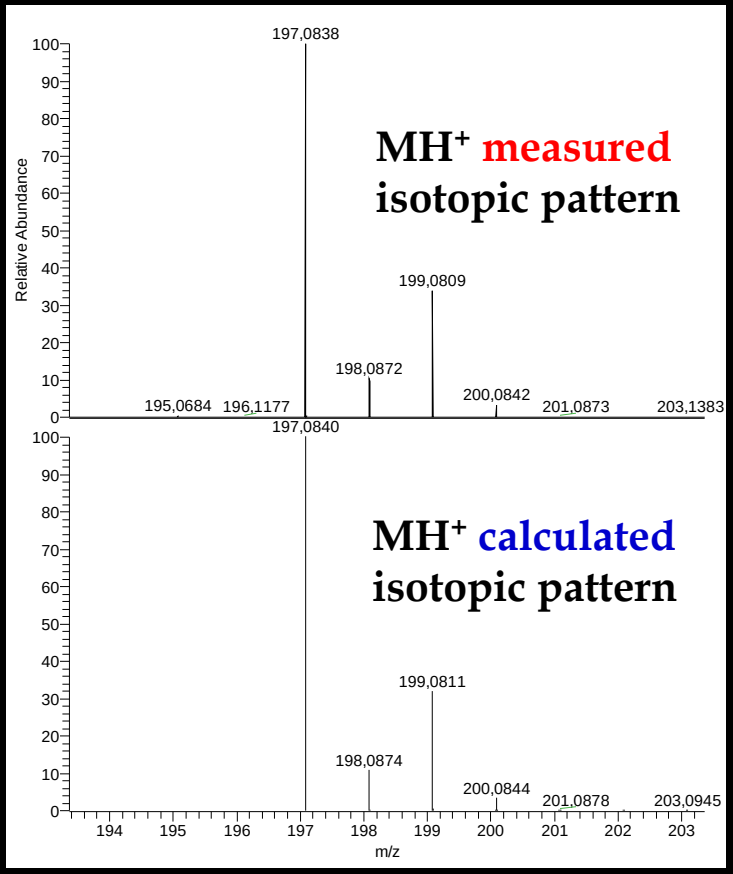
Comparison of measured and calculated MH^+ isotopic patterns

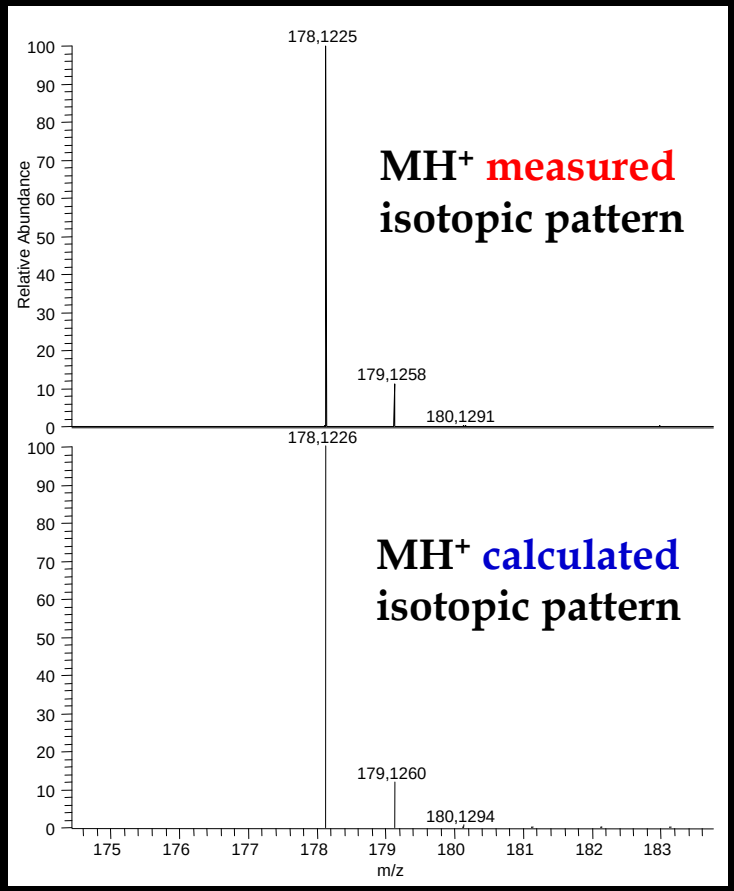


Cl-PhPip



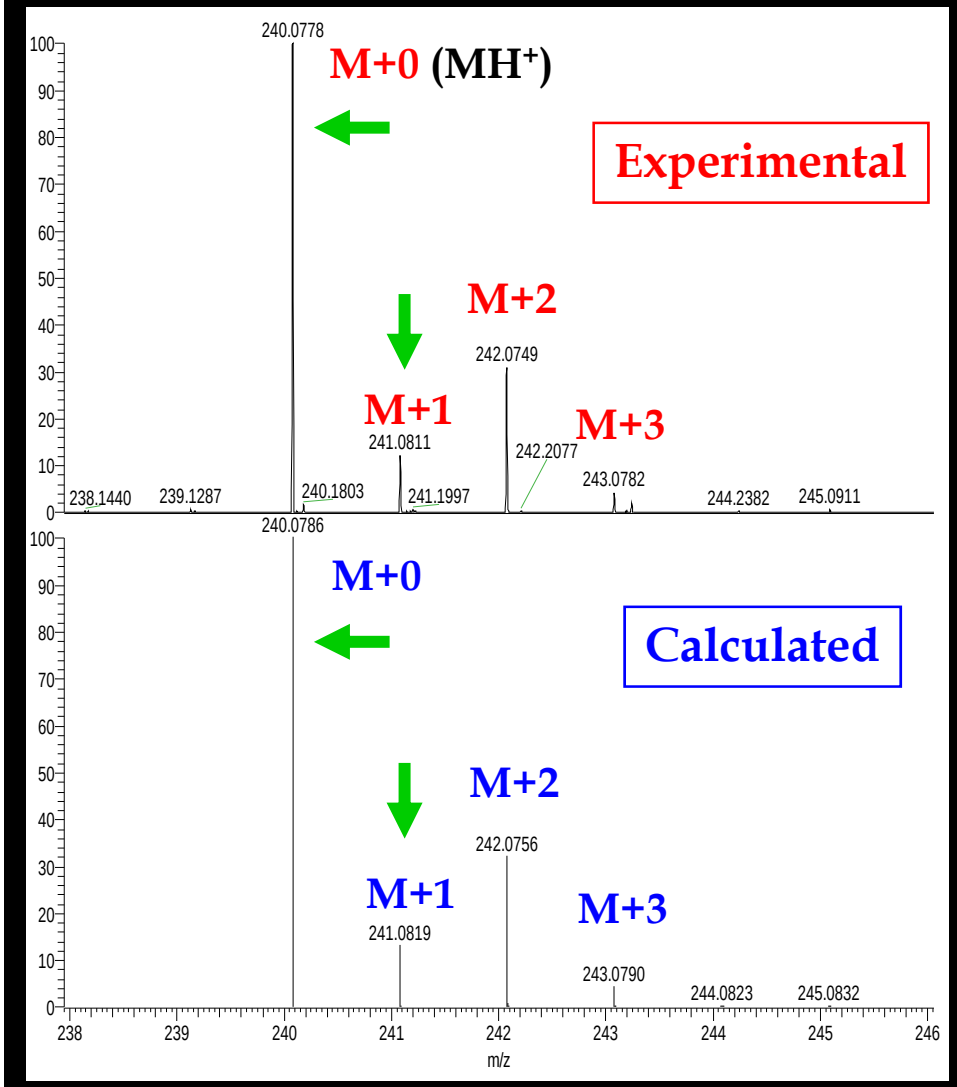
Mephedrone





4. MH^+ isotopic patterns – RIA error

Comparison of experimental and calculated MH^+ isotopic patterns



RIA

Relative Isotopic Abundance

$M+1_{ab}$ / $M+0_{ab}$ (^{13}C / ^{12}C)

RIA error (%)

$$\frac{RIA_{meas} - RIA_{calc}}{RIA_{calc}} \times 100$$

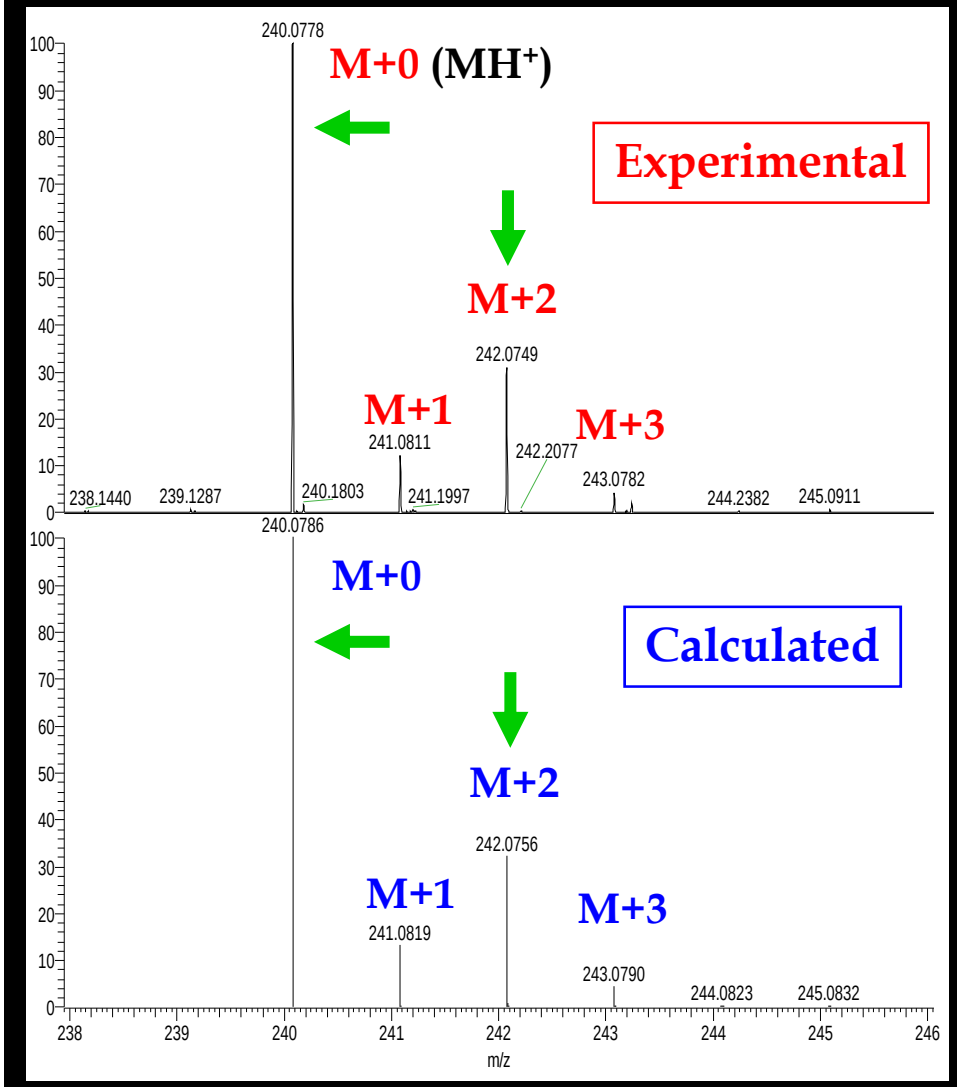
4. MH^+ isotopic patterns – RIA error

Substance	RIA error (%) (M+1/M+0)
4-Fluoro-Amphetamine	0,53
5-APB	0,88
BenzylPiperazine	-0,14
Mephedrone (4-MMC)	1,56
3-MMC	0,68
Buphedrone	1,73
Dimethyltryptamine (DMT)	-0,34
Methyl-Benzyl-Piperazine	-1,34
4-MEC	1,50
Pentedrone	0,55
Chloro-Phenyl-Piperazine	-0,20
Methylone	-0,94
2C-E	-0,28

Substance	RIA error (%) (M+1/M+0)
Diethyltryptamine (DET)	-0,18
Butylone	-0,43
2,4,5-Trimethoxyamphetamine	-0,89
Trifluoromethylphenylpiperazine	0,07
α-PVP	-0,25
4-AcO-DMT	-0,27
Methoxethamine	0,32
2C-B	-4,42
Bk-2C-B	-3,70
MDPV	-0,03
25B-NBOMe	0,62
25I-NBOMe	-2,11

4. MH^+ isotopic patterns – RIA error

Comparison of experimental and calculated MH^+ isotopic patterns



RIA

Relative Isotopic Abundance

$M+2_{ab}$ / $M+0_{ab}$ (Br or Cl / ^{12}C)

RIA error (%)

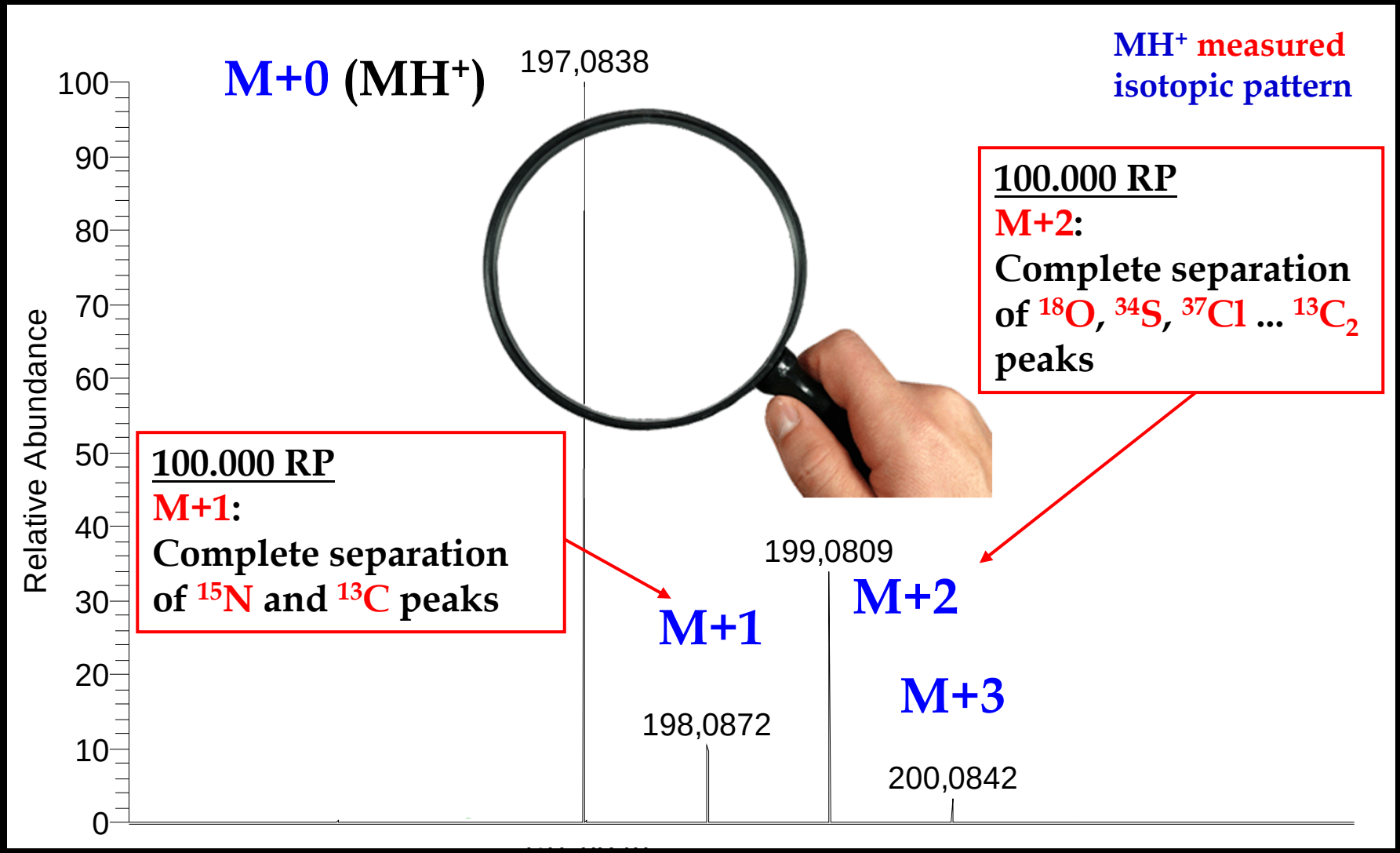
$$\frac{RIA_{meas} - RIA_{calc}}{RIA_{calc}} \times 100$$

4. MH^+ isotopic patterns – RIA error

Substance	Halogen atom	RIA error (%) (M+2/M+0)
Chloro-Phenyl-Piperazine	Cl	0,40
2C-B	Br	-0,27
bk-2C-B	Br	2,22
25B-NBOMe	Br	1,23

5. Fine Structure of MH^+ isotopic patterns

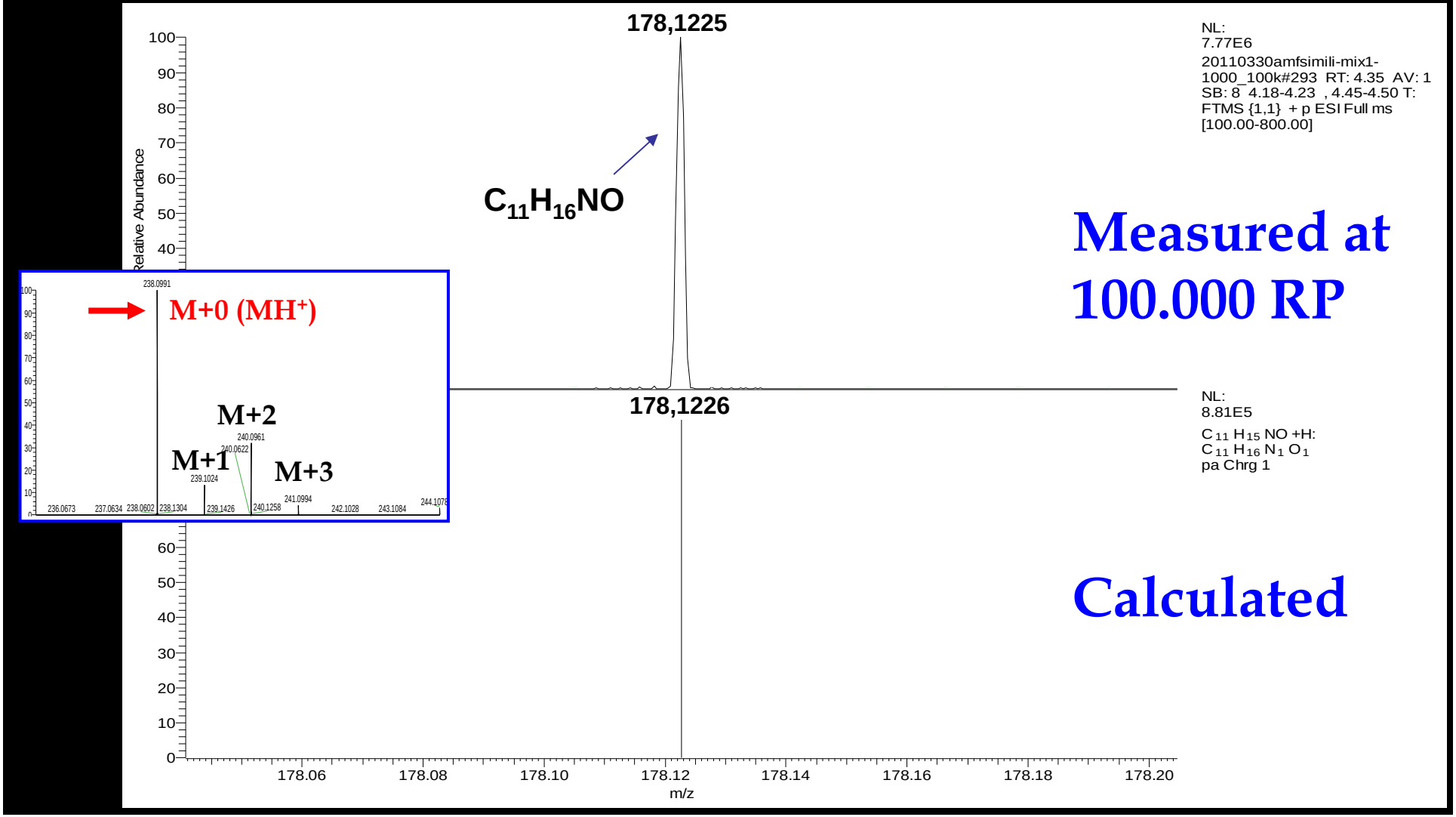
Isotopic Fine Structure of M+1, M+2, M+3 peaks vs. M+0 peak



5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

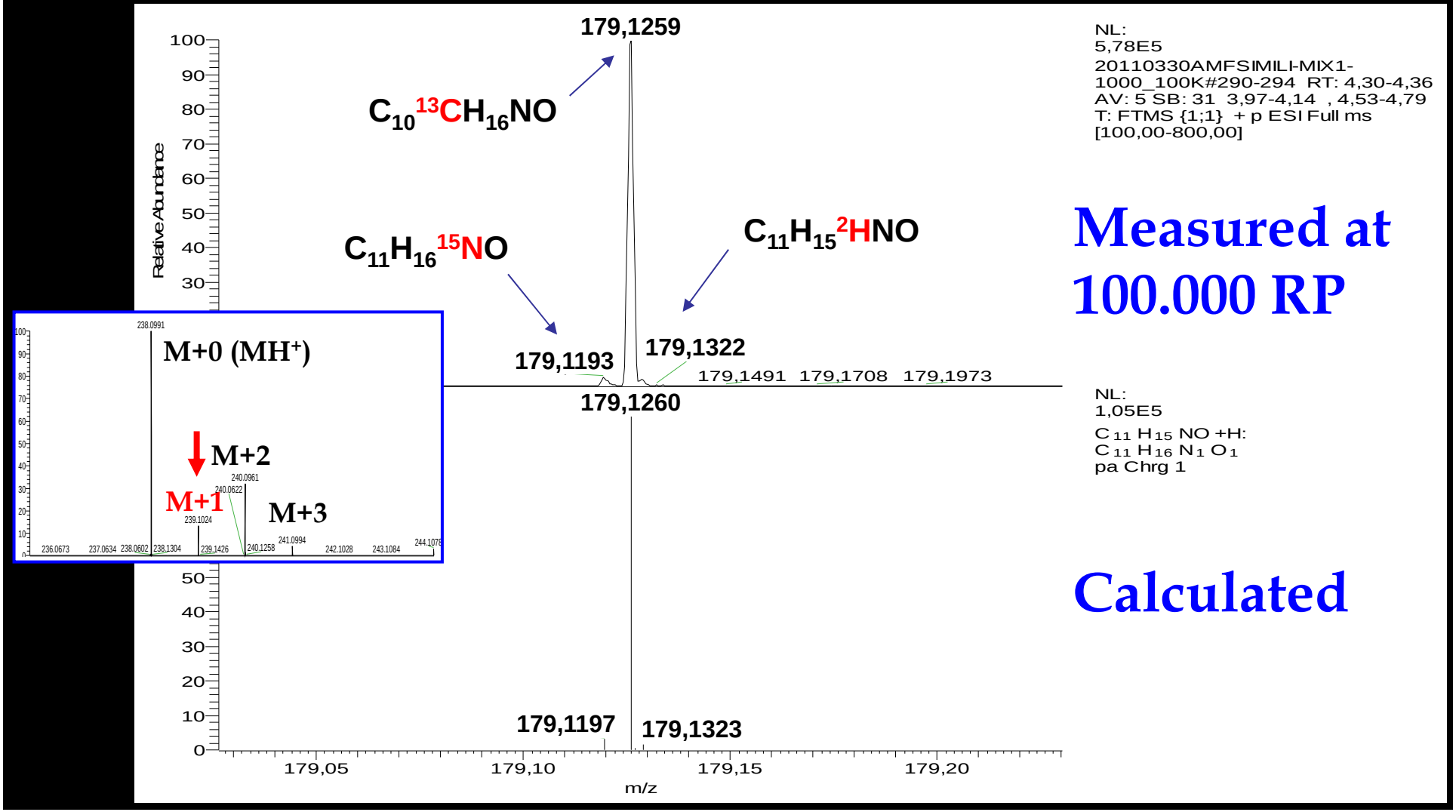
M+0 of Mephedrone (monoisotopic)



5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

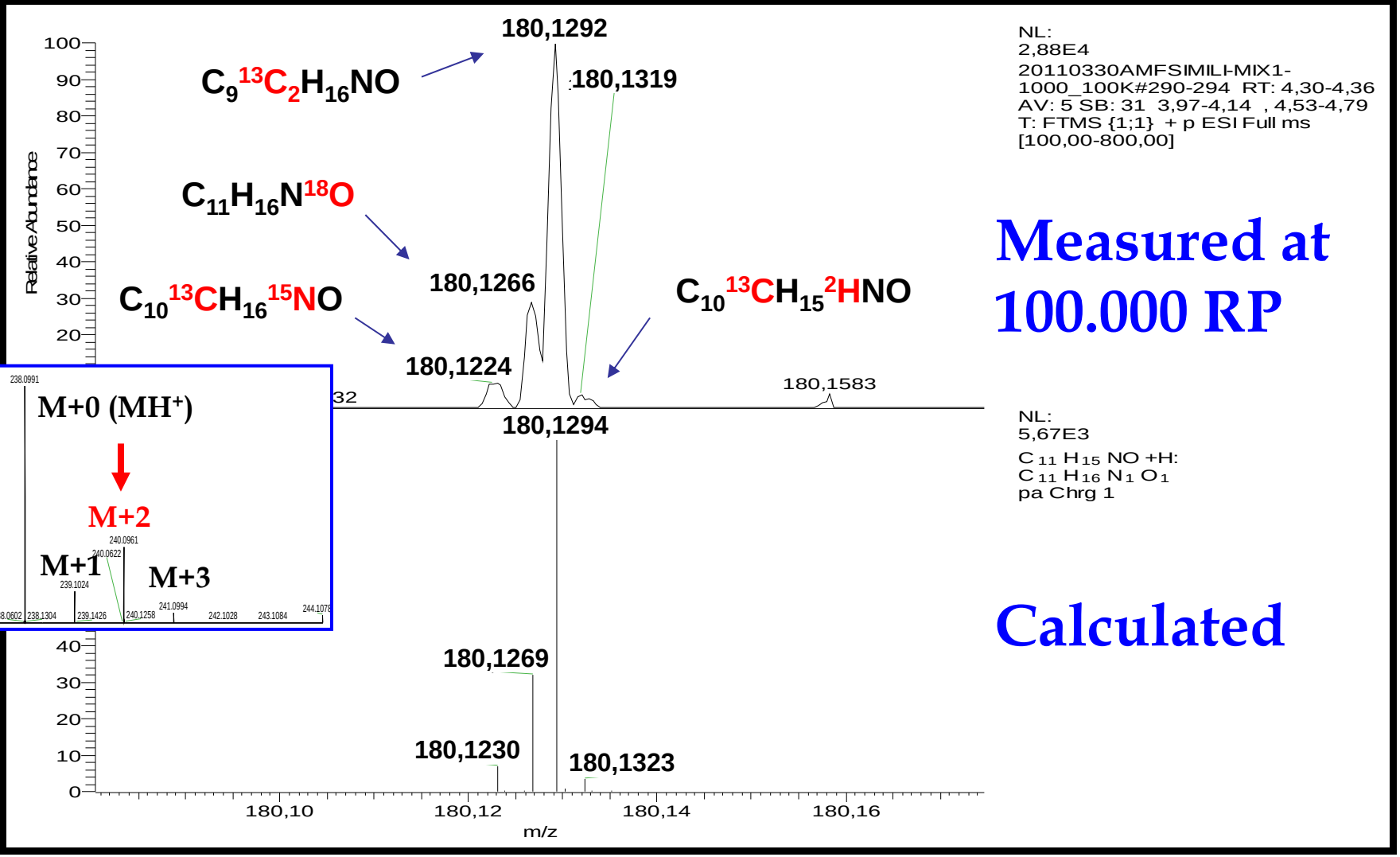
M+1 of Mephedrone isotopic pattern (12%M+0)



5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

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



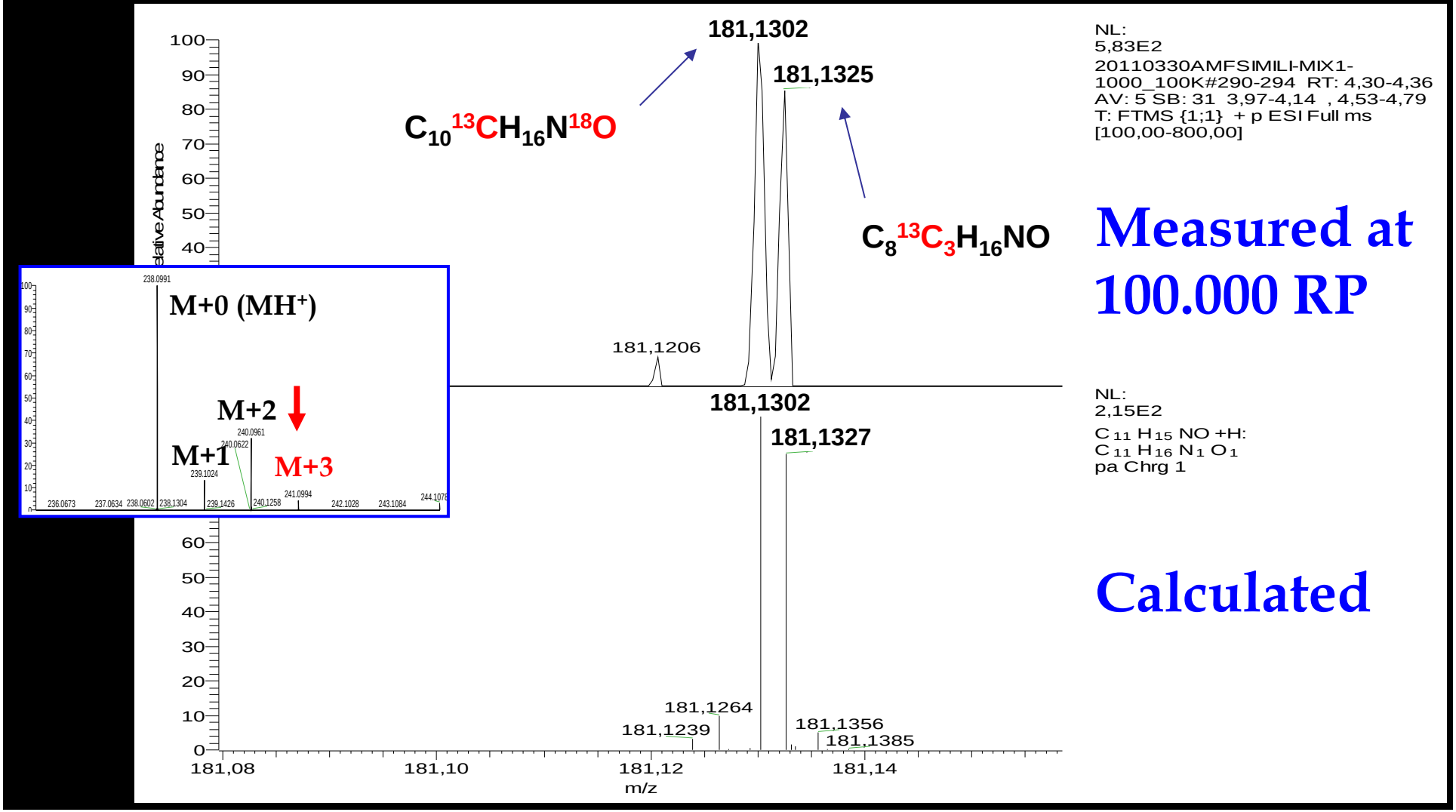
Measured at 100.000 RP

Calculated

5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

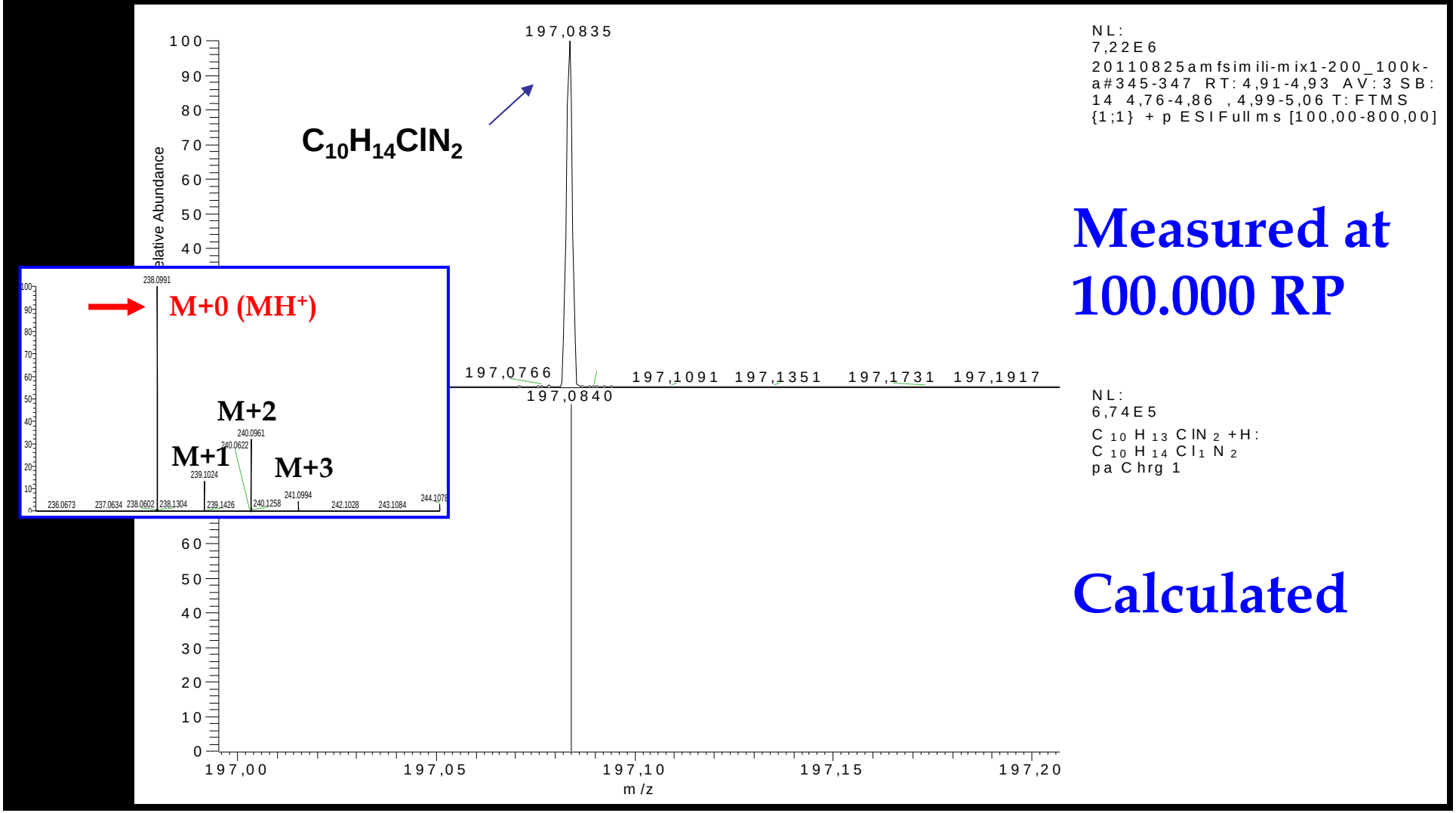
M+3 of Mephedrone isotopic pattern (0.01%M+0)



5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

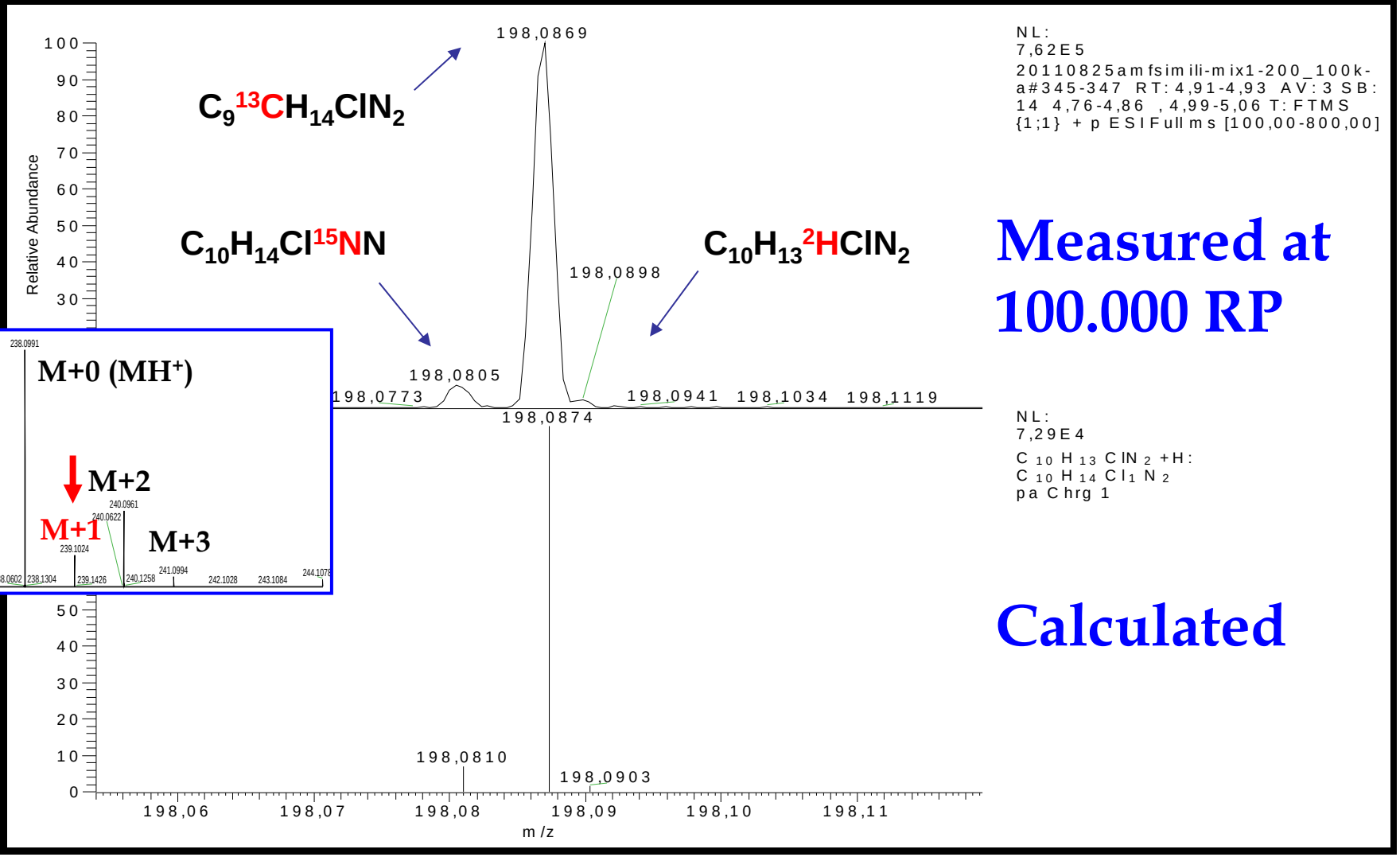
M+0 of Cl-Phenyl-Piperazine (monoisotopic)



5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

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



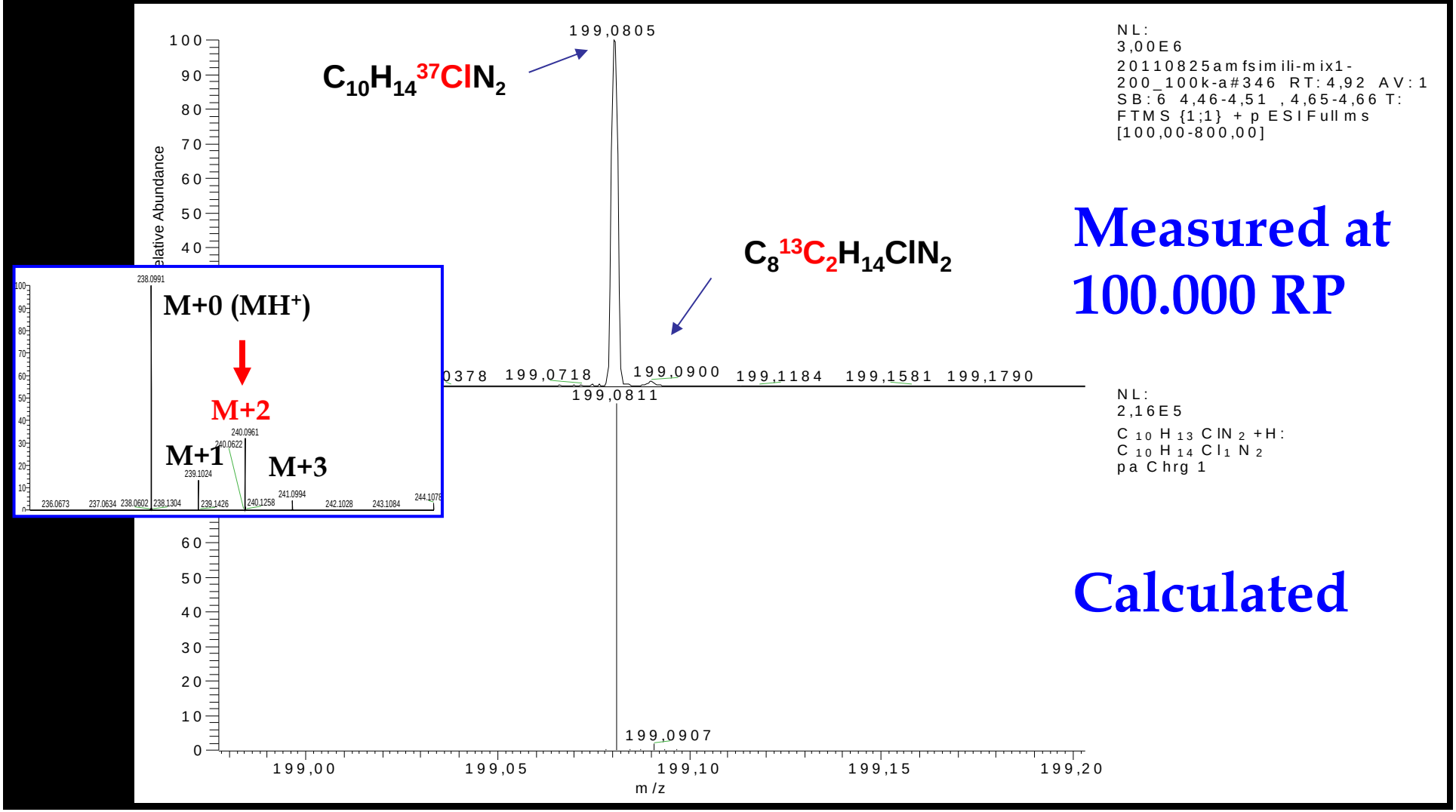
Measured at 100.000 RP

Calculated

5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

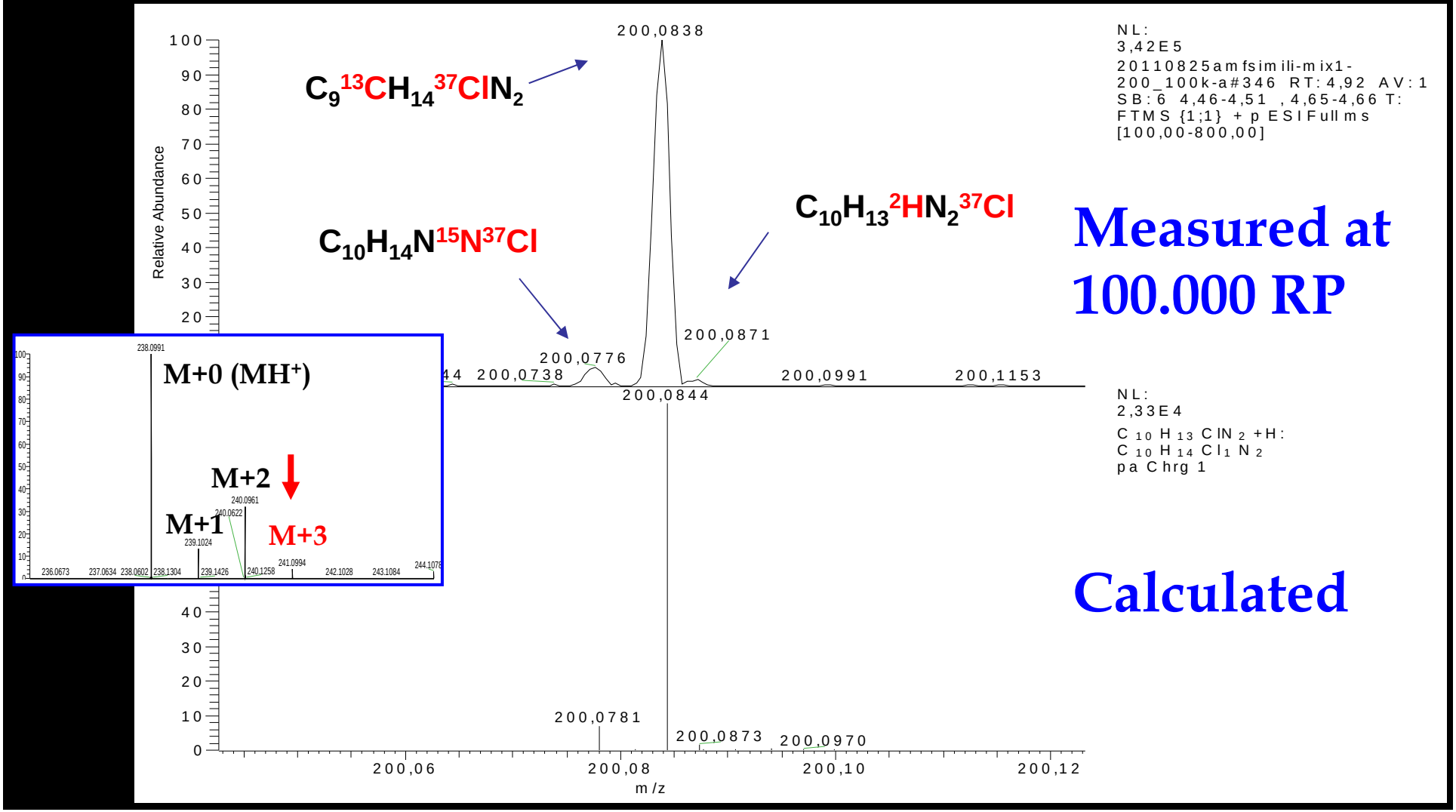
M+2 of Cl-Phenyl-Piperazine isotopic pattern (33%M+0)



5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

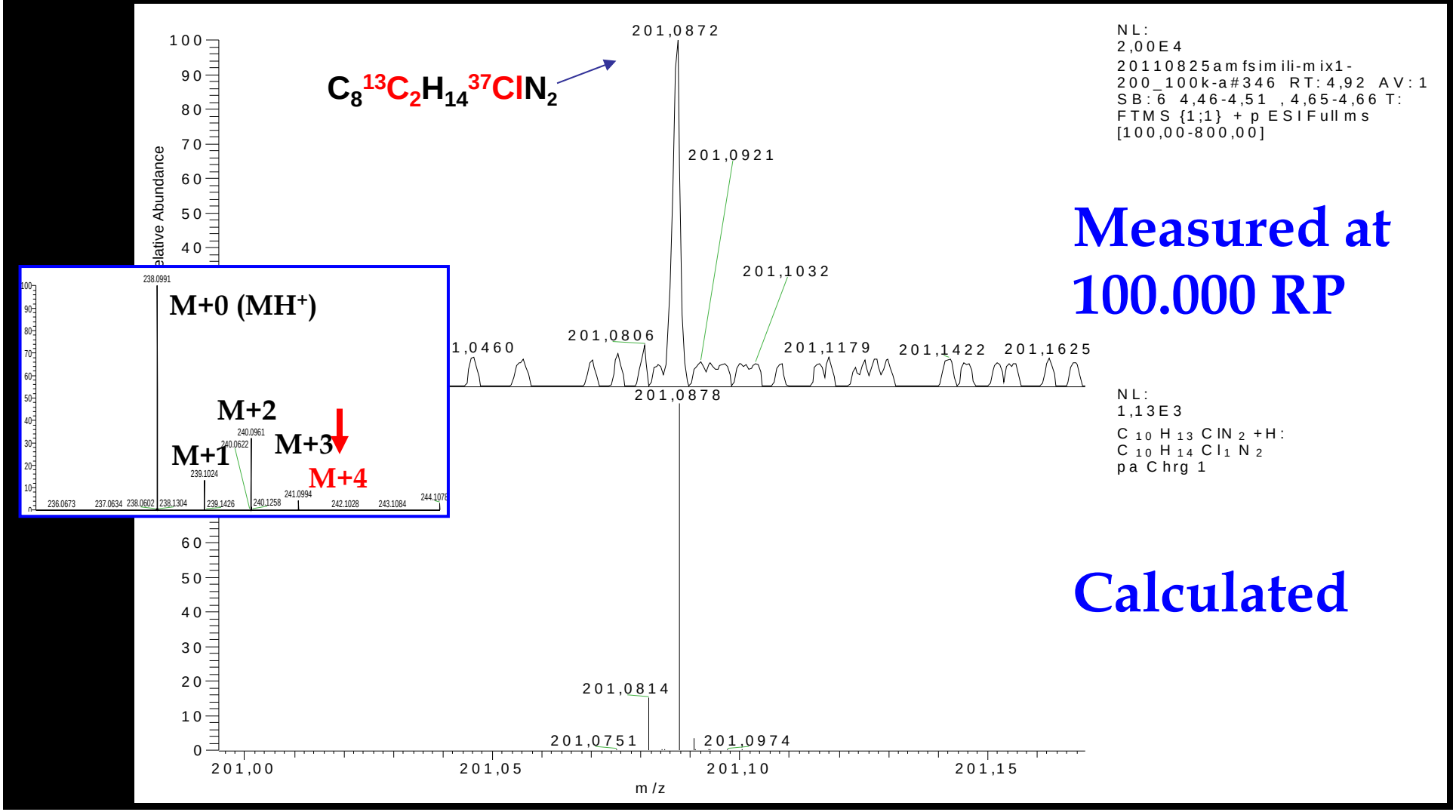
M+3 of Cl-Phenyl-Piperazine isotopic pattern (3%M+0)



5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3, M+4 isotopic peaks vs. M+0 peaks

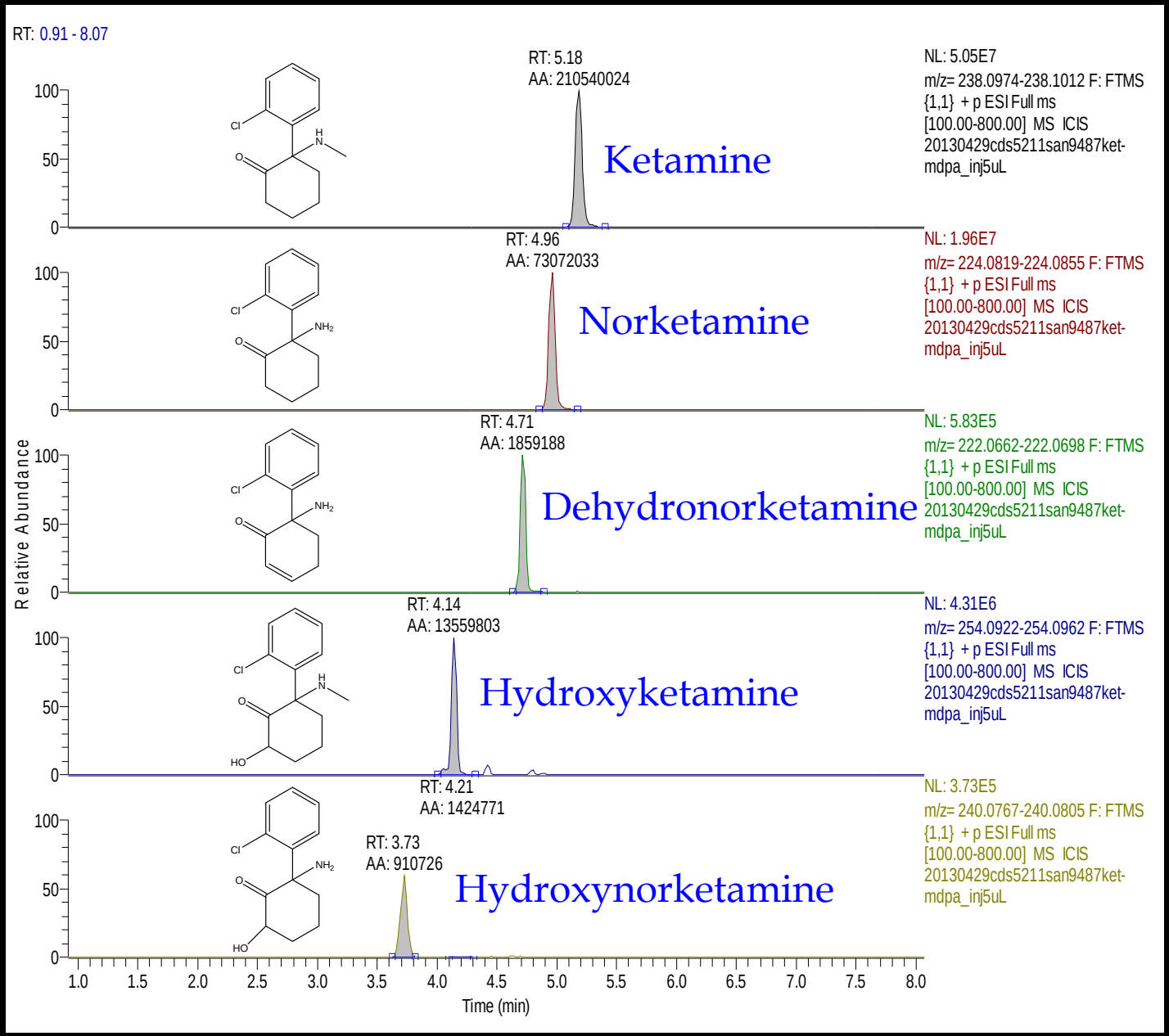
M+4 of Cl-Phenyl-Piperazine isotopic pattern (0.2%M+0)



Measured at 100.000 RP

Calculated

UHPLC/HRMS – Blood sample



Ketamine: 230 ng/mL
Norketamine: 85 ng/mL

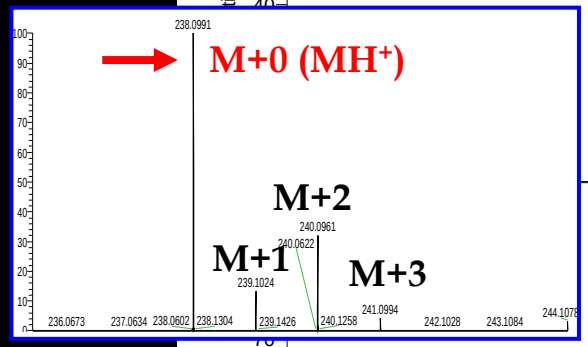
Identification of metabolites:

- Chromatographic behaviour
- Accurate mass measurements of MH^+ ionic species
- Study of MH^+ collision-induced product ions in MS/MS experiments
- Comparison of experimental and calculated MH^+ isotopic clusters

5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

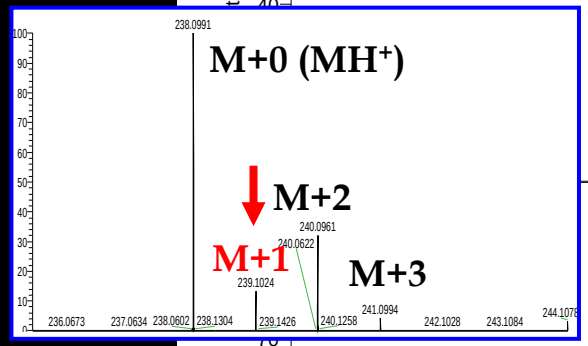
Ketamine: M+0 (monoisotopic)



5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

Ketamine: M+1 (13% of M+0)



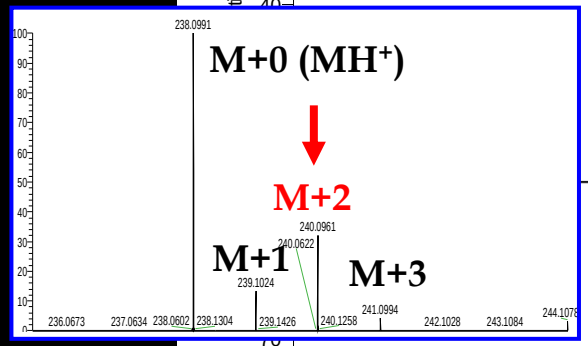
Measured at 100.000 RP

Calculated

5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

Ketamine: M+2 (32% of M+0)



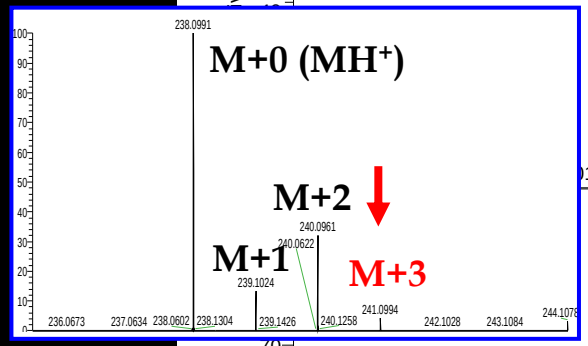
Measured at 100.000 RP

Calculated

5. Fine Structure of MH^+ isotopic patterns

M+1, M+2, M+3 isotopic peaks vs. M+0 peaks

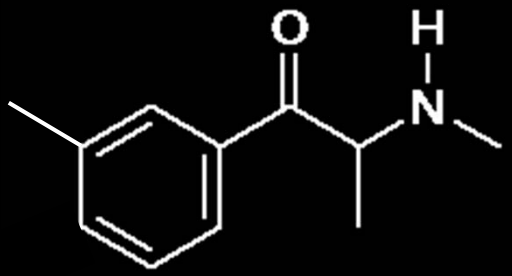
Ketamine: M+3 (5% of M+0)



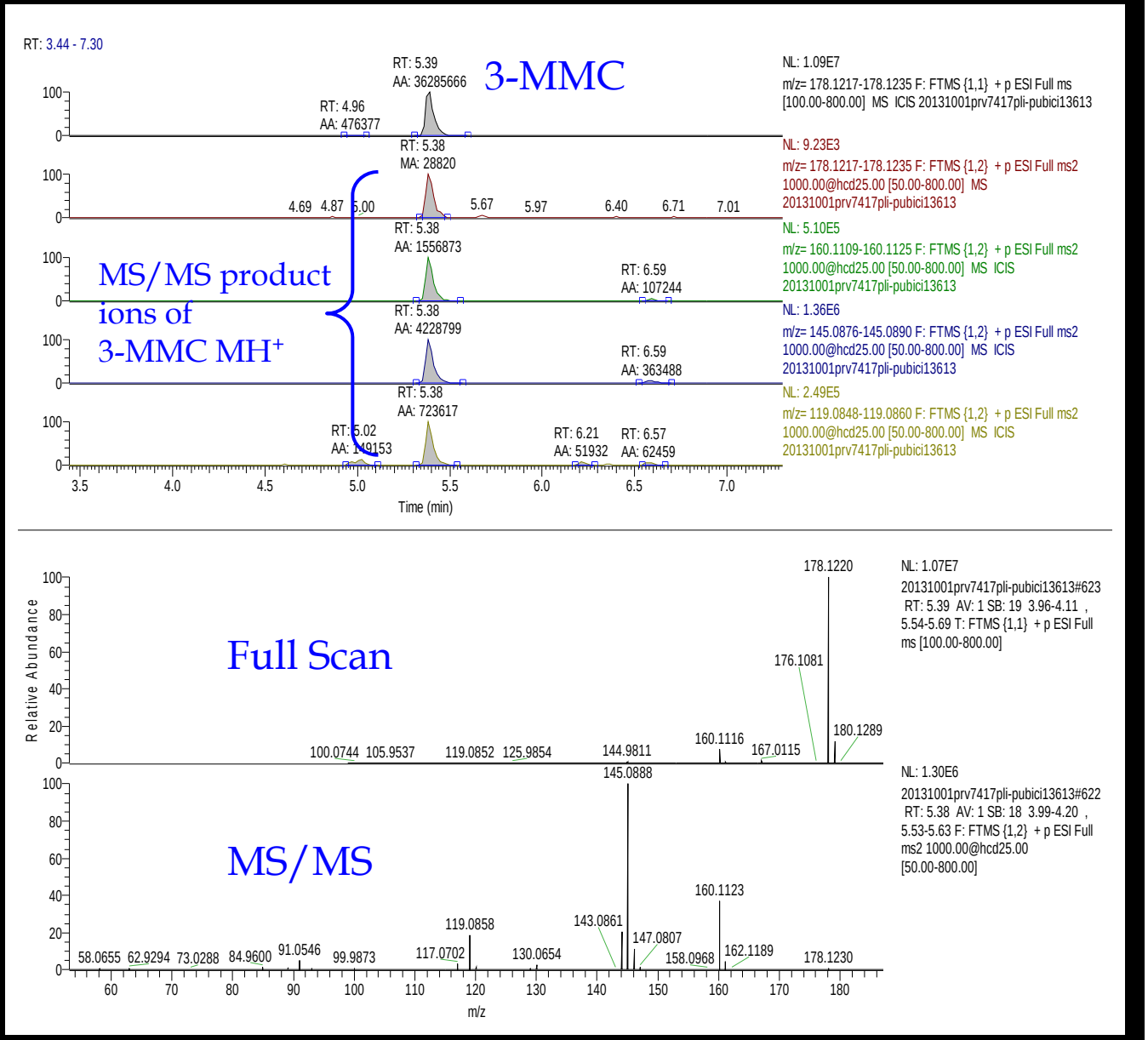
Measured at 100.000 RP

Calculated

3-MMC in pubic hair

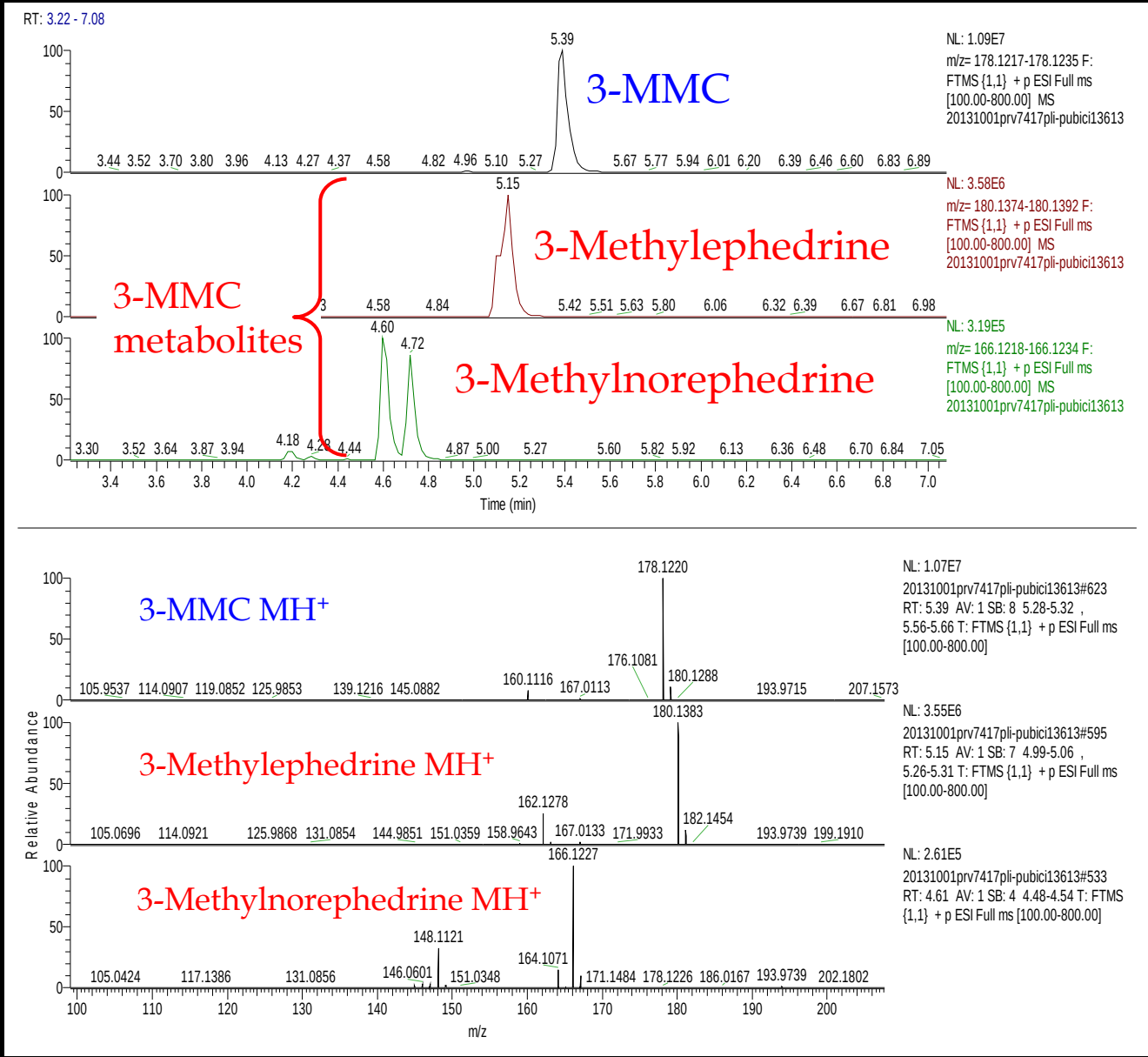


- Pubic hair sample from a regular user of 3-MMC
- UHPLC/HRMS analysis
- Identification of 3-MMC



3-MMC metabolites in pubic hair

- Identification of 3-MMC metabolites in pubic hair:
  3-Methylephedrine
  3-Methylnorephedrine
- Distinction between external contamination of hair sample and intake of drug(s)



Conclusions

1. Efficient chromatographic separation of A-R drugs
2. Accurate mass measurements of MH^+ ionic species with a mass accuracy < 2-3 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 patterns (RIA error < 5% for all A-R drugs)
5. Isotopic fine structure of the M+1, M+2, M+3 isotopic peaks completely in accordance with theoretical patterns

⬇

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

The Forensic Toxicology People at LIATF

Thank you very much for your kind attention!