Exact mass measurement of product ions using a Q-TOF: a simplified approach using combined tandem mass spectrometric functions

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Outline

- Introduction
  - Accurate mass measurement
  - Classical methods
  - Equipment
- Objective
- Study
  - Set-up
  - Experimental
- Results
- Conclusion

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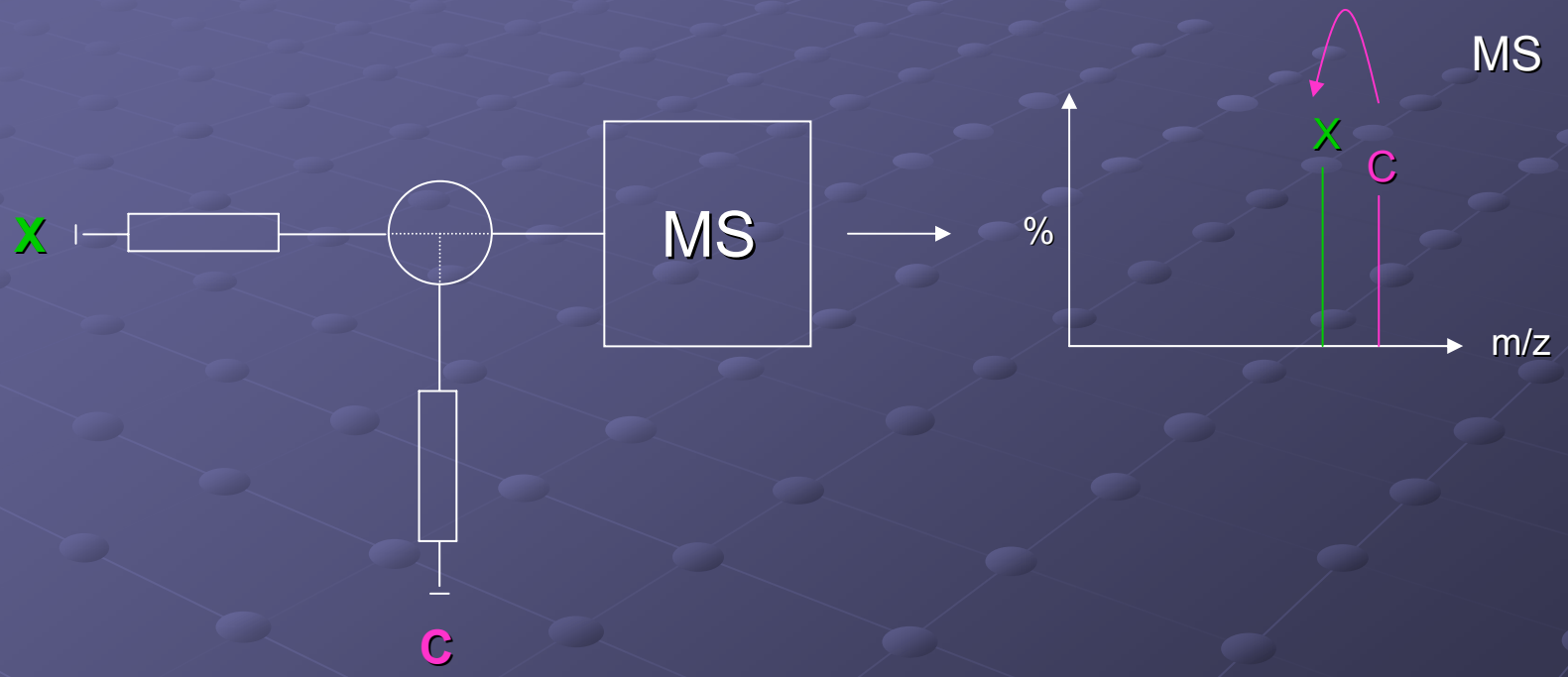
Accurate Mass Measurement

- Structural confirmation and identification of unknown compound
- Used in several domains
  - Pharmaceutical industry
  - Toxicology
  - …

Calibration for accurate mass

- Hardware calibration
- Lteff value: Flight tube length
- Software calibration: adjust the axes
- ‘on-line’ calibration (lockmass correction) = to adjust drifts by e.g. temperature fluctuations

Classical approach - MS-Mode



X: Unknown compound
C: Mass calibrator

Classical approach - MS-Mode

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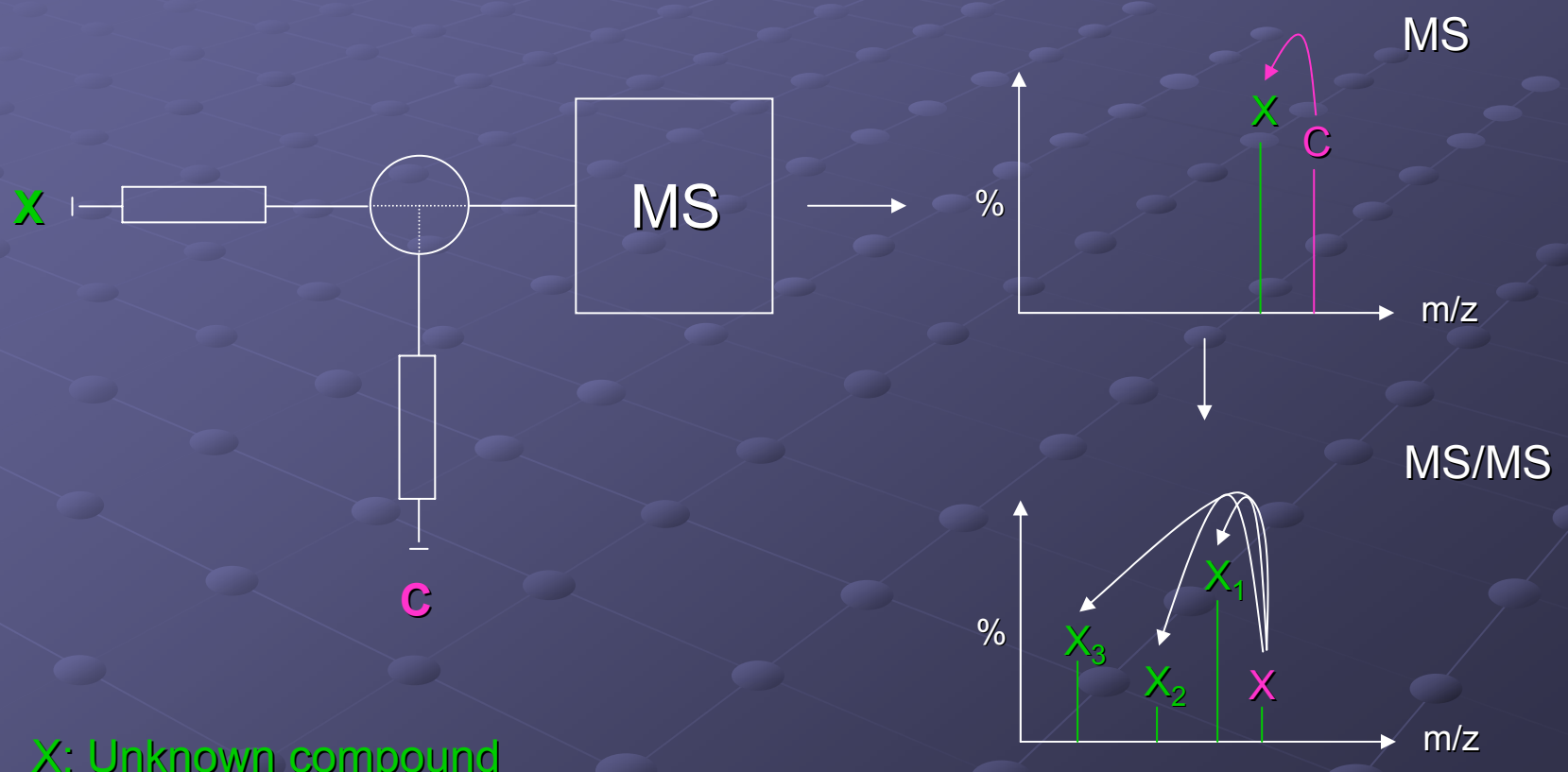
C: Mass calibrator

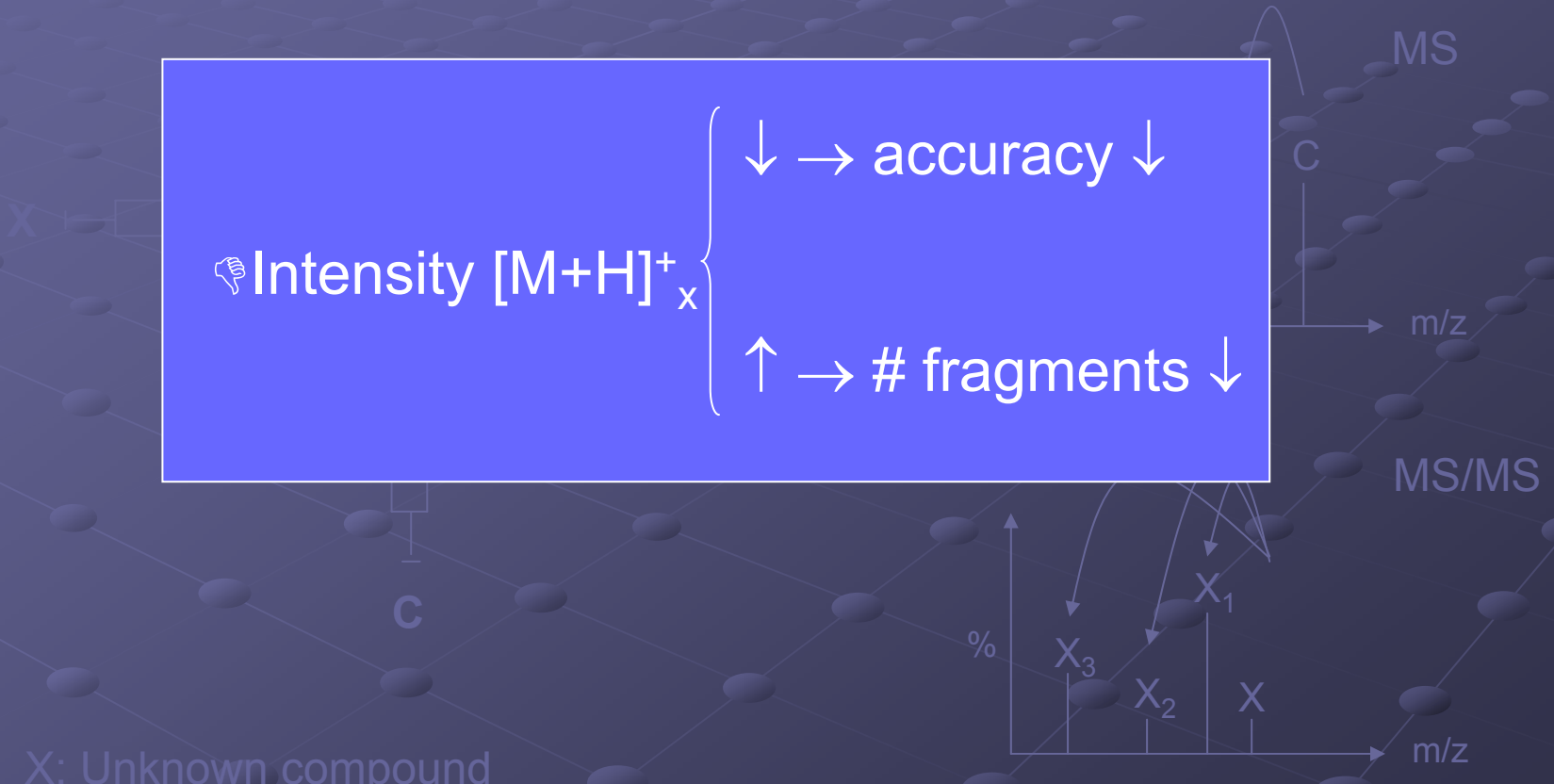
Classical approach – MS/MS-Mode (1)



X: Unknown compound
C: Mass calibrator

Classical approach – MS/MS-Mode (1)

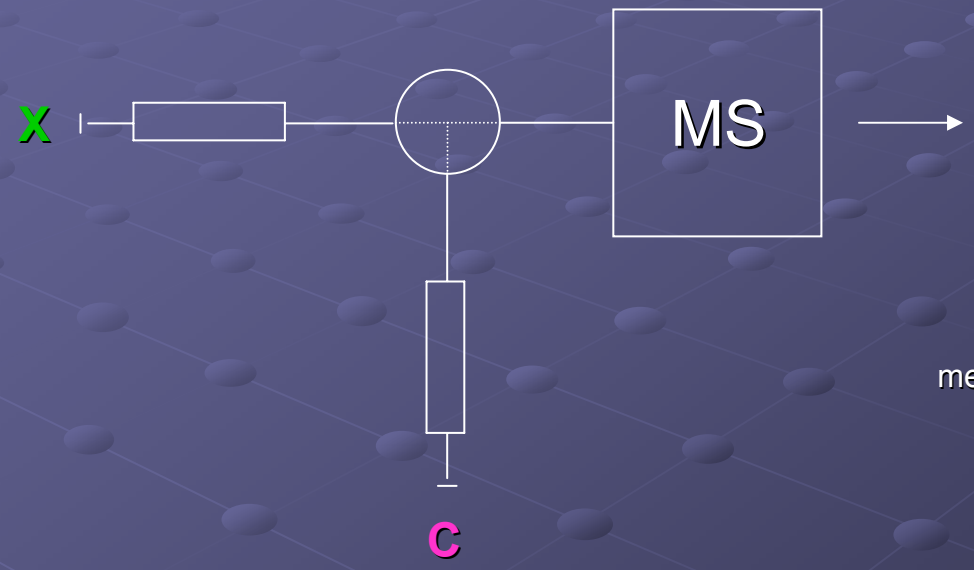
- Intensity $[M+H]^+_x$
  - $\downarrow \rightarrow$ accuracy $\downarrow$
  - $\uparrow \rightarrow$ # fragments $\downarrow$

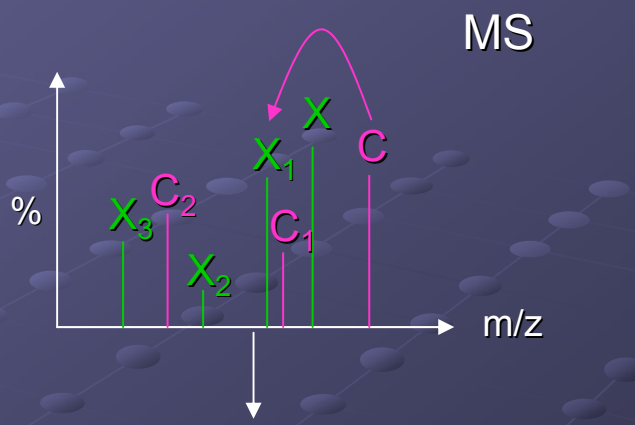


X: Unknown compound
C: Mass calibrator

Classical approach – MS/MS-Mode (2)

⇨ In-source CID

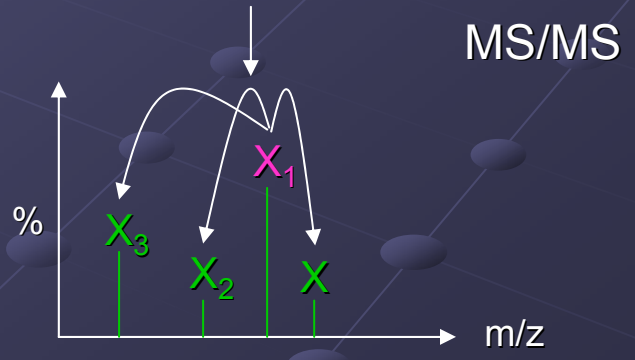




X1
measured

X1
calculated

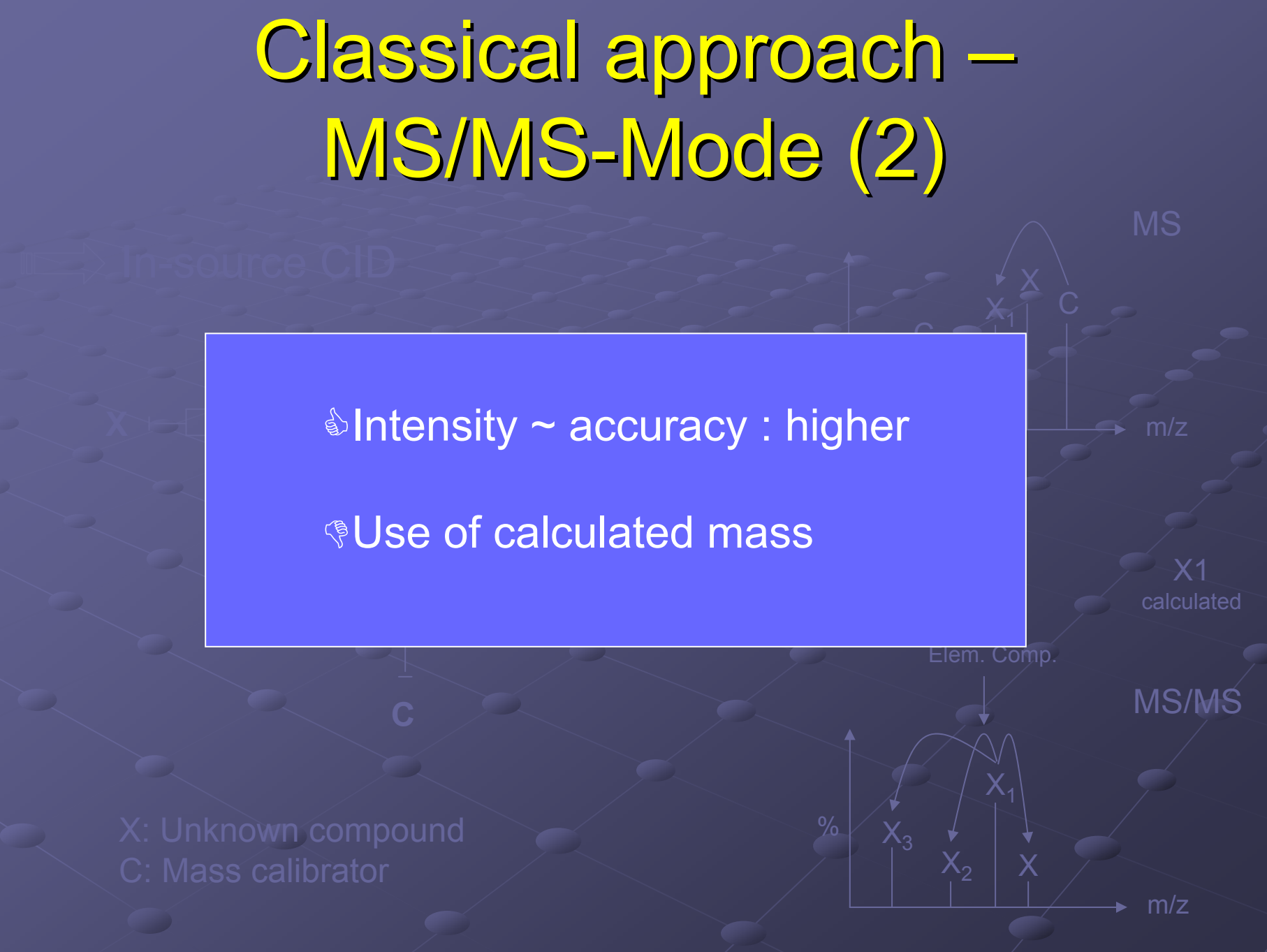
Elem. Comp.



X: Unknown compound
C: Mass calibrator

Classical approach – MS/MS-Mode (2)

- Intensity ~ accuracy : higher
- Use of calculated mass



Equipment – MS (1)

- Fourier Transform
  - High resolution
  - Mass errors below 1mDa
  - Not available for everyone
- Alternative equipment
  - Sector instrument
  - Q-TOF

} Multisprayer equipment (Lockmass!)

Equipment – Spray (2)

- An electrospray dual sprayer
- Pro's
  - Separate acquisition channels
  - No suppression of ionization
  - No clustering of the analyte and reference
- Con's
  - Too expensive if not the main application
  - Not available for all existing instruments

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Objective

- Alternative approach
  - 1-step accurate mass MS/MS
    - Combination of separate acquisition channels
  - ~~Dual spray~~
    - traditional source

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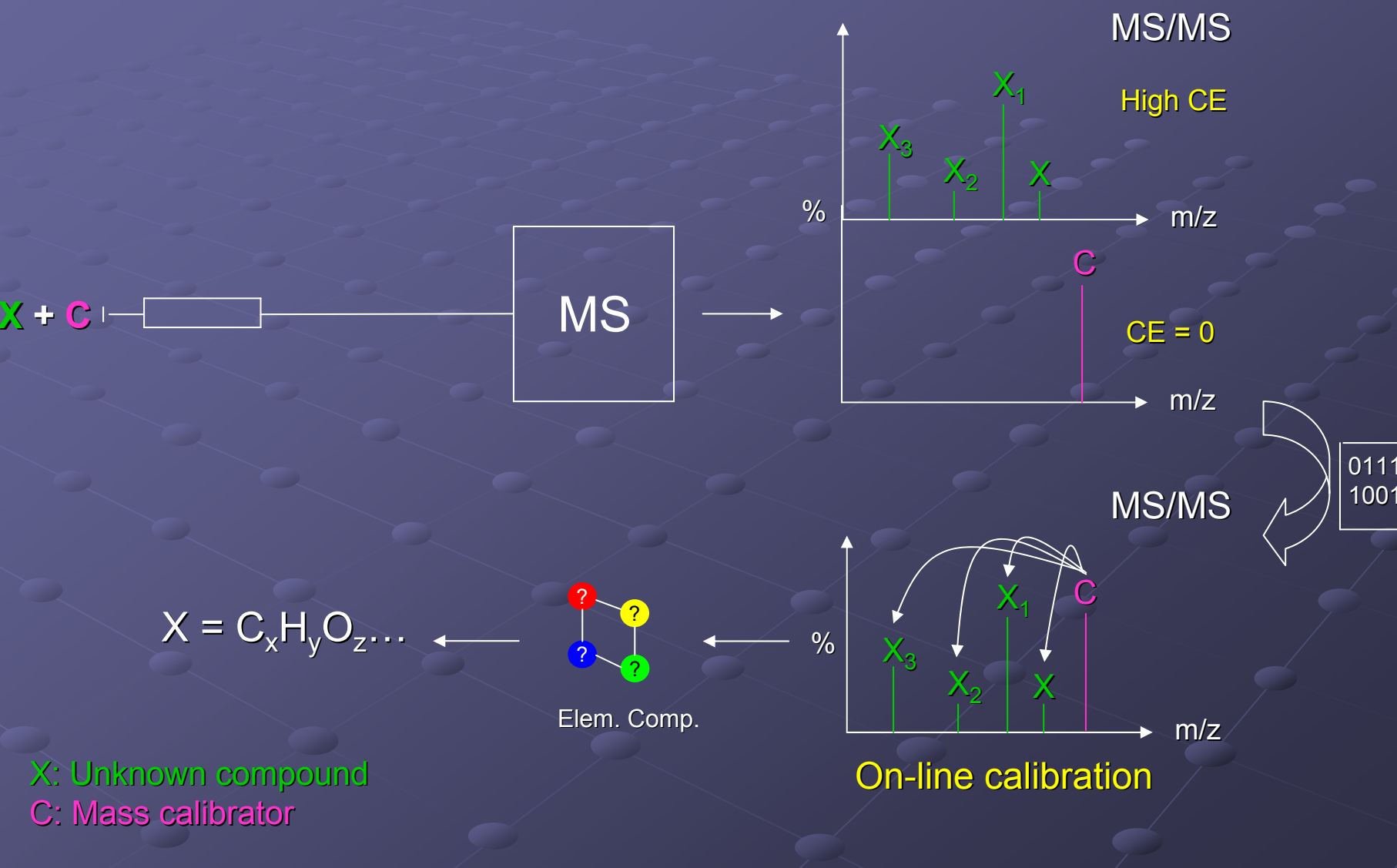
Set-up (1)


MS/MS
High CE
X1
X2
X3
X
%
m/z
C
CE = 0
m/z
X + C
MS
MS/MS
X1
C
X2
X3
X
%
m/z
On-line calibration
X = CxHyOz…
Elem. Comp.
X: Unknown compound
C: Mass calibrator

Set-up (2)

- Critical points
  - Combining the data
  - Continuous fast switching between precursor ions ~ stability

Evaluation !

Experimental (1)

- 10 compounds – 10 mass calibrators
- m/z 150-800
- m/z $[M+H]^+_x$ ~ m/z $[M+H]^+_c$ *
- Accurate mass limits
  - m/z values <200 : a mass tolerance of 3 mDa
  - m/z values >200 : a mass tolerance of 10 ppm

* Jiang L, Moini M. Anal. Chem. 2000; 72, 20-24

Experimental (2)

- Q-TOF (ESI+) - MassLynx 3.5
- Optimized for each individual compound
  - ESI capillary voltage: 2000-3000 V
  - Cone Voltage: 14-34 V
  - Collision energy: 11-40 eV
- Preparation of the samples: MeOH/H_2O (50/50)
- Concentration ~ avoid detector saturation

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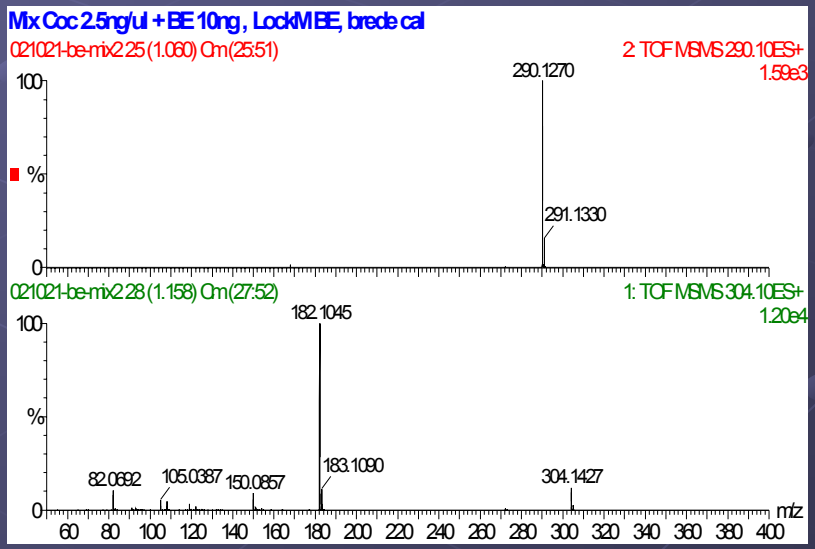
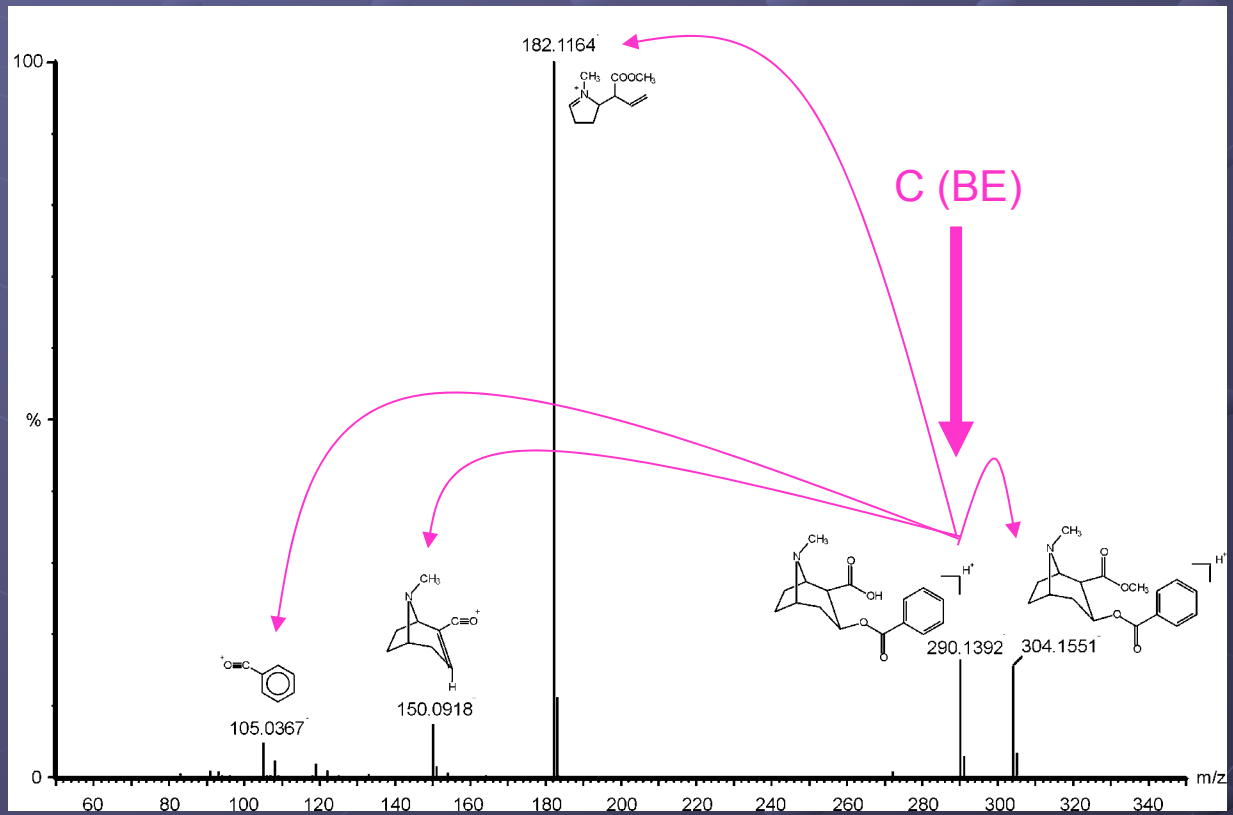
Results (1)

	Compound	Type	CE (eV)
Function 1	Cocaine	X	20
Function 2	BE	C	none



Results (2)

- Lock mass correction using $[M+H]^+$ ion of the mass calibrator (Benzoylecgonine)



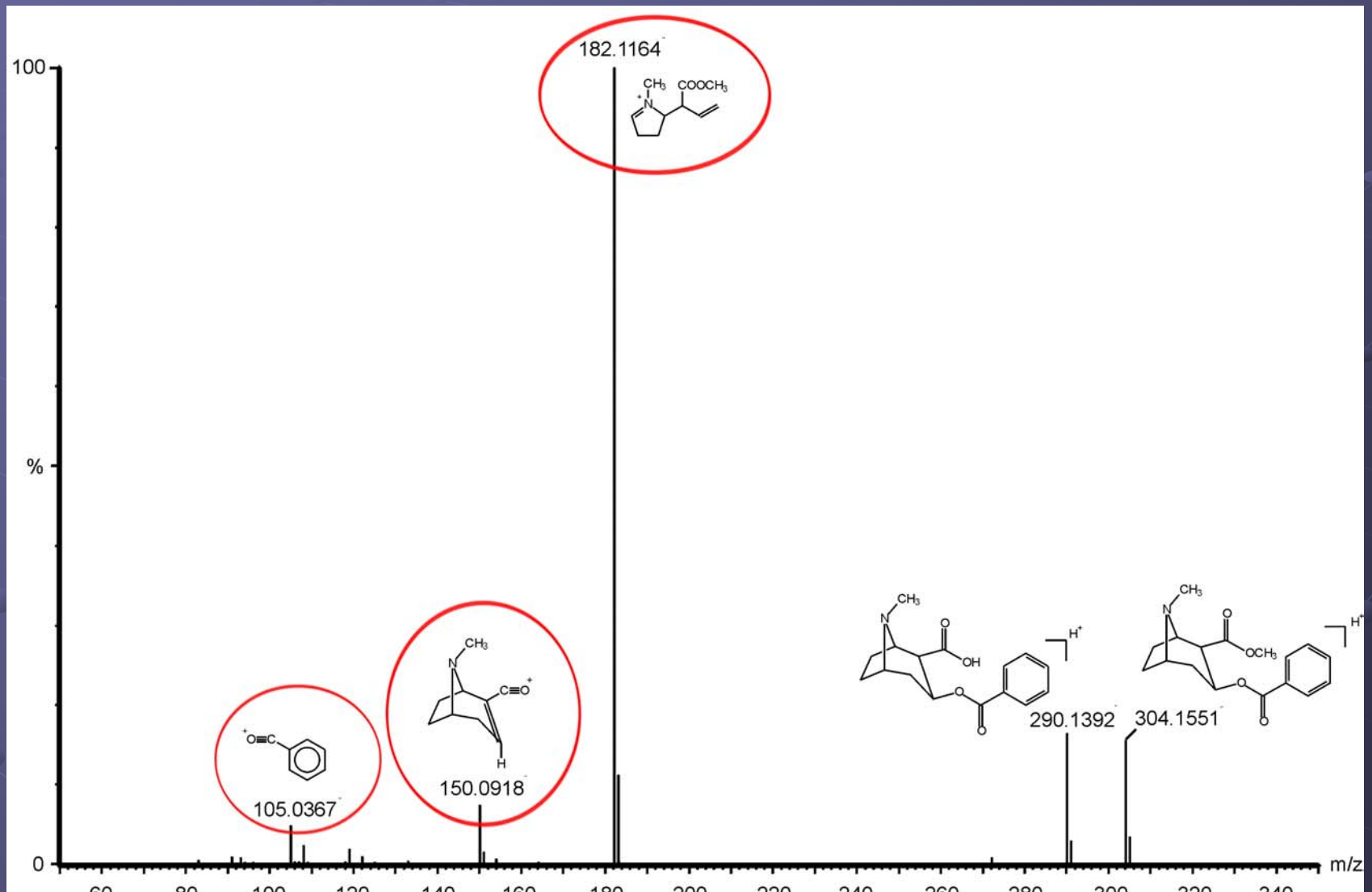
Elemental composition report
mass 304.1551

Single Mass Analysis
Tolerance = 200.0 mDa / DBE: min = -0.5, max = 50.0

Monoisotopic Mass, Odd and Even Electron Ions
172 formula(e) evaluated with 159 results within limits (up to 50 closest results for each mass)

Minimum:			-0.5		
Maximum:		200.0	5.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
304.1551	**304.1549**	**0.2**	**0.7**	**7.5**	**C17 H22 N O4**
	304.1562	-1.1	-3.7	12.5	C18 H18 N5
	304.1535	1.6	5.1	8.0	C15 H20 N4 O3
	304.1576	-2.5	-8.1	12.0	C20 H20 N2 O
	304.1522	2.9	9.5	3.0	C14 H24 O7
	304.1509	4.2	13.9	3.5	C12 H22 N3 O6
	304.1621	-7.0	-23.0	3.5	C11 H22 N5 O5
	304.1468	8.3	27.2	-0.5	C7 H22 N5 O8
	304.1634	-8.3	-27.4	3.0	C13 H24 N2 O6

Fragment ions of cocaine



Elemental composition report
of the fragment ions

Monoisotopic Mass, Odd and Even Electron Ions
109 formula(e) evaluated with 83 results within limits (up to 50 closest results for each mass)

Minimum:			-0.5		
Maximum:		200.0	5.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
182.1164	**182.1181**	**-1.7**	**-9.4**	**3.5**	**C10 H16 N O2**
	182.1141	2.3	12.7	-0.5	C5 H16 N3O4
	182.1096	6.8	37.6	8.0	C14 H14
	182.1253	-8.9	-49.0	-0.5	C4 H16 N5 O3
	182.1055	10.9	59.7	4.0	C9 H14 N2 O2
150.0918	**150.0919**	**-0.1**	**-0.6**	**4.5**	**C9 H12 N O**
	150.0905	1.3	8.4	5.0	C7 H10 N4
	150.0892	2.6	17.3	0.0	C6 H14 O4
	150.0879	3.9	26.2	0.5	C4 H12 N3 O3
	150.0991	-7.3	-48.6	0.5	C3 H12 N5 O2
105.0367	**105.0340**	**2.7**	**25.3**	**5.5**	**C7 H5 O**
	105.0327	4.0	38.1	6.0	C5 H3 N3
	105.0413	-4.6	-43.3	1.5	C H5 N4 O2
	105.0426	-5.9	-56.1	1.0	C3 H7 N O3
	105.0300	6.7	63.6	1.5	C2 H5 N2 O3

Overview of the obtained lock mass corrected accurate mass measurements

Compound	Measured Mass	Elemental Composition	Calculated Mass	Mass Error (ppm)	Mass Error mDa	Used Mass Calibrant
Amphetamine	136.1127	$C_9H_{14}N$	136.1126[a]	0.6	0.1	Methamphetamine
	119.0852	C_9H_{11}	119.0861	-7.4	-0.9	150.1282
	91.0526	C_7H_7	91.0548	-23.9	-2.2	
Methamphetamine	150.1286	$C_{10}H_{16}N$	150.1283 [a]	2.2	0.3	Amphetamine
	119.0851	C_9H_{11}	119.0861	-8.2	-1	136.1127
	91.0530	C_7H_7	91.0548	-19.5	-1.8	
Benzoylecgonine	290.1395	$C_{16}H_{20}NO_4$	290.1392 [a]	0.9	0.3	Cocaine
	168.1032	$C_9H_{14}NO_2$	168.1025	12.4	2.1	304.1549
	150.0939	$C_9H_{12}NO$	150.0919	13.4	2.0	
	105.0359	C_7H_5O	105.0340	17.7	1.9	
Cocaine	304.1551	$C_{17}H_{22}NO_4$	304.1549 [a]	0.7	0.2	Benzoylecgonine
	182.1164	$C_{10}H_{16}NO_2$	182.1181	-9.4	-1.7	290.1392
	150.0918	$C_9H_{12}NO$	150.0919	-0.6	-0.1	
	105.0367	C_7H_5O	105.0340	25.3	2.7	
Tetracycline	445.1611	$C_{22}H_{25}N_2O_8$	445.1611 [a]	0.0	0.0	Flupentixol
	428.1367	$C_{22}H_{22}NO_8$	428.1345	5.0	2.2	435.1718
	410.1215	$C_{22}H_{20}NO_7$	410.1240	-6.0	-2.5	
Flupentixol	435.1707	$C_{23}H_{26}F_3N_2OS$	435.1718 [a]	-2.5	-1.1	Tetracycline
	390.1360	$C_{21}H_{21}F_3N_2S$	390.1378	-4.5	-1.8	445.1611
	305.0583	$C_{17}H_{12}F_3S$	305.0612	-9.4	-2.9	
Reserpine	609.2827	$C_{33}H_{41}N_2O_9$	609.2812 [a]	2.5	1.5	Aconitine
	448.1996	$C_{23}H_{30}NO_8$	448.1971	5.5	2.5	646.3227
	195.0628	$C_{10}H_{11}O_4$	195.0657	-15.0	-2.9	
Aconitine	646.3176	$C_{34}H_{48}NO_{11}$	646.3227 [a]	0.3	0.2	Reserpine
	586.2976	$C_{32}H_{44}NO_9$	586.3016	-6.8	-4.0	609.2812
	526.2683	$C_{26}H_{40}NO_{10}$[b]	526.2652	5.8	3.1	
	368.1717	$C_{18}H_{26}NO_7$[b]	368.1709	2.1	0.8	
	105.0358	C_7H_5O	105.0340	16.8	1.8	
Troleandomycin	814.4602	$C_{41}H_{68}NO_{15}$	814.4589 [a]	1.6	1.3	Rifampicine
	628.3654	$C_{32}H_{54}NO_{11}$[b]	628.3697	-6.8	-4.3	823.4129
	200.1219	$C_{14}H_{16}O$[b]	200.1201	8.9	1.8	
	116.1064	$C_6H_{14}NO$[b]	116.1075	-9.8	-1.1	
Rifampicin	823.4166	$C_{43}H_{59}N_4O_{12}$	823.4129 [a]	-1.6	-1.3	Troleandomycine
	791.3866	$C_{42}H_{55}N_4O_{11}$	791.3867	-0.2	-0.1	814.4589
	399.1595	$C_{26}H_{23}O_4$[b]	399.1596	-0.3	-0.1	

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Conclusion

- Accurate mass measurement in MS/MS mode
- Easy approach
- For any Q-TOF instrument (No dual sprayer required)
- Measured values compared with theoretical values fell within predefined limits
  - m/z values <200 a mass tolerance of 3 mDa
  - m/z values >200 a mass tolerance of 10 ppm