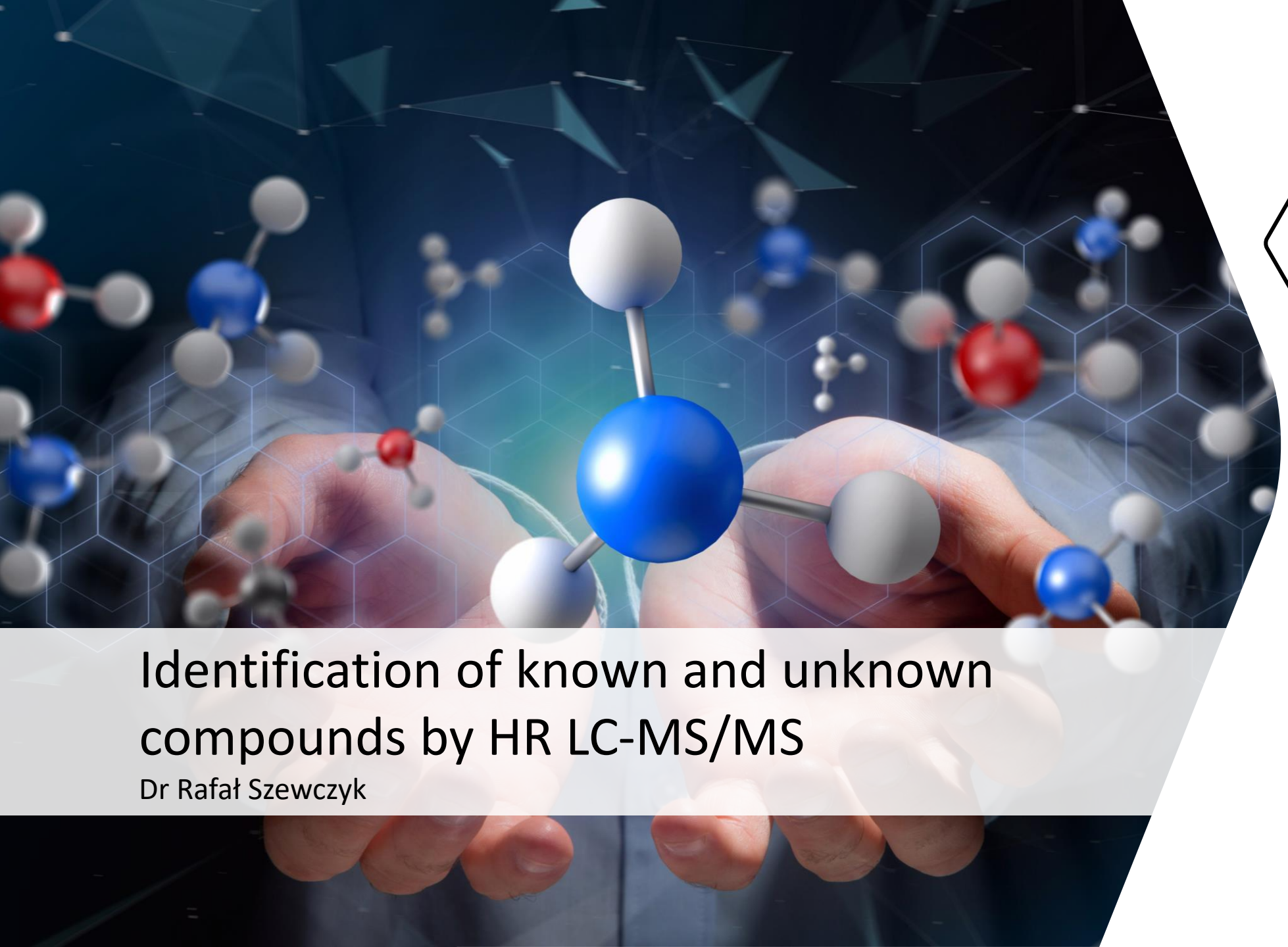

Identification of known and unknown compounds by HR LC-MS/MS

Dr Rafał Szewczyk

AGENDA

01

BRIEF INTRODUCTION

From discovery to targeted analysis
High resolution LC-MS/MS
Materials and methods

02

UNTARGETED ANALYSIS
Manual id

Diclofenac (DDA)
Hydroxyzine (DDA)

03

UNTARGETED ANALYSIS
Semi-automatic id

Loperamid (DIA)

04

TARGETED ANALYSIS
Quantitation and confirmation

DRUID (MRMhr)
Anabolic steroids (MRMhr)

05

SUMMARY

Wokflows applied summarized
What is still important?
Future developments?

06

Q&A

LabExperts
professionals in laboratory

BRIEF INTRODUCTION

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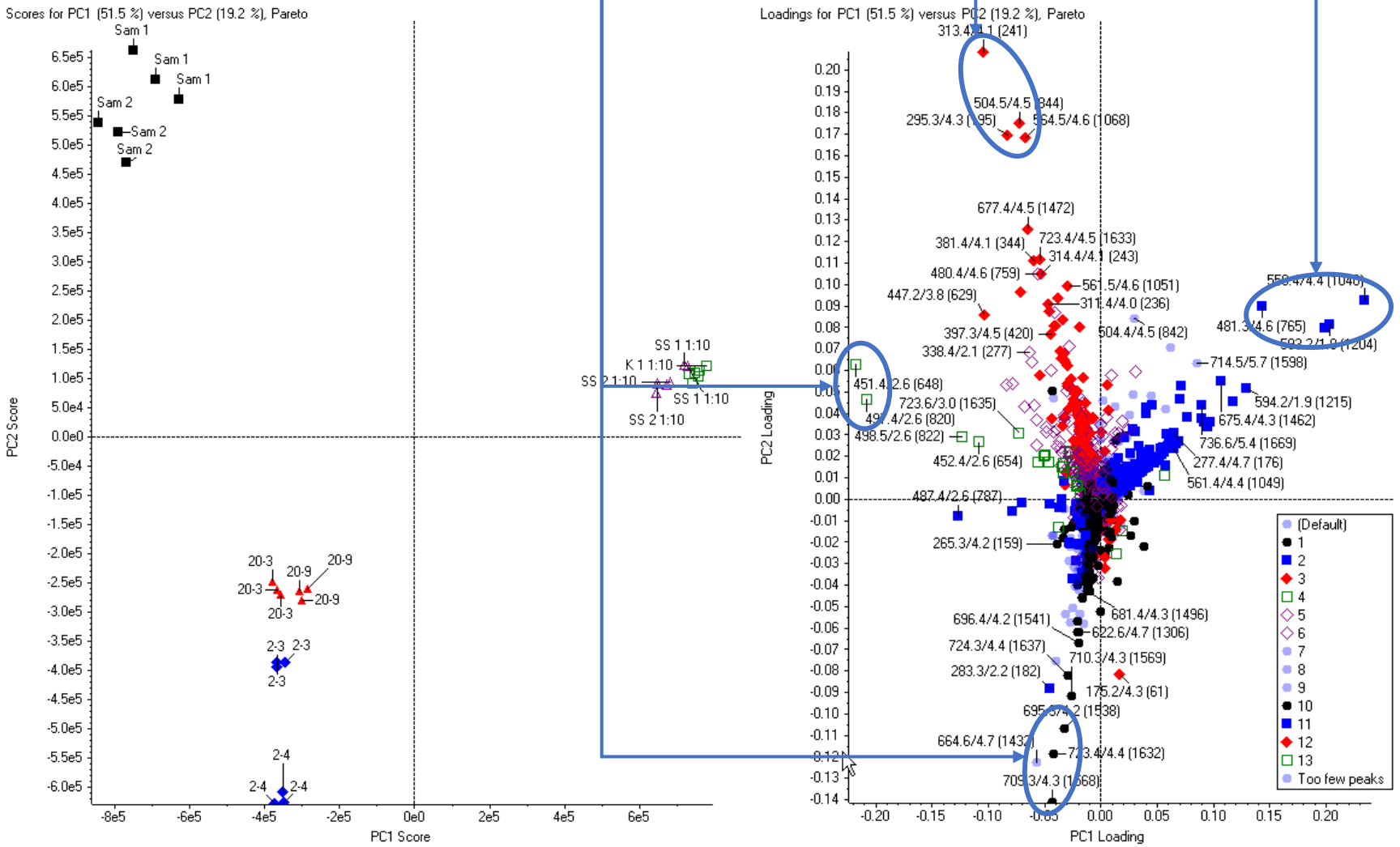
Once You have your samples separated (left) and the separation is statistically significant, big question arise – what are these?



Is it an already described compound?
Is it something completely new?
What is the chemical formula?
How the structure looks?
What are the properties of it?
What is the exact function of it?

And many more…

Instrumental methods like MS/MS

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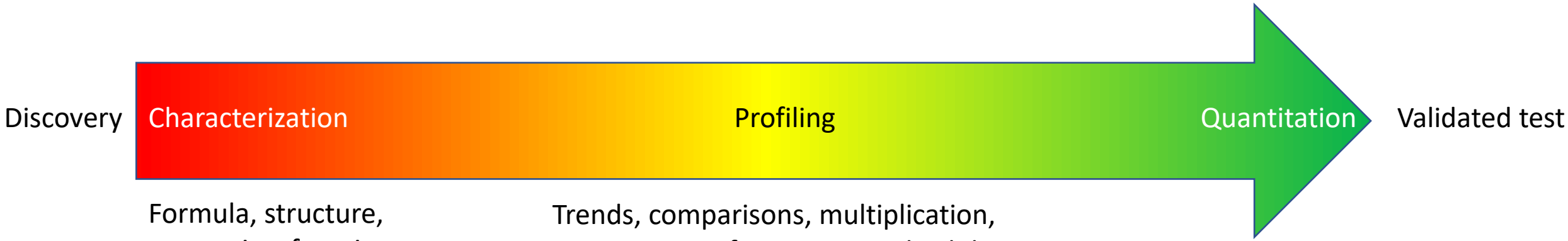
From discovery to quantitation



Formula, structure, properties, function etc.

Trends, comparisons, multiplication, statistics, significance, reproducibility, etc.

Cut-offs and what it means?

MS/MS is applicable on every step

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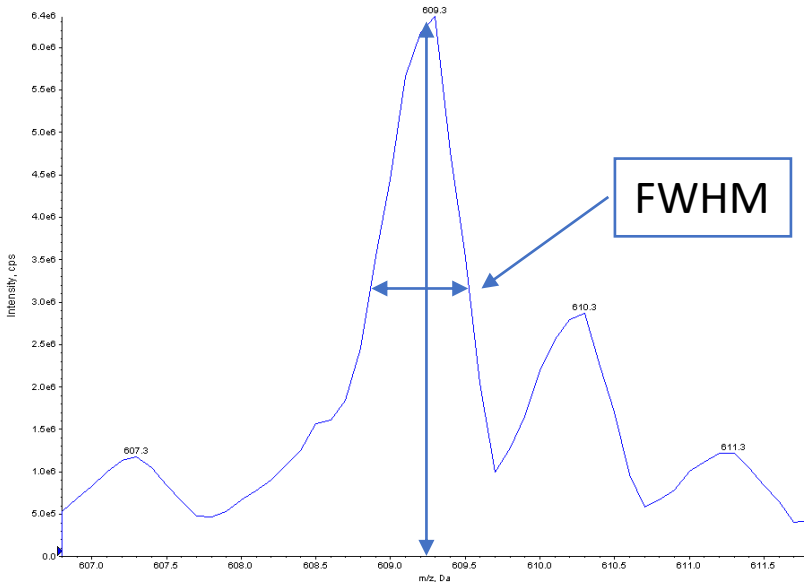
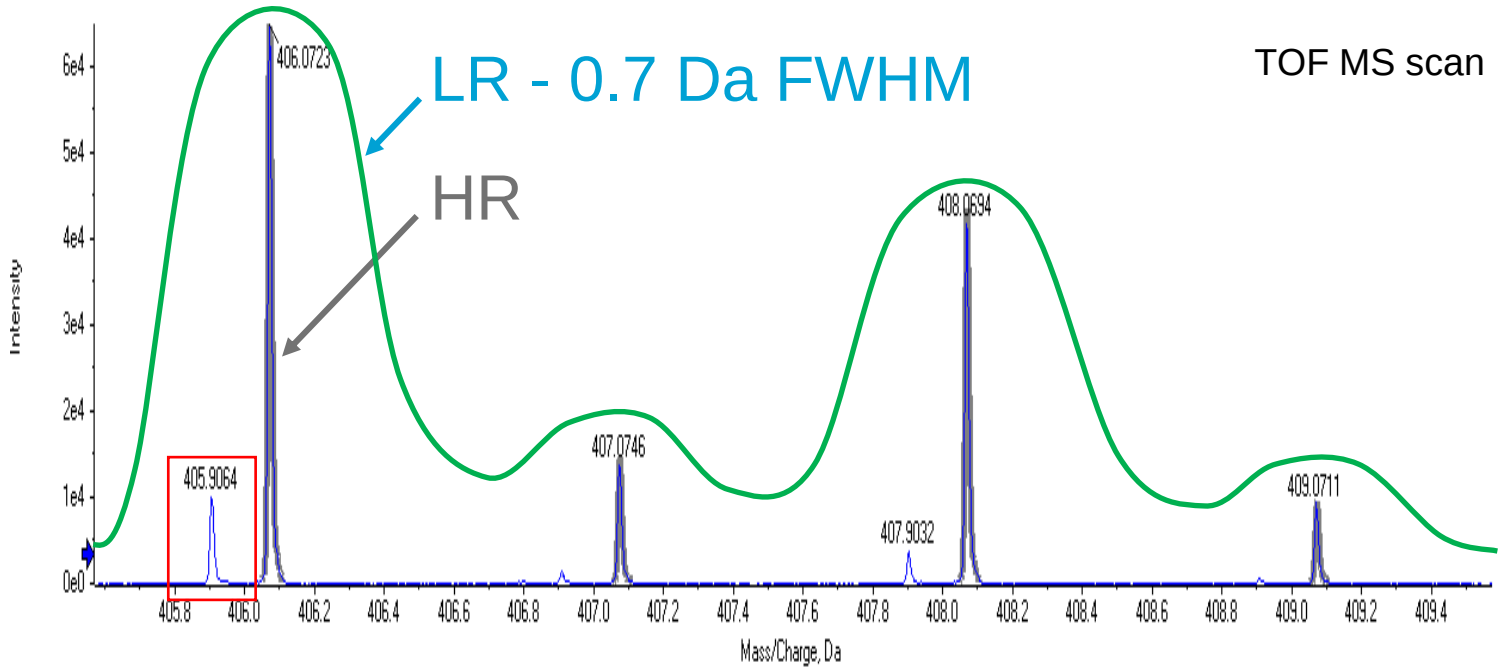
$$R = \frac{value\ m/z}{signal\ width\ at\ FWHM}$$





TOF MS scan

Low resolution mass spectrometry (LR-MS)

R < 2000

Mass accuracy – 0.1 m/z

High resolution mass spectrometry (HR-MS)

R > 20 000

Mass accuracy – 0.0001 m/z

XXXX.XXXX m/z

Nominal mass [Da, amu]

Mass defect [ppm, mDa]

LabExperts



METERIALS AND MTHODS (very briefly)

SAMPLES:

Blood, urine, cell culture, fractions, other

SAMPLE PREPARATION:

Matrix & compounds specifc – LLE, SPE with or without, dilution, concentration step

LC-MS/MS:

SCIEX ZenoTOF 7600 MS/MS, ExionAC LC

SCIEX X500R MS/MS, ExionAC LC

DDA, DIA & MRMhr scanning

SOFTWARE:

SciexOS, Molecule Profiler, ACD Labs

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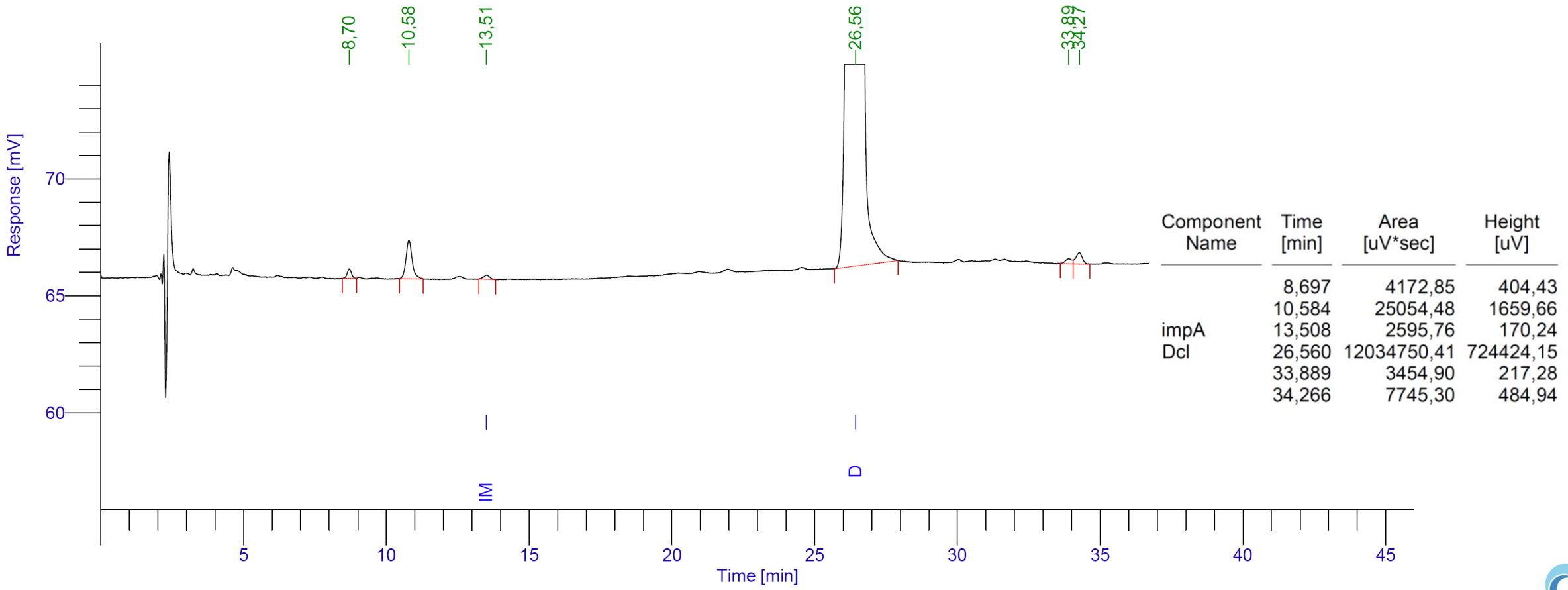
Untargeted analysis
Compound identification

Compound identification
Diclofenac

Material – liquid after chemical degradation reaction
The issue to be solved – we observe additional signals besides Diclofenac – what are these compounds?
(list and structural formulas of potential derivatives attached)



Component Name	Time [min]	Area [uV*sec]	Height [uV]
	8,697	4172,85	404,43
	10,584	25054,48	1659,66
impA	13,508	2595,76	170,24
Dcl	26,560	12034750,41	724424,15
	33,889	3454,90	217,28
	34,266	7745,30	484,94

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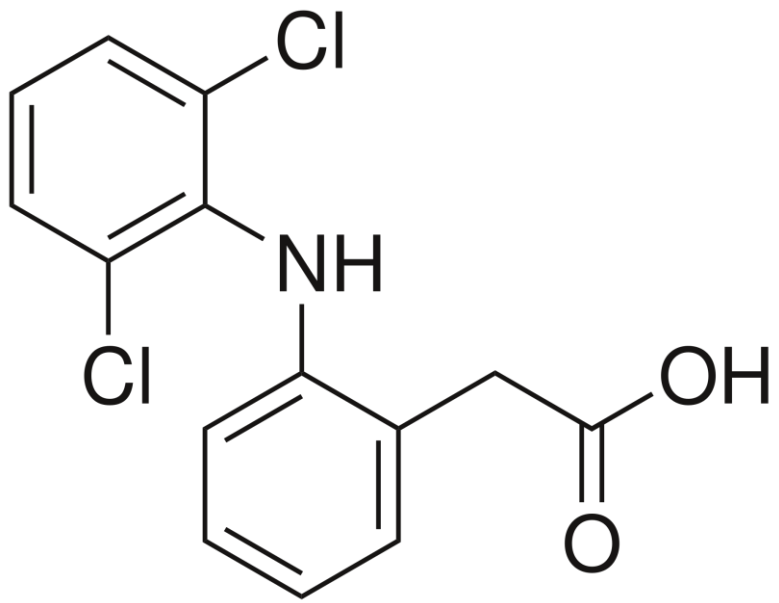
Compound identification
Diklofenak

X500R LC-MS/MS
Manual identification supported by tools available in SciexOS
Based on the available data, preliminary problems have been identified:
the part is ionized positively, the part negatively or positively and negatively,
some compounds ionize badly (poor sensitivity),
Some compounds ionize very well (risk of of detector saturation)
LC-MS/MS method (10 min.) – DDA TOF MS → TOF MS/MS (POS & NEG)
Analysis of samples in several dilutions



Chemical formula: $C_{14}H_{11}Cl_2NO_2$
MW: 296,148 g/mol

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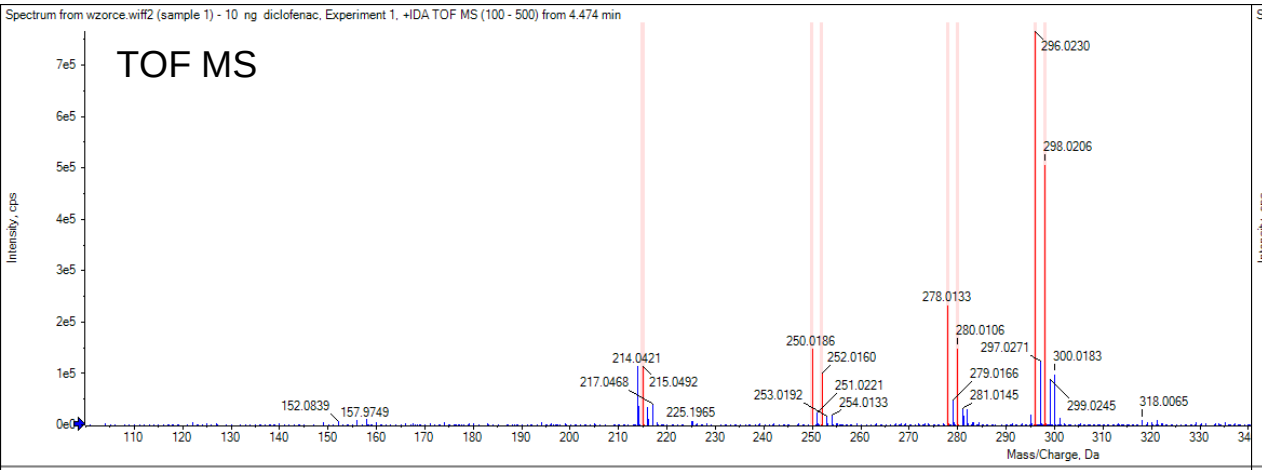
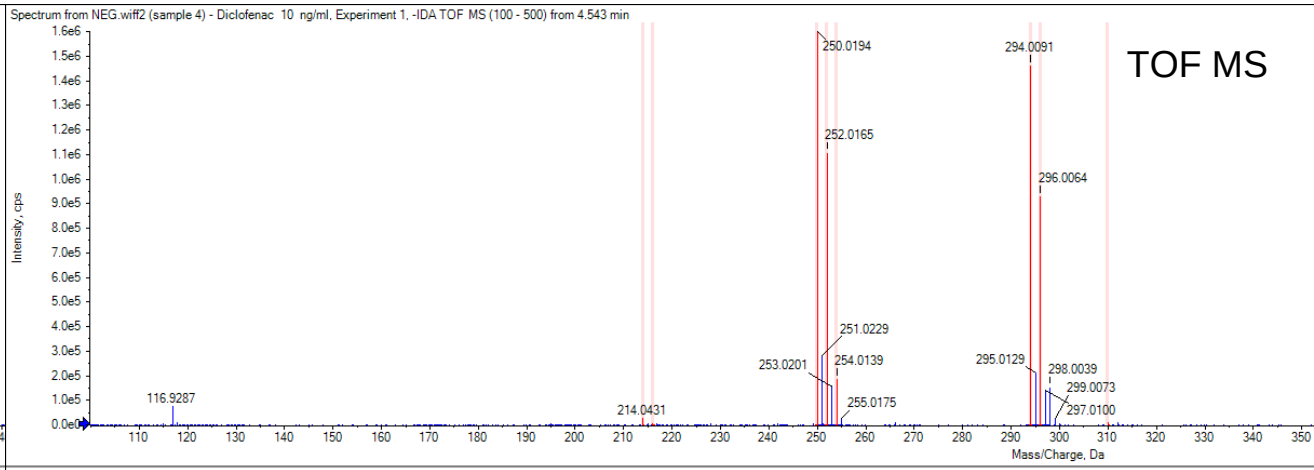
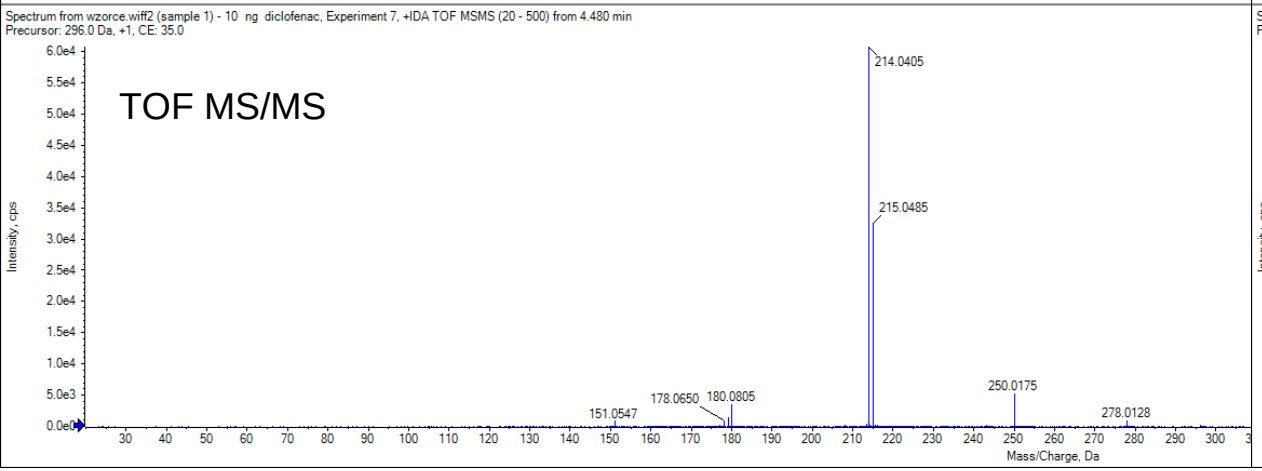
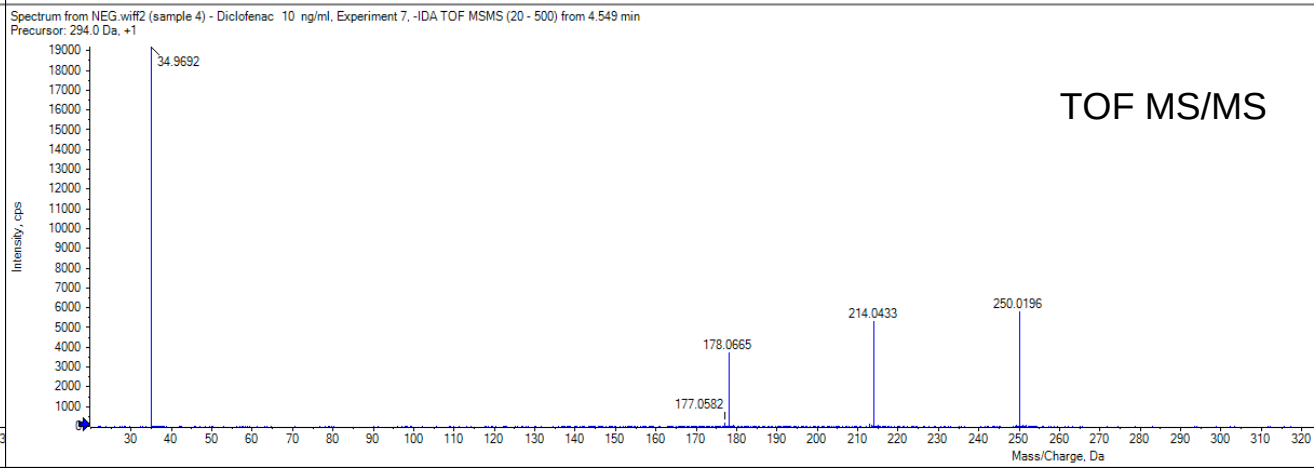
Compound identification
Diclofenac



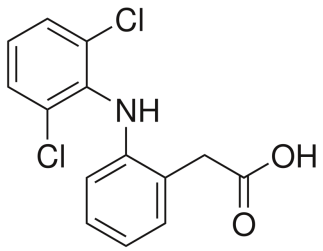






POS

NEG



Diclofenac ionizes positively and negatively
Fragmentation occurs in the ion source (!)
Fragmentation in the collision cell (CAD) does not deliver a large number of fragments

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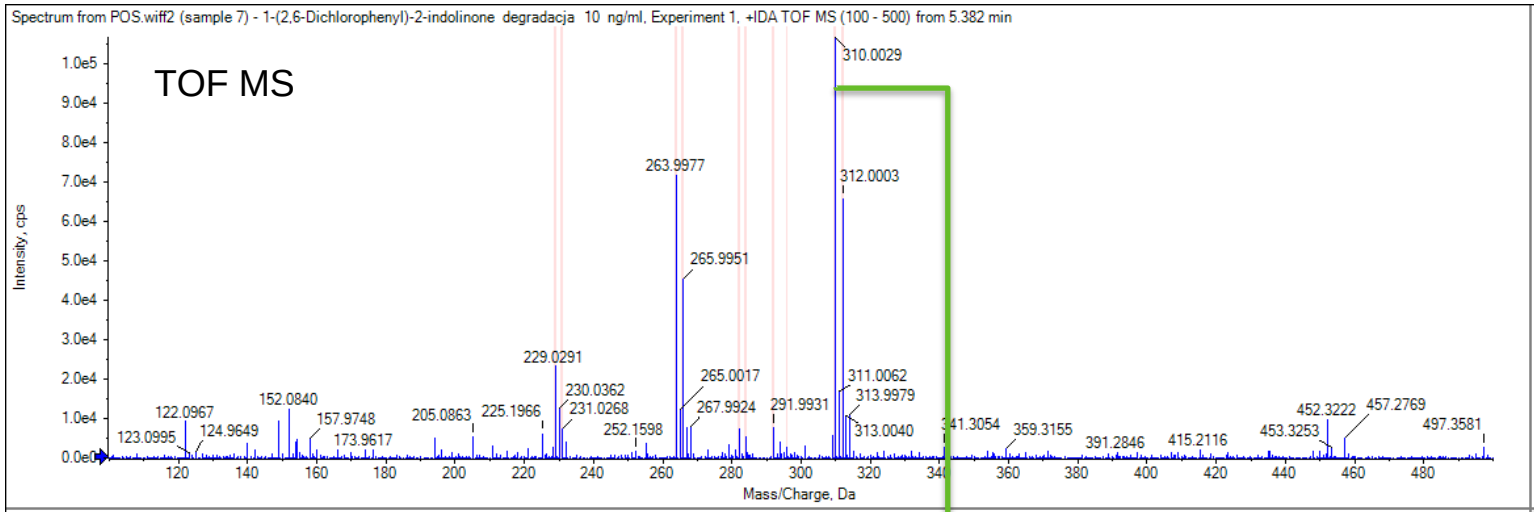
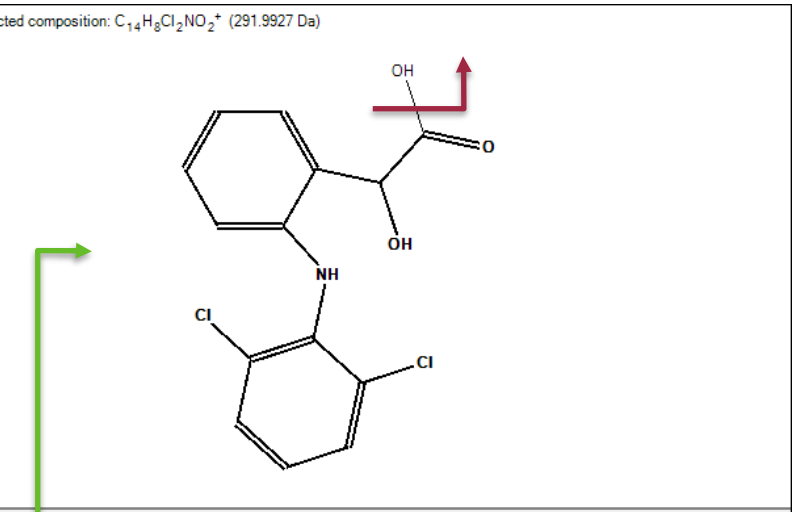
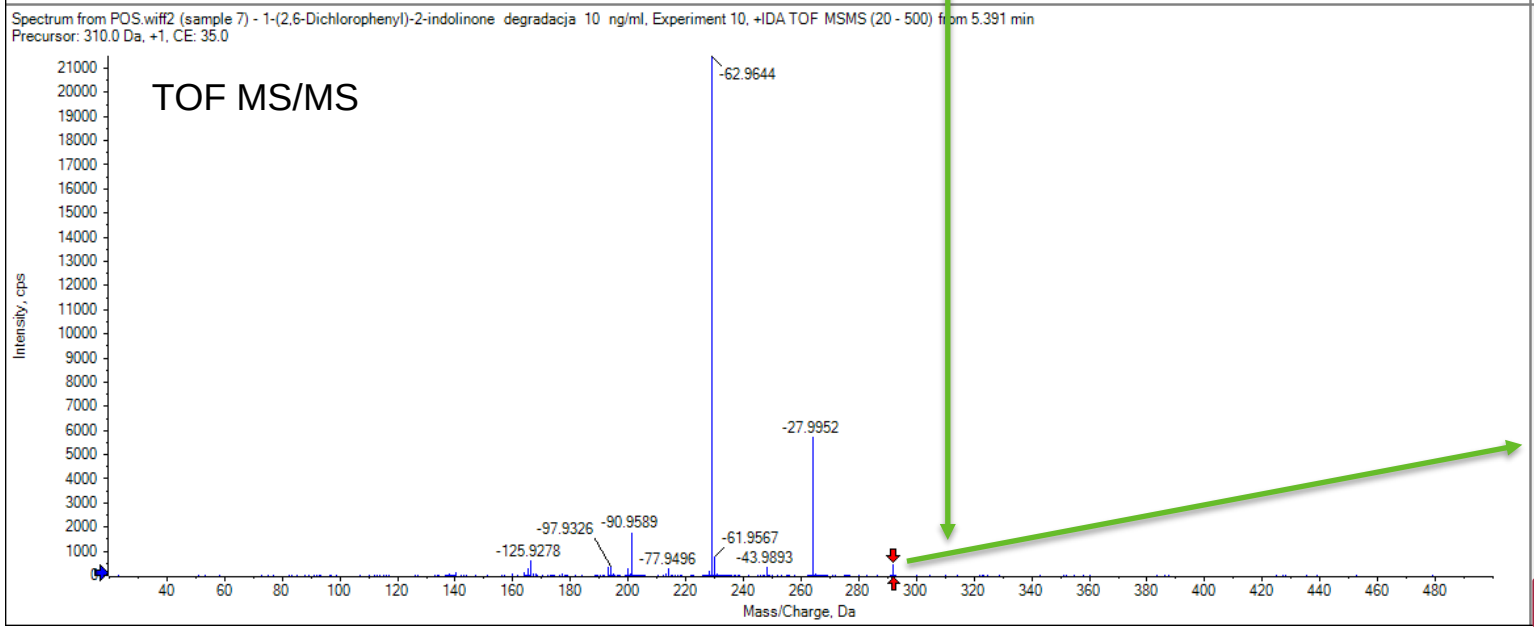
Compound identification
Diclofenac



C	N
O	P
S	F
Cl	Br
I	Na
K	Ca





Fragments | Peaks

Mass/Charge	Intensity (%)	Assigned	Error (ppm)	Radical
140.0496	0.56	☑	0.7	☐
164.0498	0.65	☑	2.0	☐
165.0572	1.49	☑	0.8	☑
166.0649	2.92	☑	1.5	☐
193.0536	1.49	☑	7.3	☑
194.0601	1.64	☑	0.2	☐
200.0263	1.30	☑	0.8	☐
201.0338	8.34	☑	0.9	☑
214.0431	1.32	☑	6.0	☐
228.0230	0.74	☑	8.3	☐
229.0282	100.00	☑	2.9	☑
230.0360	3.68	☑	3.2	☐
248.0034	1.61	☑	2.1	☐
263.9974	26.58	☑	1.3	☐
291.9930	2.12	☑	1.3	☐

Matches: 15 of 15 peaks, 100.0% of total intensity

Compound A - MS; MS/MS; fragmentation analysis → 2-(2,6-dichloroanilino)phenylglycolic acid

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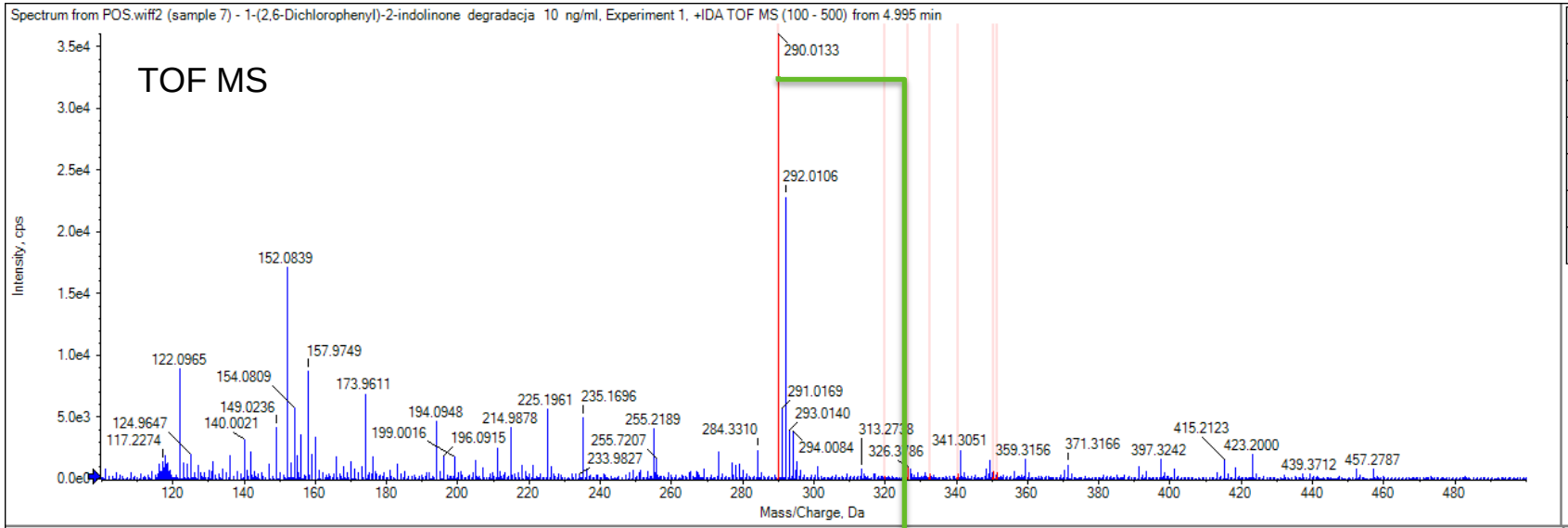
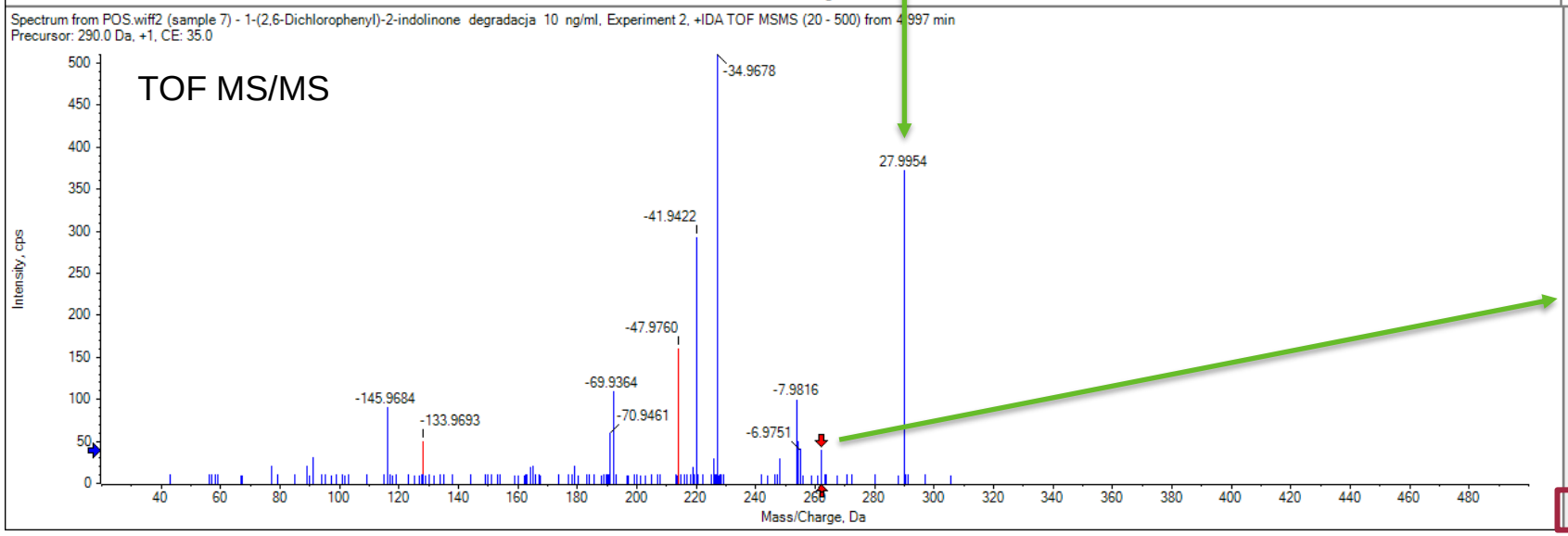
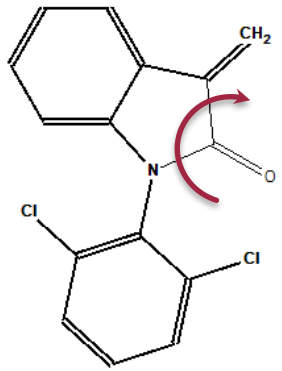
Compound identification
Diclofenac





C	N
O	P
S	F
Cl	Br
I	Na
K	Ca

C, selected composition: $C_{14}H_{10}Cl_2N^+$ (262.0185 Da)



Fragments | Peaks

Mass/Charge	Intensity (%)	Assigned	Error (ppm)	Radical
116.0501	17.65	☑	5.1	☐
128.0492	9.80	☐		■
191.0724	11.76	☑	2.7	☑
192.0820	21.57	☑	6.6	☐
214.0425	31.37	☐		■
220.0763	57.25	☑	2.8	☐
227.0507	100.00	☑	4.7	☑
254.0369	19.61	☑	0.6	☐
255.0434	7.84	☑	4.5	☑
262.0179	7.84	☑	2.3	☐
290.0139	72.94	☑	1.6	☐

Matches: 9 of 11 peaks, 88.5% of total intensity

Compound C - MS; MS/MS ; fragmentation analysis → 1-(2,6-dichlorophenyl)-3-methylideneindol-2-one

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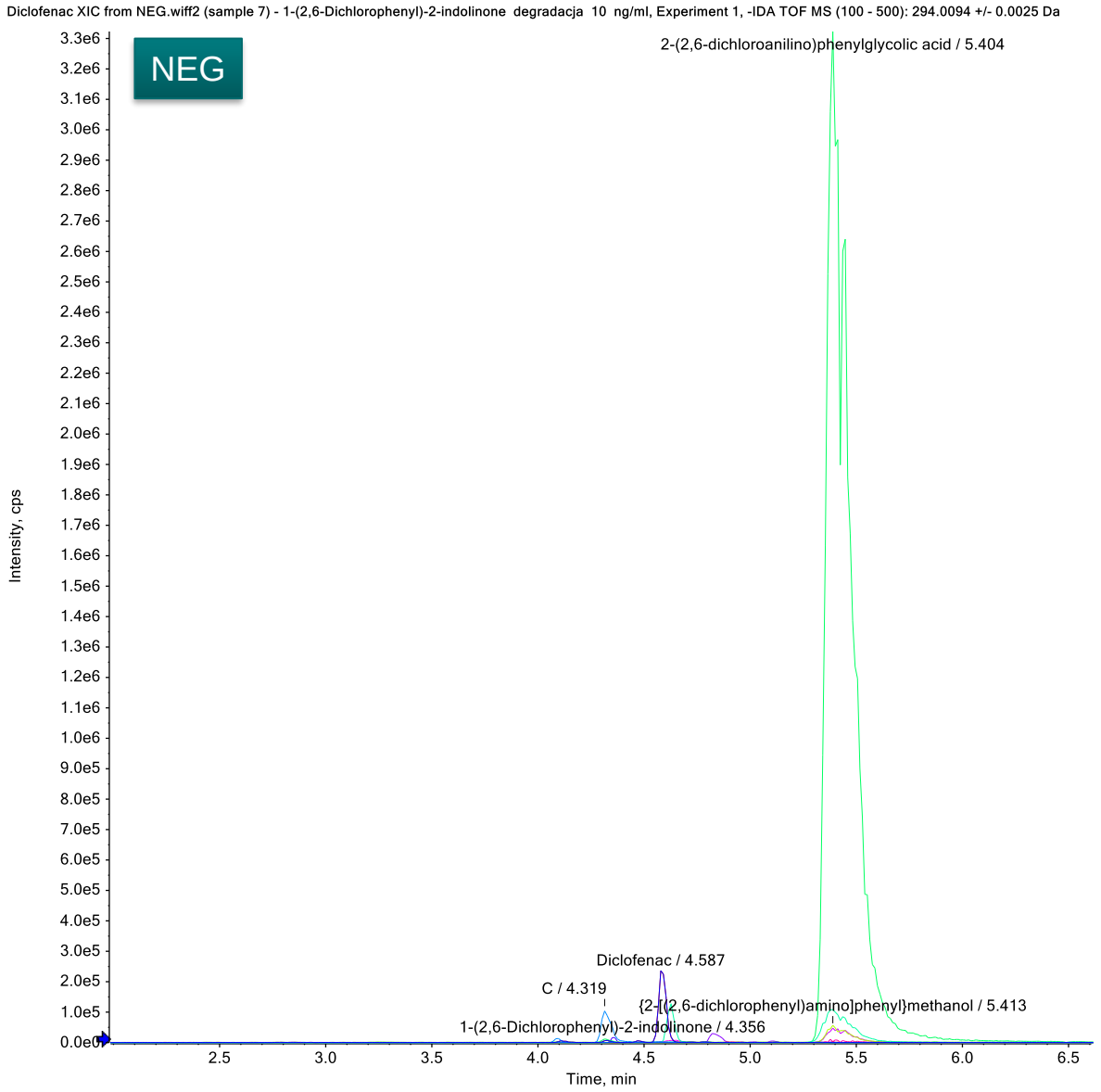
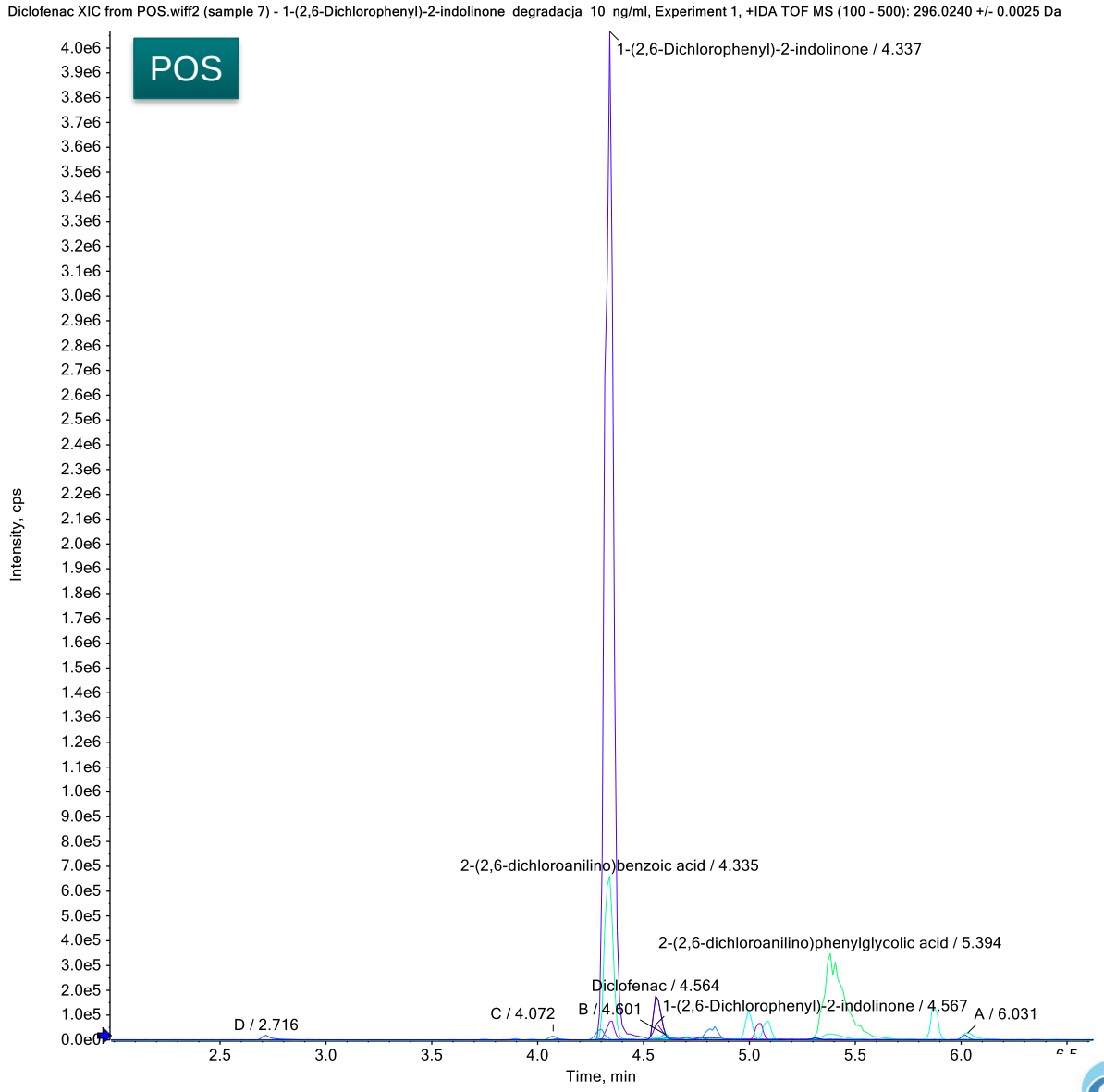
Compound identification
Diclofenac





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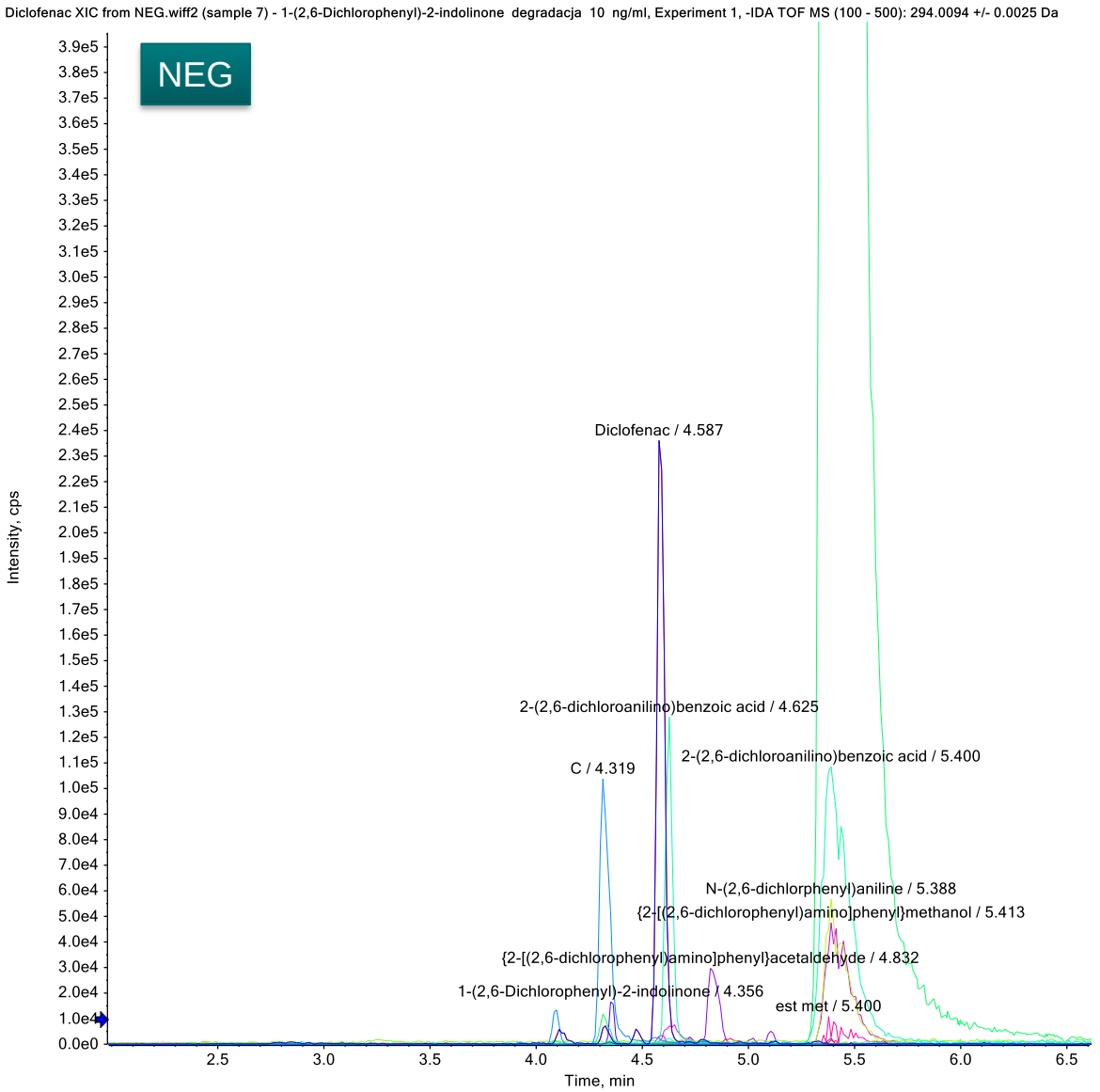
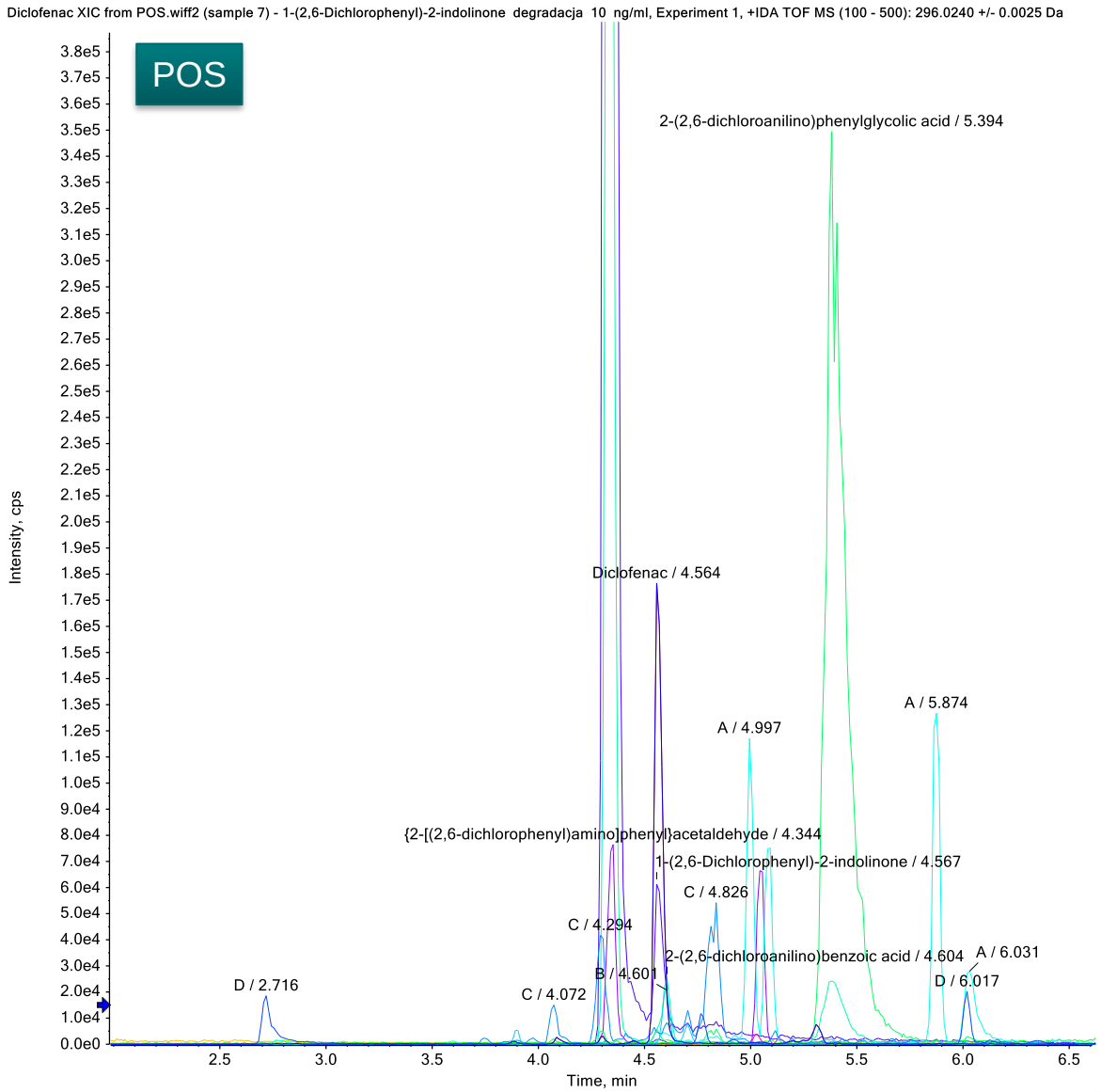
Compound identification
Diclofenac





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Compound identification
Diclofenac

Nazwa	RT	Wzór sumaryczny	m/z		Dane potwierdzające				ID
			POS	NEG	POS		NEG		
					MS	MS/MS	MS	MS/MS	
Diclofenac	4.56	C14H11Cl2NO2	296.024	294.0094	+	+	+	+	Diclofenac
1-(2,6-Dichlorophenyl)-2-indolinone	4.34	C14H9Cl2NO	278.0134	275.9988	+	+	-	-	1-(2,6-Dichlorophenyl)-2-indolinone
{2-[(2,6-dichlorophenyl)amino]phenyl}acetaldehyde	5.05	C14H11Cl2NO	280.029	278.0145	+	+	+/-	-	{2-[(2,6-dichlorophenyl)amino]phenyl}acetaldehyde
{2-[(2,6-dichlorophenyl)amino]phenyl}methanol	-	C13H11Cl2NO	268.029	266.0145	-	-	-	-	
Diclofenac ester etylowy	4.64	C16H15Cl2NO2	324.0553	322.047	-	-	+/-	-	Diclofenac ester etylowy
Diclofenac ester metylowy	-	C15H13Cl2NO2	310.0396	308.0251	-	-	-	-	
Diclofenac ester propylowy	-	C17H17Cl2NO2	338.0709	336.0564	-	-	-	-	
2,6-Dichloroaniline	-	C6H5Cl2N	161.9872	159.9726	-	-	-	-	
N-Phenyl-(2,6-dichlorophenoxy)acetamide	5.312	C14H11Cl2NO2	296.024	294.0094	+	+/-	-	-	N-Phenyl-(2,6-dichlorophenoxy)acetamide
2,6-dichlorobenzoic acid	-	C7H4Cl2O2	190.9661	188.9516	-	-	-	-	
2,6-dichlorobenzamide	-	C7H5Cl2NO	189.9821	187.9675	-	-	-	-	
N-(2,6-dichlorphenyl)aniline	-	C12H9Cl2N	238.0185	236.0039	-	-	-	-	
Phenylacetic acid	-	C8H8O2	137.0597	135.0452	-	-	-	-	
1,3-Dichlorobenzen	-	C6H4Cl2	146.9763	144.9617	-	-	-	-	
(2-aminophenyl)acetic acid	-	C8H9NO2	152.0706	150.0561	-	-	-	-	
A	5.39	C14H9Cl2NO3	310.0032	307.9888	+	+	+	+	2-(2,6-dichloroanilino)phenylglycolic acid
B	4.6	C13H9Cl2NO2	282.0083	279.9938	+	+	+	+/-	2-(2,6-dichloroanilino)benzoic acid
C	5.0; 5.08; 5.87; 6.03	C15H9Cl2NO	290.0134	287.9988	+	+	-	-	1-(2,6-dichlorophenyl)-3-methylideneindol-2-one (3Z)-3-[(2,6-dichlorophenyl)methylidene]-1H-indol-2-one
D	3.9; 4.6; 4.7	C16H11Cl2NO2	320.024	318.094	+	+/-	-	-	ethynyl {2-[(2,6-dichlorophenyl)amino]phenyl}acetate
E	4.07	C15H9Cl2NO2	306.0083	303.9938	+	+/-	+	+/-	1-(2,6-dichlorophenyl)-1H-indole-3-carboxylic acid
F	2.72; 4.77	C16H11Cl2NO	304.029	302.0145	+	+/-	-	-	?

Compounds not present in the customer list

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Compound identification Hydroxyzine

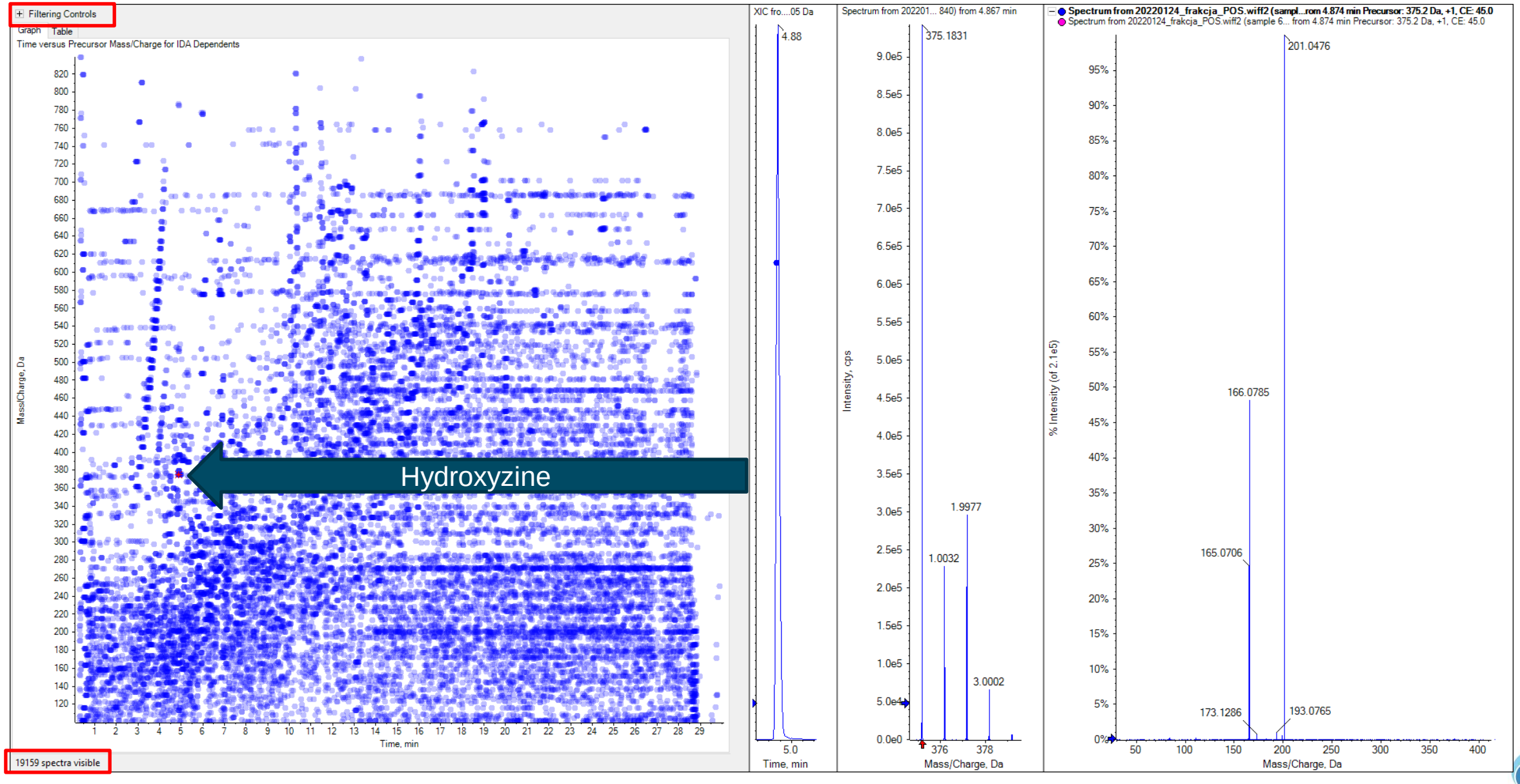
Material – liquid – HPLC sample/extract and HPLC fraction.
The problem to be solved – identification of contamination, which in the 55 min. isocratic method occurs every second analysis from the injection of the test sample → RT = 90 min.

Manual identification supported by tools available in SciexOS
Literature and other available materials → XIC list

ZenoTOF LC-MS/MS
LC-MS/MS method (30 min.) – DDA TOF MS → TOF MS/MS (POS & NEG)

Key results were obtained in collected fraction analysis 90 min +/- 1 min
Samples were concentrated and dissolved in LC mobile phase

Compound identification
Hydroxyzine



Each dot is the MS/MS spectrum – under each dot are XIC data (chromatographic peak, TOF MS, TOF MS/MS)

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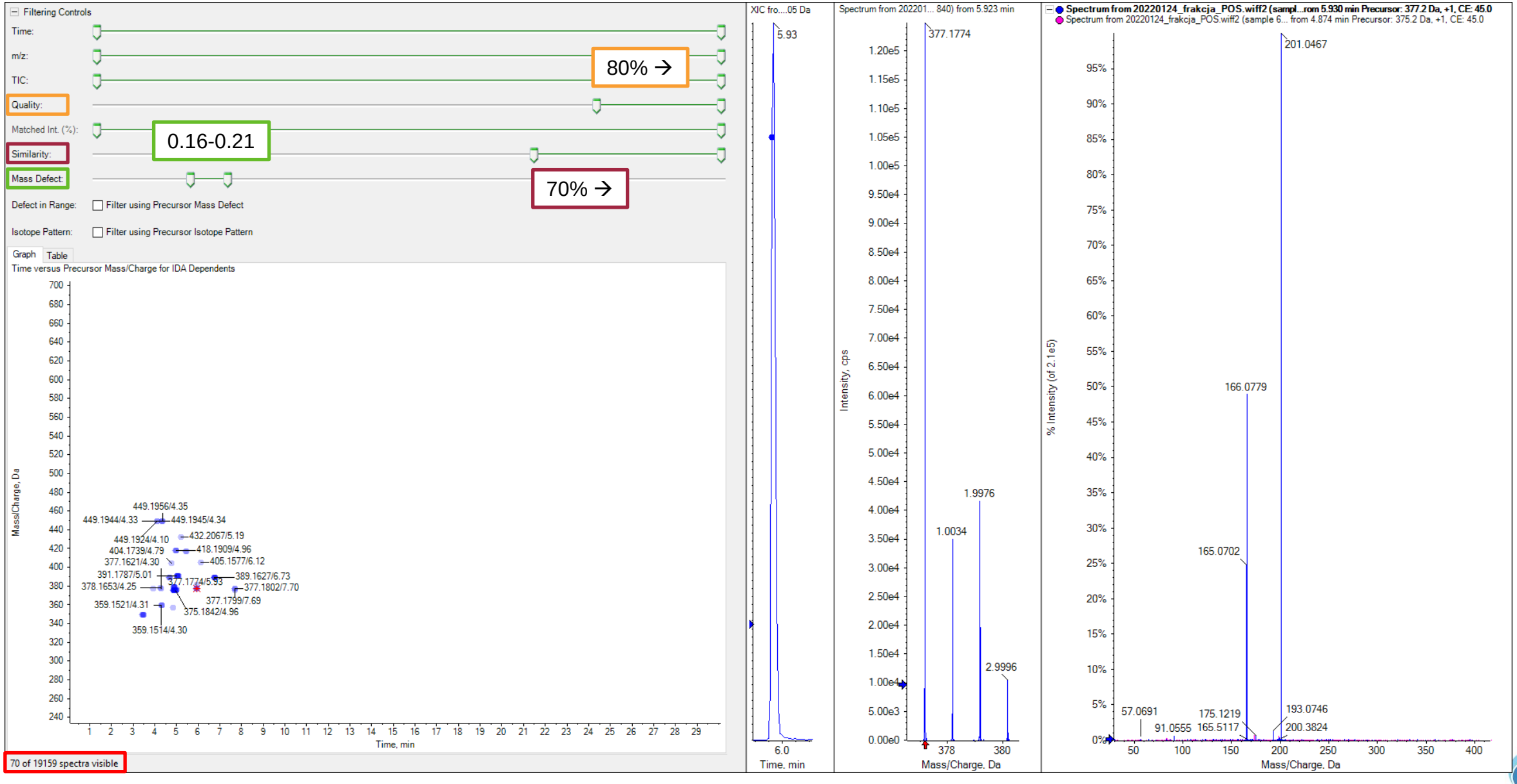
Compound identification
Hydroxyzine



After applying filters - the results are narrowed down to compounds with a similar mass defect and similar fragmentation to hydroxyzine.

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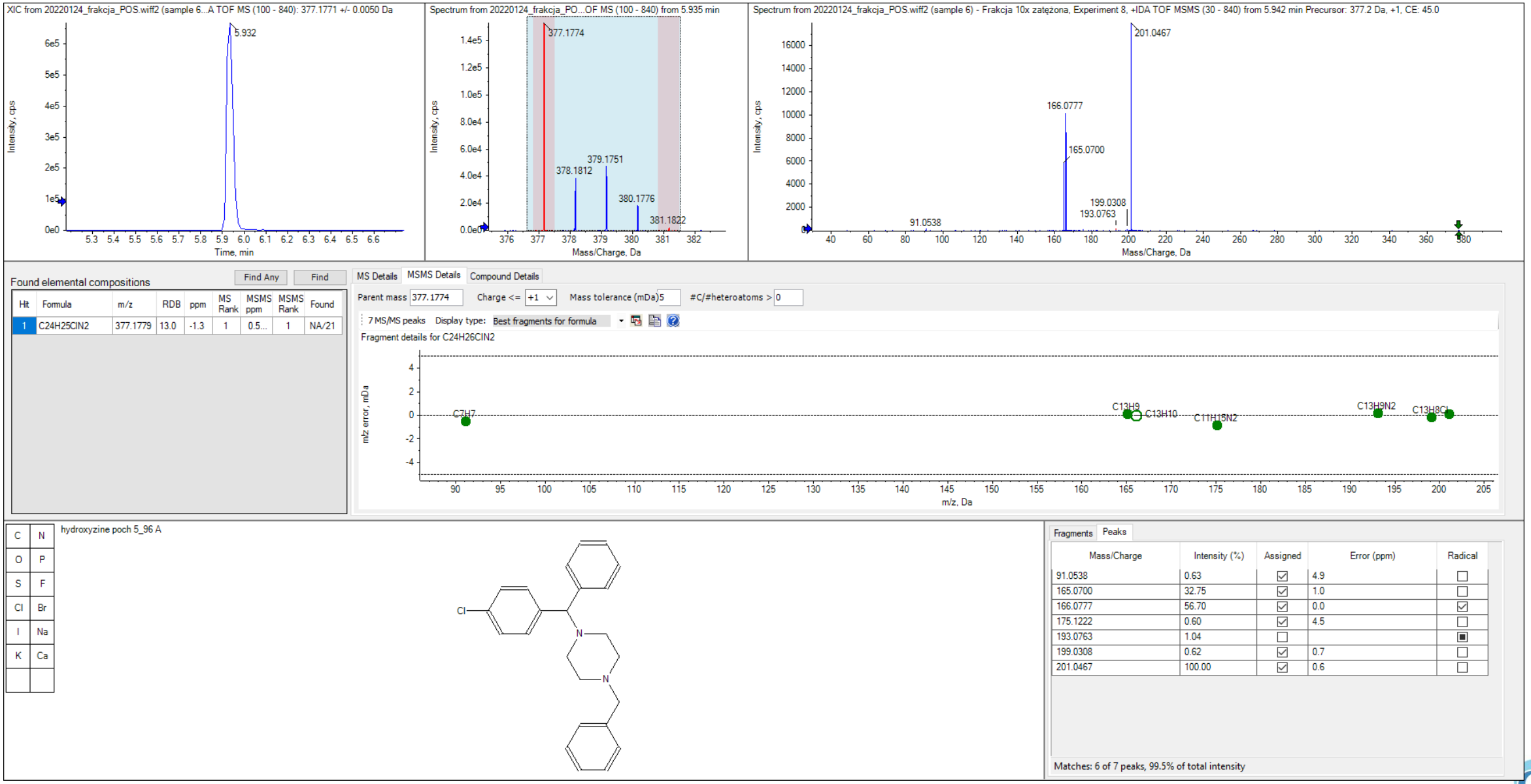
Compound identification
Hydroxyzine



Of particular interest was 1-Benzyl-4-[(4-chlorophenyl)-phenylmethyl]piperazine (a hydroxyzine derivative).

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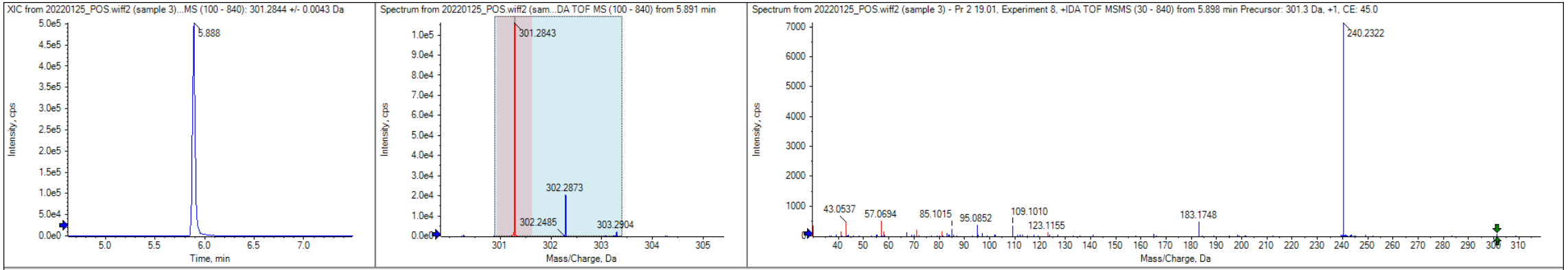
Compound identification
Hydroxyzine



Found elemental compositions

Find Any | Find

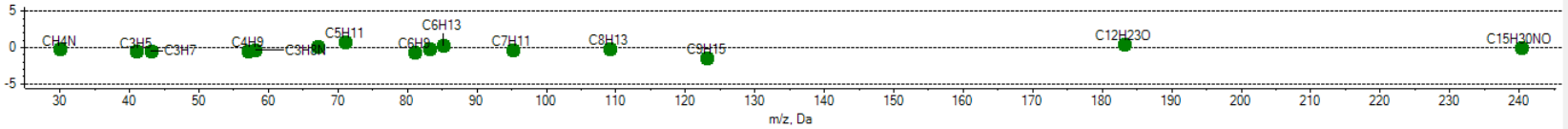
Hit	Formula	m/z	RDB	ppm	MS Rank	MSMS ppm	MSMS Rank	Found
1	C17H36N2O2	301.2850	1.0	-2.2	1	1.4...	1	NA/24

MS Details | MSMS Details | Compound Details

Parent mass 301.2843 | Charge <= +1 | Mass tolerance (mDa) 5 | #C/#heteroatoms > 0

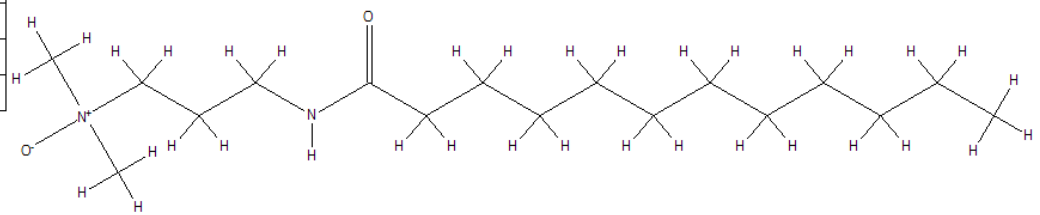
15 MS/MS peaks | Display type: All

Fragment details for C17H37N2O2



C	N
O	P
S	F
Cl	Br
I	Na
K	Ca

LAPAO



Fragments | Peaks

Mass/Charge	Intensity (%)	Assigned	Error (ppm)	Radical
30.0336	4.54	☐		■
41.0380	1.94	☐		■
43.0537	6.52	☐		■
57.0694	7.17	☐		■
58.0647	2.01	☐		■
67.0544	1.60	☑	2.1	☐
71.0864	2.72	☐		■
81.0692	1.82	☐		■
83.0854	1.36	☑	1.5	☐
85.1015	3.25	☑	3.4	☐
95.0852	5.17	☑	3.5	☐
109.1010	4.56	☑	1.2	☐
123.1155	1.50	☐		■
183.1748	6.66	☑	2.5	☐
240.2322	100.00	☑	0.1	☐

Matches: 7 of 15 peaks, 81.3% of total intensity

POS/NEG – many compounds of this type in the tested fraction.

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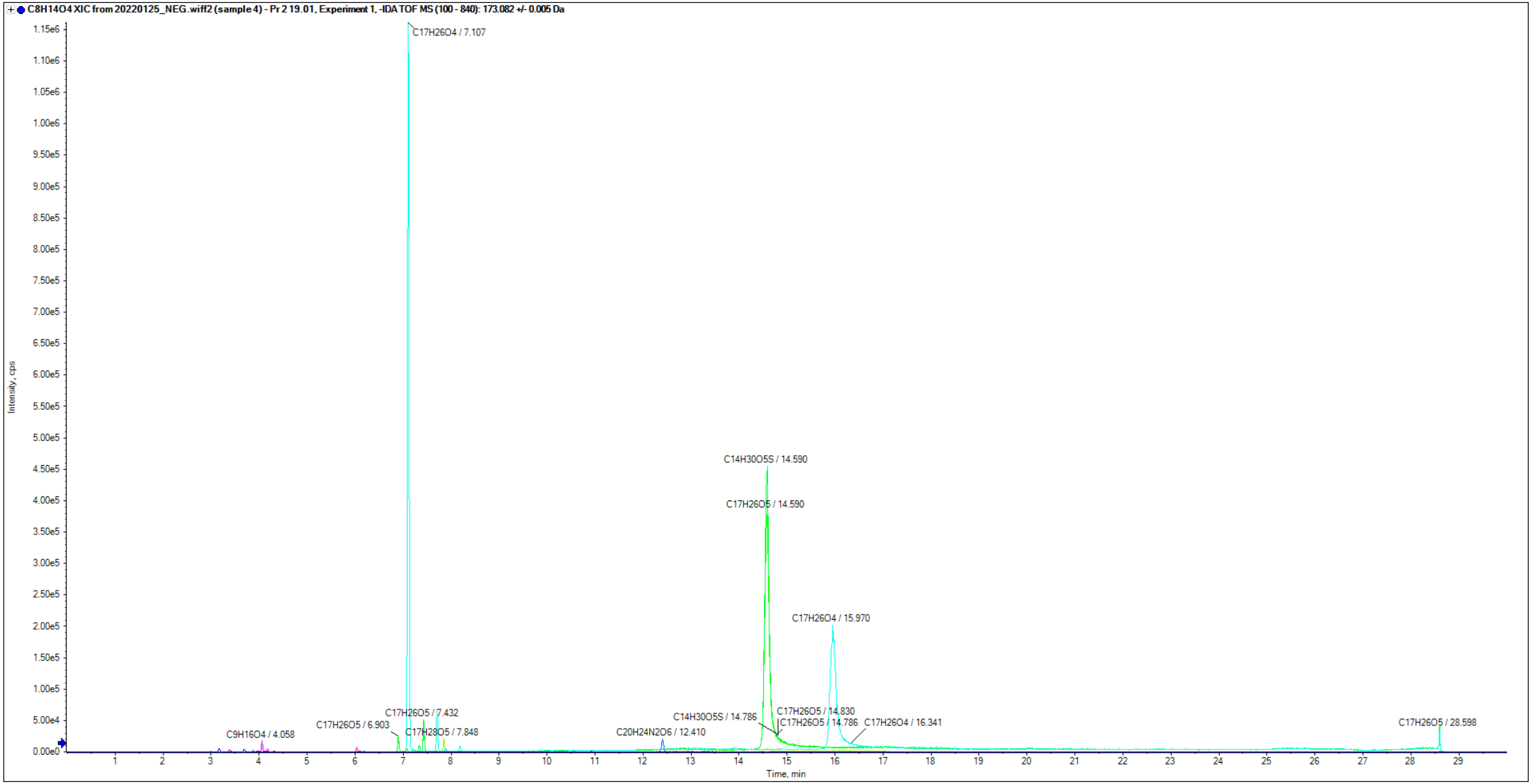
Compound identification
Hydroxyzine



XIC NEG – Analysis of HPLC 90 min. fraction.

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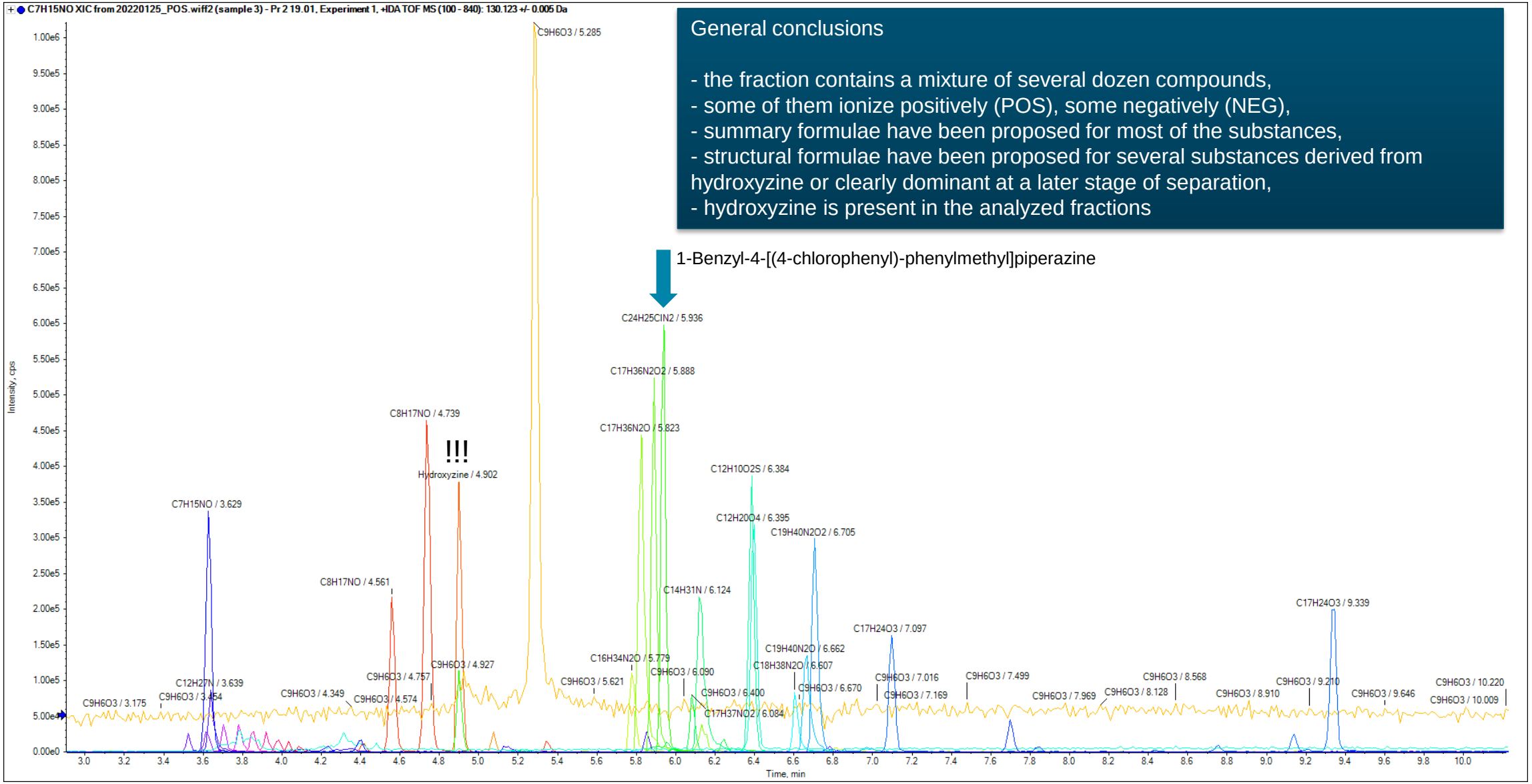
Compound identification
Hydroxyzine



General conclusions

- the fraction contains a mixture of several dozen compounds,
- some of them ionize positively (POS), some negatively (NEG),
- summary formulae have been proposed for most of the substances,
- structural formulae have been proposed for several substances derived from hydroxyzine or clearly dominant at a later stage of separation,
- hydroxyzine is present in the analyzed fractions

1-Benzyl-4-[(4-chlorophenyl)-phenylmethyl]piperazine

XIC POS – Analysis of HPLC 90 min. fraction.

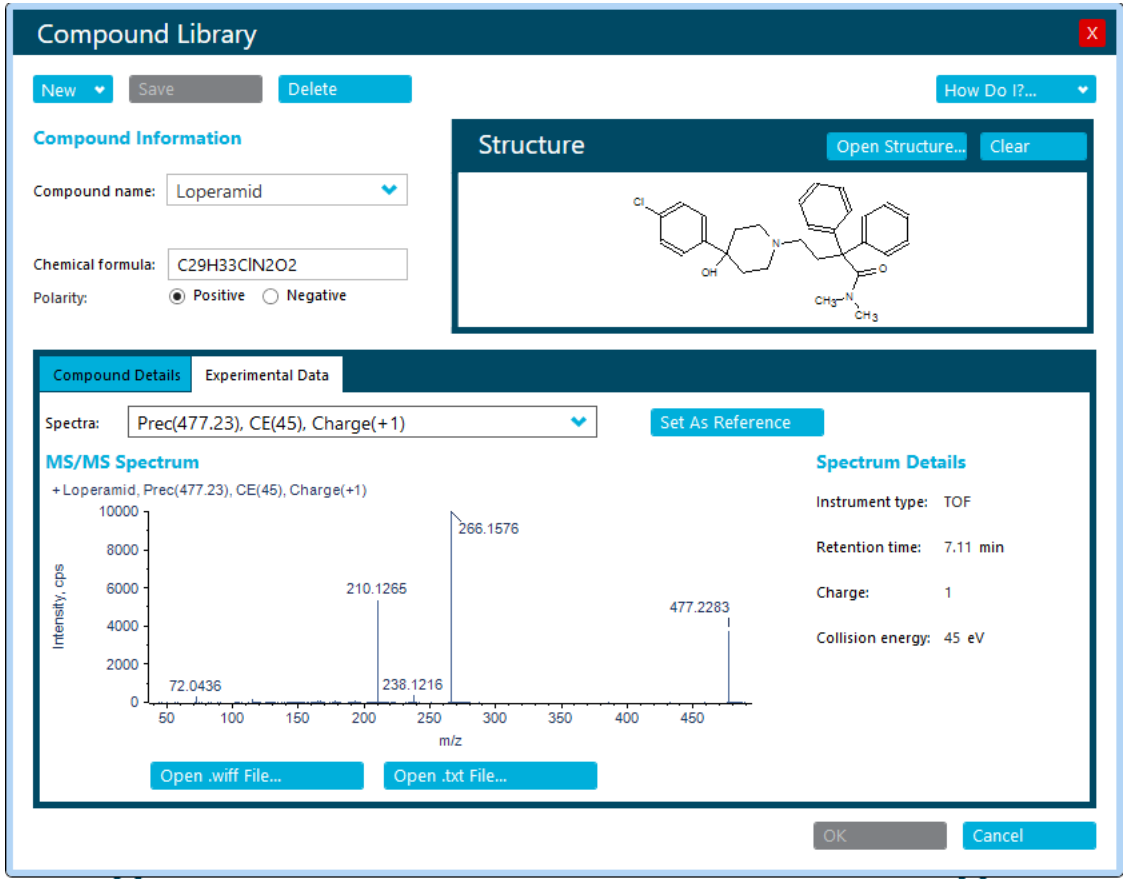
Compound identification
Loperamid – semi-automatic approach

MoleculeProfiler – software supported semi-automatic metabolite identification:

- Substrate knowledge needed (structure, MS & MS/MS data)
- Identification, interpretation, visualisation and profiling:
  - Small-molecule metabolites
  - Molecular weight catabolites (e.g. peptides & PTMs)
  - ADC – "antibody-drug-conjugates" – metabolites, catabolites and structure

Material: LLC extracts form cell line culture diluted with mobile phase

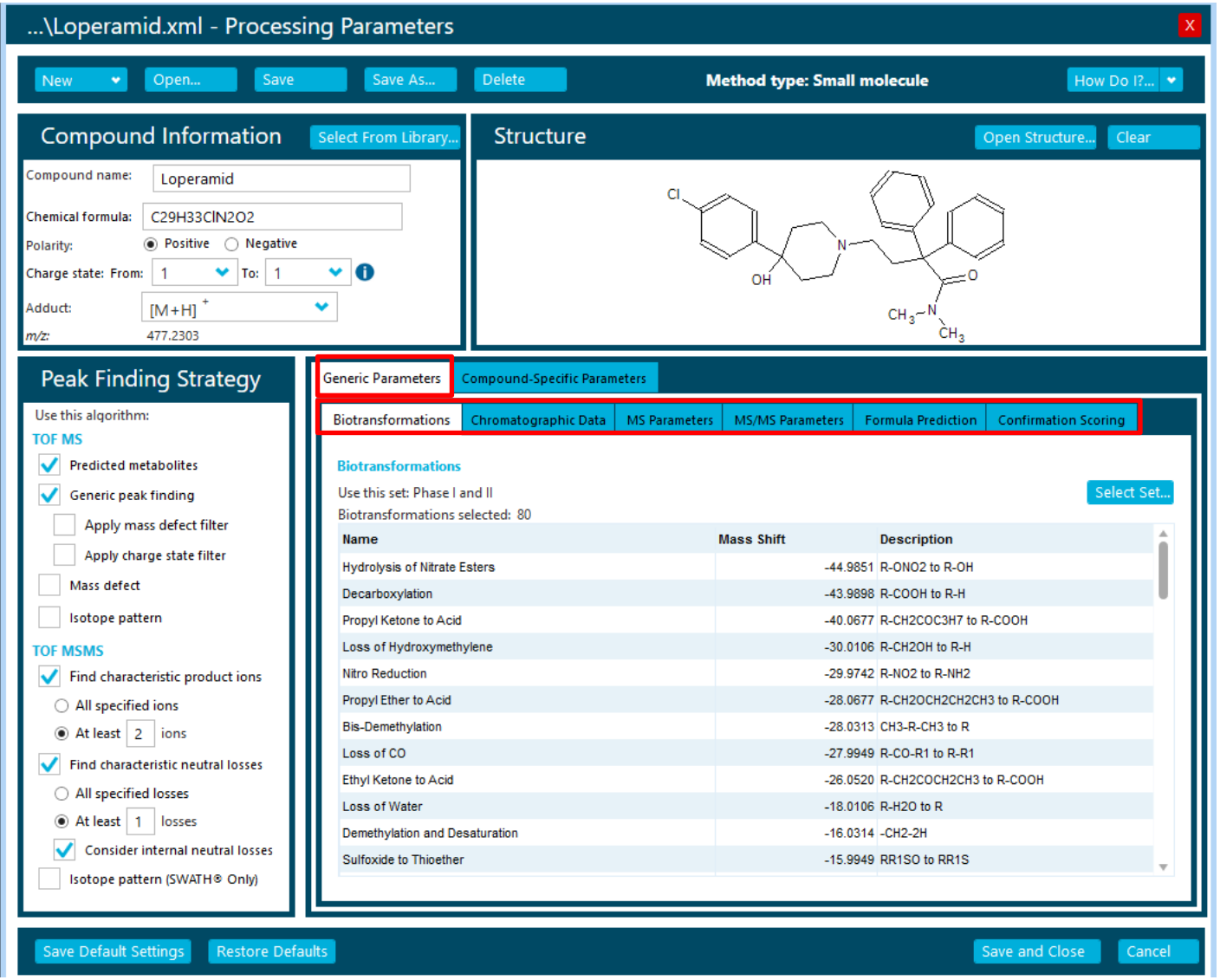
X500R LC-MS/MS
LC-MS/MS method (15 min.) – DIA TOF MS → SWATH MS/MS (POS)
30 SWATH MS/MS windows (100 – 1000 m/z range covered)

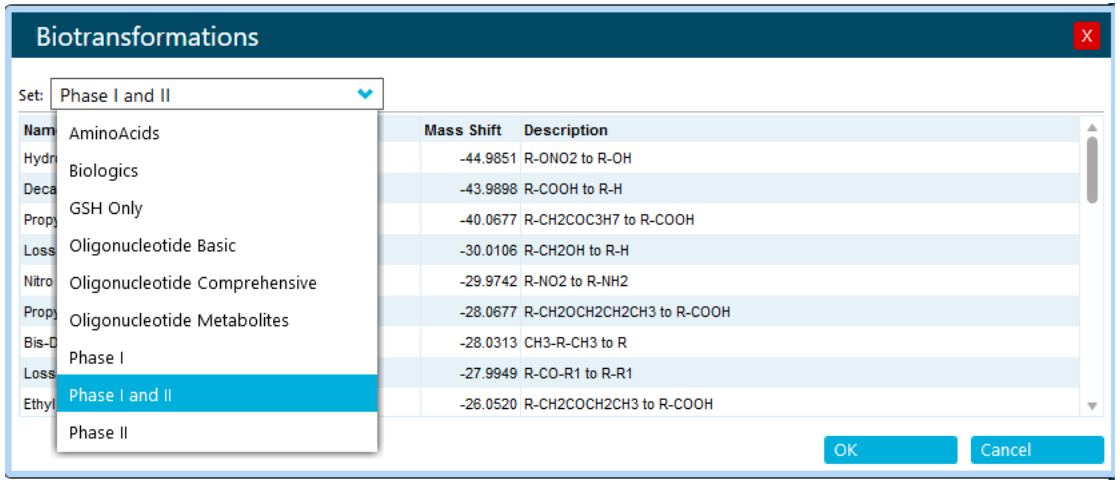


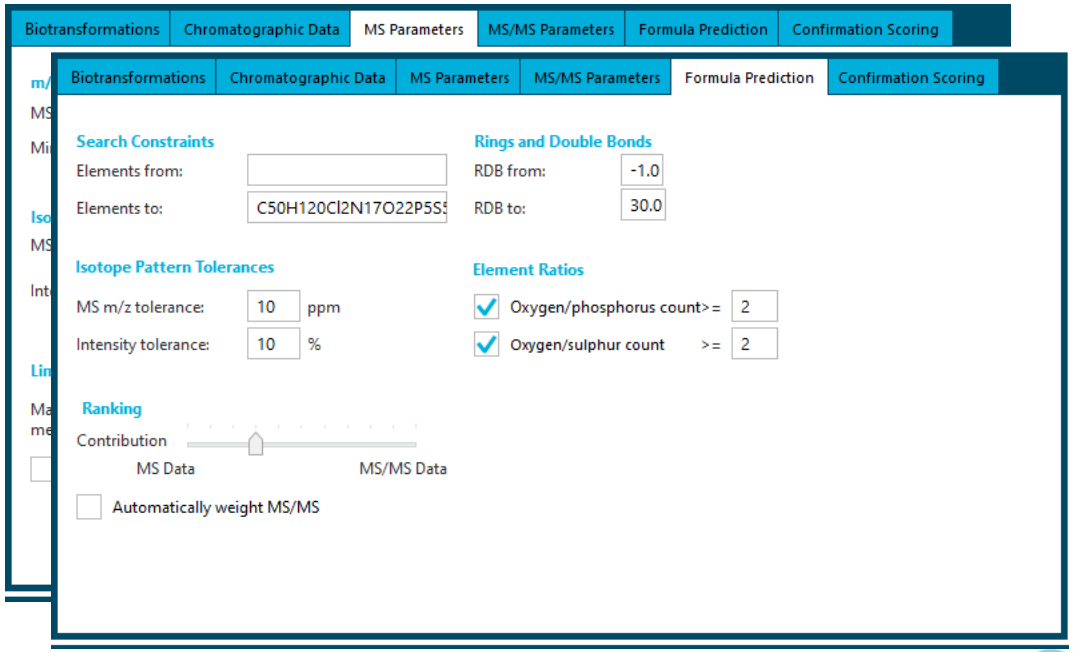
Step 1 – Chemical formula & structure - MS, MS/MS data.

LabExperts
professionals in laboratory

Compound identification
Loperamid – semi-automatic approach



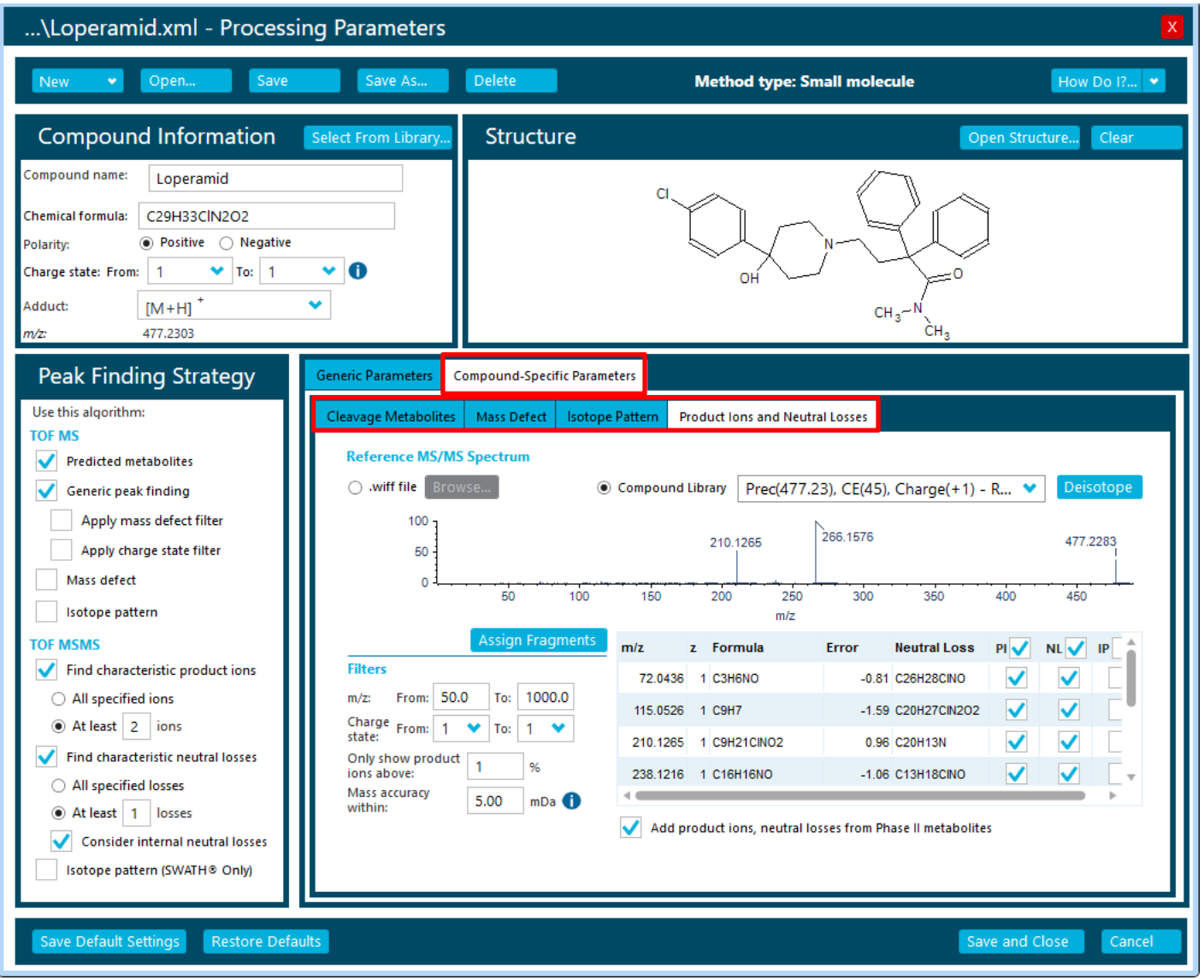


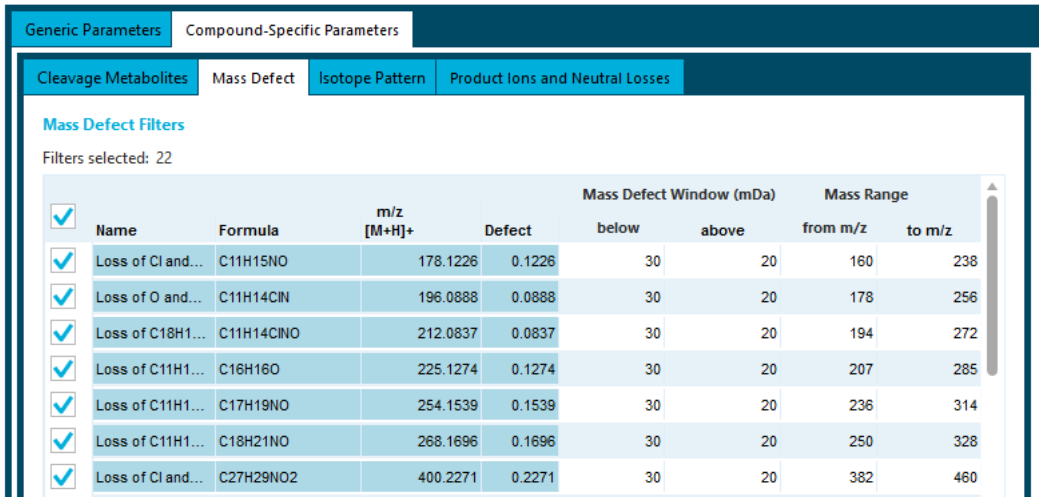


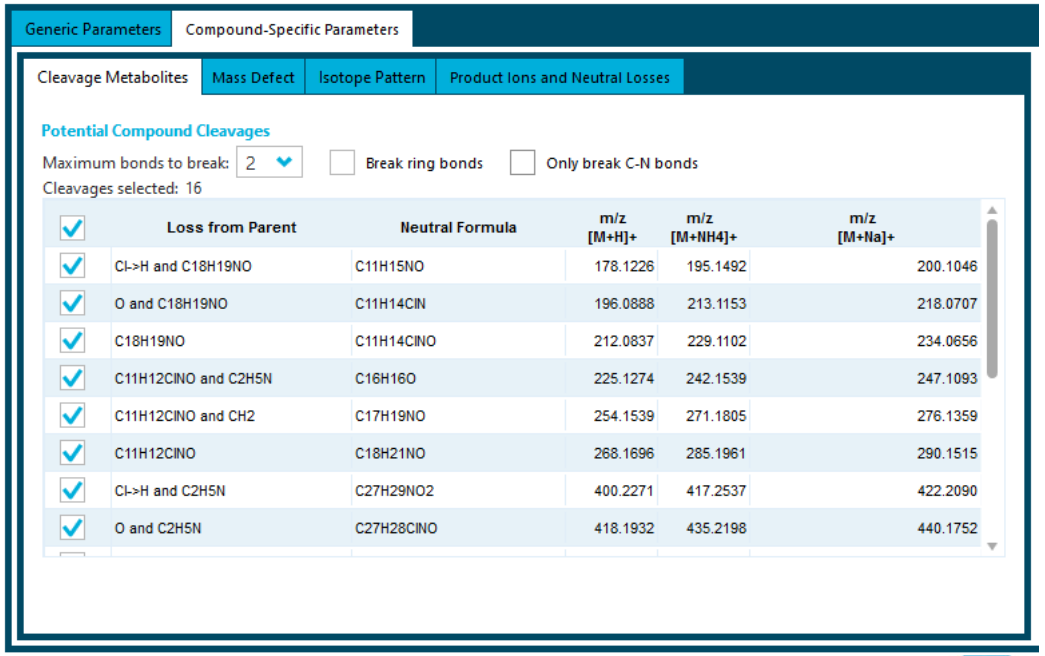
Set-up of parameters of the method of searching for metabolites.

LabExperts
professionals in laboratory

Compound identification
Loperamid – semi-automatic approach



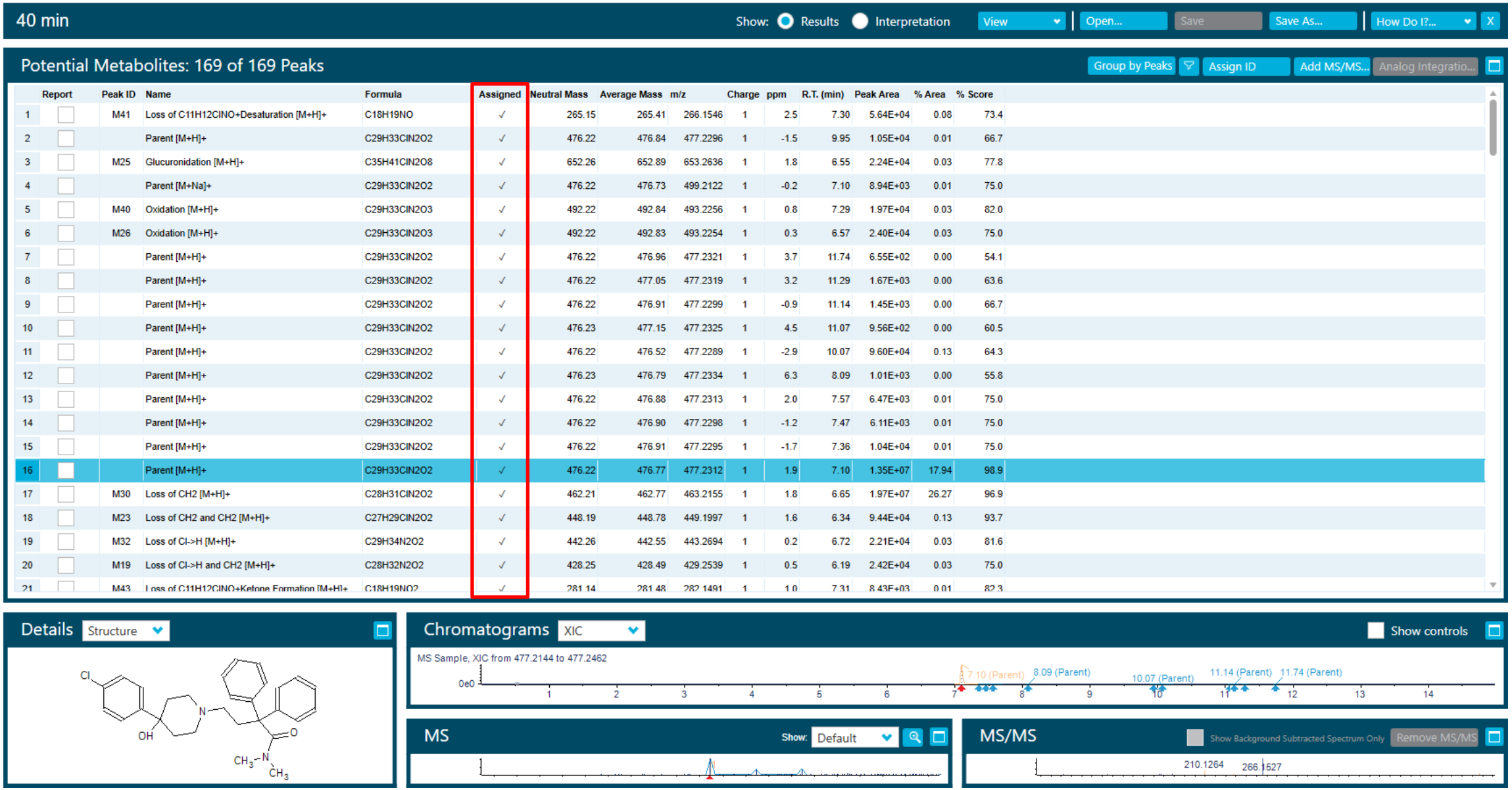




Set-up of parameters of the method of searching for metabolites.

LabExperts
professionals in laboratory

Compound identification
Loperamid – semi-automatic approach



	Report	Peak ID	Name	Formula	Assigned	Neutral Mass	Average Mass	m/z	Charge	ppm	R.T. (min)	Peak Area	% Area	% Score
1		M41	Loss of C11H12ClNO+Desaturation [M+H]+	C18H19NO	✓	265.15	265.41	266.1546	1	2.5	7.30	5.64E+04	0.08	73.4
2			Parent [M+H]+	C29H33ClN2O2	✓	476.22	476.84	477.2296	1	-1.5	9.95	1.05E+04	0.01	66.7
3		M25	Glucuronidation [M+H]+	C35H41ClN2O8	✓	652.26	652.89	653.2636	1	1.8	6.55	2.24E+04	0.03	77.8
4			Parent [M+Na]+	C29H33ClN2O2	✓	476.22	476.73	499.2122	1	-0.2	7.10	8.94E+03	0.01	75.0
5		M40	Oxidation [M+H]+	C29H33ClN2O3	✓	492.22	492.84	493.2256	1	0.8	7.29	1.97E+04	0.03	82.0
6		M26	Oxidation [M+H]+	C29H33ClN2O3	✓	492.22	492.83	493.2254	1	0.3	6.57	2.40E+04	0.03	75.0
7			Parent [M+H]+	C29H33ClN2O2	✓	476.22	476.96	477.2321	1	3.7	11.74	6.55E+02	0.00	54.1
8			Parent [M+H]+	C29H33ClN2O2	✓	476.22	477.05	477.2319	1	3.2	11.29	1.67E+03	0.00	63.6
9			Parent [M+H]+	C29H33ClN2O2	✓	476.22	476.91	477.2299	1	-0.9	11.14	1.45E+03	0.00	66.7
10			Parent [M+H]+	C29H33ClN2O2	✓	476.23	477.15	477.2325	1	4.5	11.07	9.56E+02	0.00	60.5
11			Parent [M+H]+	C29H33ClN2O2	✓	476.22	476.52	477.2289	1	-2.9	10.07	9.60E+04	0.13	64.3
12			Parent [M+H]+	C29H33ClN2O2	✓	476.23	476.79	477.2334	1	6.3	8.09	1.01E+03	0.00	55.8
13			Parent [M+H]+	C29H33ClN2O2	✓	476.22	476.88	477.2313	1	2.0	7.57	6.47E+03	0.01	75.0
14			Parent [M+H]+	C29H33ClN2O2	✓	476.22	476.90	477.2298	1	-1.2	7.47	6.11E+03	0.01	75.0
15			Parent [M+H]+	C29H33ClN2O2	✓	476.22	476.91	477.2295	1	-1.7	7.36	1.04E+04	0.01	75.0
16			Parent [M+H]+	C29H33ClN2O2	✓	476.22	476.77	477.2312	1	1.9	7.10	1.35E+07	17.94	98.9
17		M30	Loss of CH2 [M+H]+	C28H31ClN2O2	✓	462.21	462.77	463.2155	1	1.8	6.65	1.97E+07	26.27	96.9
18		M23	Loss of CH2 and CH2 [M+H]+	C27H29ClN2O2	✓	448.19	448.78	449.1997	1	1.6	6.34	9.44E+04	0.13	93.7
19		M32	Loss of Cl->H [M+H]+	C29H34N2O2	✓	442.26	442.55	443.2694	1	0.2	6.72	2.21E+04	0.03	81.6
20		M19	Loss of Cl->H and CH2 [M+H]+	C28H32N2O2	✓	428.25	428.49	429.2539	1	0.5	6.19	2.42E+04	0.03	75.0
21		M43	Loss of C11H12ClNO+Ketone Formation [M+H]+	C18H19NO2	✓	281.14	281.48	282.1491	1	1.0	7.31	8.43E+03	0.01	82.3

Example result – sample 40 min.

LabExperts
professionals in laboratory

Compound identification
Loperamid – semi-automatic approach

40 min

Show: Results Interpretation | View | Open... | Save | Save As... | How Do I?... | X

Potential Metabolites: 169 of 169 Peaks

Group by Peaks | Assign ID | Add MS/MS... | Analog Integratio...

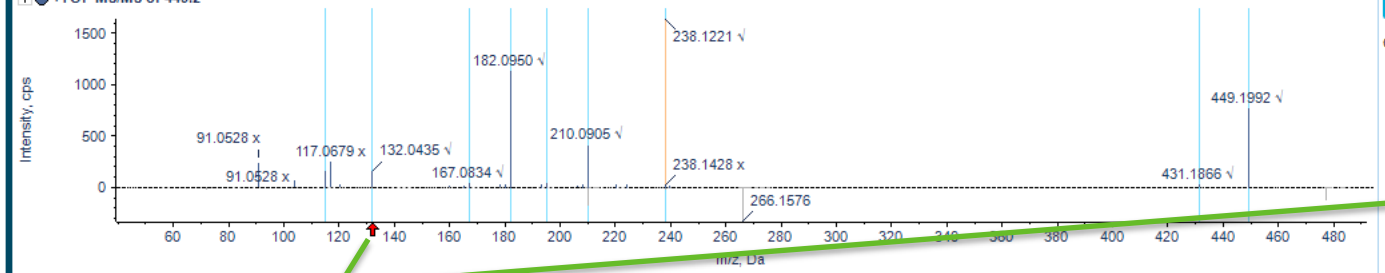
	Report	Peak ID	Name	Formula	Assigned	Neutral Mass	Average Mass	m/z	Charge	ppm	R.T. (min)	Peak Area	% Area	% Score
17		M30	Loss of CH2 [M+H]+	C28H31ClN2O2	✓	462.21	462.77	463.2155	1	1.8	6.65	1.97E+07	26.27	96.9
18		M23	Loss of CH2 and CH2 [M+H]+	C27H29ClN2O2	✓	448.19	448.78	449.1997	1	1.6	6.34	9.44E+04	0.13	93.7
19		M32	Loss of Cl->H [M+H]+	C29H34N2O2	✓	442.26	442.55	443.2694	1	0.2	6.72	2.21E+04	0.03	81.6
20		M19	Loss of Cl->H and CH2 [M+H]+	C28H32N2O2	✓	428.25	428.49	429.2539	1	0.5	6.19	2.42E+04	0.03	75.0

Interpretation

Deisotope | Prepare... | Options... | Generate | Apply | Remove

Selected neutral formula: C27H29ClN2O2

+TOF MS/MS of 449.2



Assigned: 10 of 27 peaks, score for 10 proposed assignments: 385.5

Fragments: 10 of 15 Proposed Formulae

	Use	Mass (m/z)	Ion Formula	Error (ppm)	Intensity (cps)	RDB	Proposed Structures	Score
1	✓	115.0532	C9H7	-8.5	160.0	7.0	5	37.0
2	✓	132.0435	C8H6NO	-6.9	162.0	7.0	2	38.0
3	✓	167.0834	C7H16ClO2	0.3	41.0	0.0	0	8.0
4	✓	182.0950	C13H12N	-7.7	1133.0	9.0	1	28.0
5	✓	182.1062	C9H14N2O2	6.7	41.0	4.5	1	18.0
6	✓	195.1160	C15H15	-4.5	41.0	9.0	1	44.5
7	✓	210.0905	C14H12NO	-4.1	408.0	10.0	1	45.0
8	✓	238.1221	C16H16NO	-2.4	1633.0	10.0	2	56.0
9	✓	431.1866	C27H28ClN2O	-4.4	31.0	15.0	1	51.0
10	✓	449.1992	C27H30ClN2...	0.3	761.0	14.0	1	60.0

Structure Details for C8H5NO

	Use	Broken E	Delta H	Score
1	✓	2	-4	38.0
2		2	-4	38.0

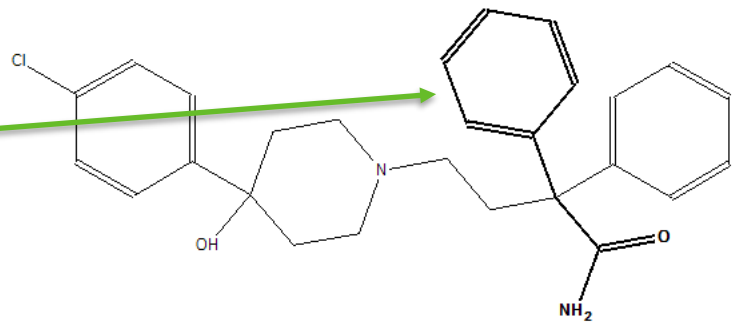
Contained Neutral Losses

Use	Mass	Formula

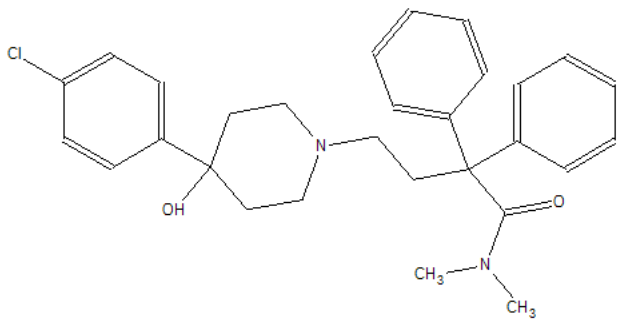
Load | Delete | Save As... | Assign

Structure 1 of 1, rank = 1

Composition: C27H29ClN2O2, Mass: 448.1918, Selected: C8H7NO, Mass: 133.0528



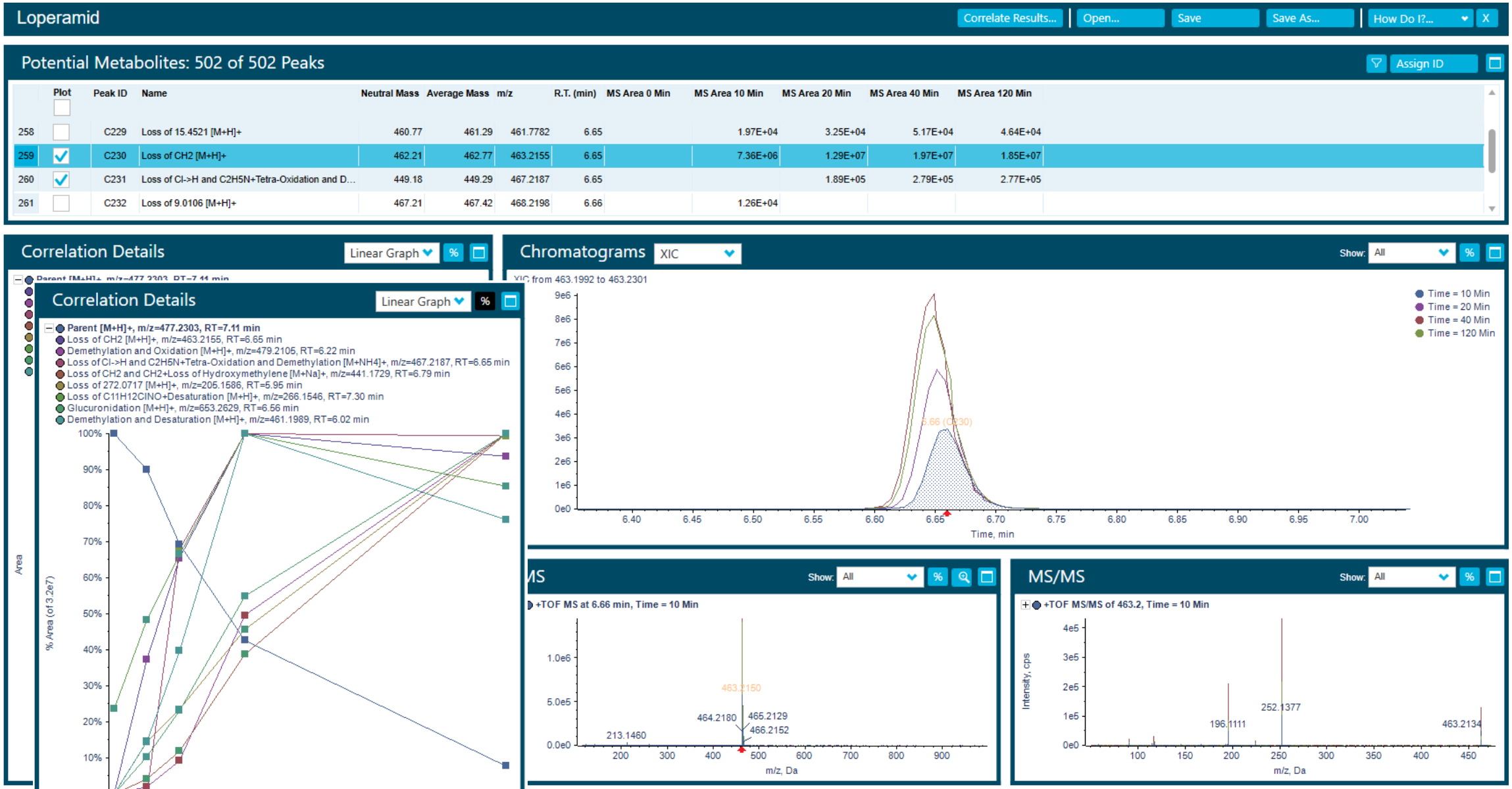
Parent Structure | Structure Candidates



Example result – sample 40 min.

LabExperts
professionals in laboratory

Compound identification
Loperamid – semi-automatic approach



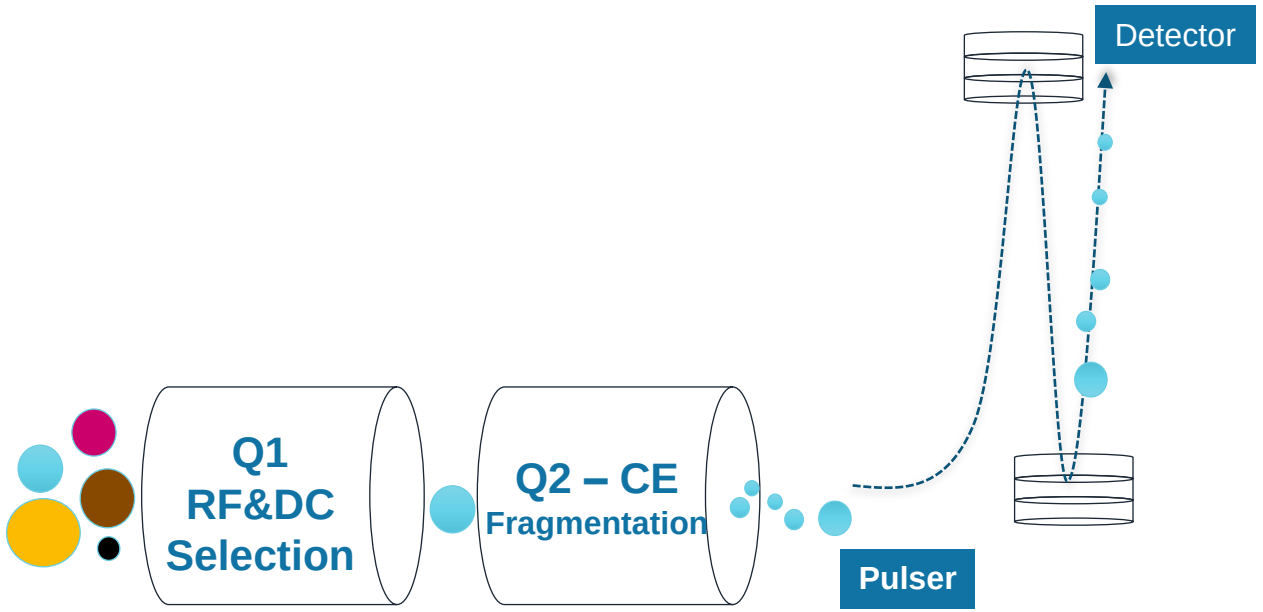
Profiling time series.

LabExperts
professionals in laboratory

Targeted analysis
Compound presence confirmation

DRUID – Driving Under the Influence of Drugs, Alcohol and Medicines in Europe

Common and nowadays obligatory test after a car accident,
Material to test – blood,
LC-MS/MS covers quantitative analysis of 27 most common compounds,
Typically MRM on triplequad instruments



YES – initially we did it on triplequad LC-MS/MS, but especially at low concentrations we had problem with compound presence confirmation – poor MRM ratio, signal specificity or matrix interferences.

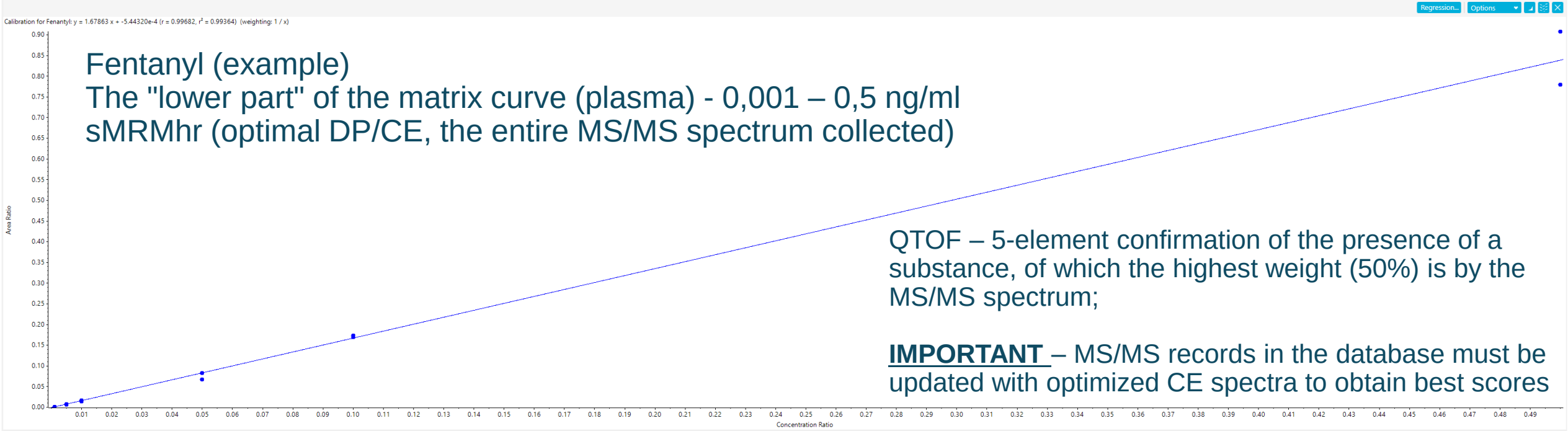
Maybe we should try HR-MS/MS?

MRMhr – TOF MS/MS → Optimized DP, EP, CE. Scanning in a narrow window around the selected fragment ion or the entire MS/MS spectrum.

Target confirmation
Example I - DRUID

13 rows | Filters: 0 | Qualify for Rules Filters

Index	Sample Name	Sample T...	Acquisition Date & Time	Component Name	Component Type	Component Group Name	Actual Concentration	Expected RT	Area	Retent... Time	Retenti... Time D...	Signal / Noise	U...	Calculated Concentrat...	Adduct / Charge	Accuracy	Formula	Precursor Mass	Found At Mass	Mass Error (...	Fragment Mass	Found At Fra...	Fragm... Mass E...	Ac... Ac...	Co... Ac...	Reporta...	Mass Error...	Frag... Mass...	RT Confi...	Isotope Confi...	Library Confi...	Library Hit	Library Score	Combi... Score	Isotope Ratio...
20	Blank osocze	Unknown	5/10/2022 11:45:32 PM	Fenantyl	Quantifiers	Fenantyl_group	N/A	3.63	N/A	N/A	N/A	N/A	☑	N/A	[M+H]+	N/A	C22H28N...	337.227	N/A	N/A	188.1436	N/A	N/A			☑							N/A	N/A	N/A
52	Druid osocze [0.001 ng/ml]	Standard	5/10/2022 11:57:27 PM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	3.503e2	3.71	0.09	29.3	☑	0.001	[M+H]+	110.67	C22H28N...	337.227	337.2444	50.3	188.1436	188.1416	-10.9			☑	●	▲	✓	●	●	Fentanyl (NIST) [Smart Confirmati...	28.2	27.888	Infinity
84	Druid osocze [0.001 ng/ml]	Standard	5/11/2022 12:09:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	4.428e2	3.75	0.13	36.1	☑	0.001	[M+H]+	128.77	C22H28N...	337.227	337.2417	42.2	188.1436	188.1416	-10.4	!		☑	●	▲	▲	●	●	Fentanyl (NIST) [Smart Confirmati...	12.7	20.462	Infinity
116	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:23:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	1.610e3	3.78	0.15	101.8	☑	0.004	[M+H]+	80.41	C22H28N...	337.227	337.2352	23.0	188.1436	188.1427	-4.9	!		☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	87.1	63.129	Infinity
148	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:35:17 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	2.109e3	3.74	0.12	1004.7	☑	0.005	[M+H]+	101.86	C22H28N...	337.227	337.2353	23.3	188.1436	188.1418	-9.5			☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	83.1	56.613	Infinity
180	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 12:47:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	4.093e3	3.71	0.08	1932.7	☑	0.010	[M+H]+	102.02	C22H28N...	337.227	337.2301	7.7	188.1436	188.1438	1.3			☑	▲	✓	✓	●	✓	Fentanyl (NIST) [Smart Confirmati...	99.8	77.800	23.4
212	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 1:01:13 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	3.940e3	3.72	0.10	397.3	☑	0.009	[M+H]+	89.69	C22H28N...	337.227	337.2320	13.6	188.1436	188.1435	-0.7	!		☑	●	✓	▲	●	▲	Fentanyl (NIST) [Smart Confirmati...	66.3	57.102	24.9
244	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:39:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	2.054e4	3.73	0.10	10094.7	☑	0.049	[M+H]+	98.67	C22H28N...	337.227	337.2276	0.6	188.1436	188.1434	-1.3			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.2	96.027	3.2
276	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:51:04 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	1.832e4	3.73	0.11	1438.2	☑	0.041	[M+H]+	81.33	C22H28N...	337.227	337.2274	-0.1	188.1436	188.1433	-1.3	!		☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.9	95.964	8.4
308	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:02:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.883e4	3.75	0.12	3439.6	☑	0.104	[M+H]+	103.81	C22H28N...	337.227	337.2272	-0.8	188.1436	188.1435	-0.4			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	100.0	96.560	3.7
340	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:16:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.513e4	3.72	0.09	3046.3	☑	0.102	[M+H]+	101.58	C22H28N...	337.227	337.2278	1.0	188.1436	188.1428	-4.3			☑	✓	✓	▲	▲	✓	Fentanyl (NIST) [Smart Confirmati...	98.7	89.128	14.3
372	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:47:22 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.850e5	3.70	0.07	14316.7	☑	0.541	[M+H]+	108.22	C22H28N...	337.227	337.2271	-1.0	188.1436	188.1436	0.1			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.0	96.783	1.6
404	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:59:14 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.785e5	3.71	0.09	101809.8	☑	0.465	[M+H]+	92.96	C22H28N...	337.227	337.2272	-0.6	188.1436	188.1438	0.9			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.4	96.668	2.1



Fentanyl (example)
The "lower part" of the matrix curve (plasma) - 0,001 – 0,5 ng/ml
sMRMhr (optimal DP/CE, the entire MS/MS spectrum collected)

QTOF – 5-element confirmation of the presence of a substance, of which the highest weight (50%) is by the MS/MS spectrum;

IMPORTANT – MS/MS records in the database must be updated with optimized CE spectra to obtain best scores

LabExperts
professionals in laboratory

Target confirmation
Example I - DRUID

13 rows | Filters: 0 | Qualify for Rules Filters

Index	Sample Name	Sample T...	Acquisition Date & Time	Component Name	Component Type	Component Group Name	Actual Concentration	Expected RT	Area	Retent... Time	Retenti... Time D...	Signal / Noise	U...	Calculated Concentrat...	Adduct / Charge	Accuracy	Formula	Precursor Mass	Found At Mass	Mass Error (...	Fragment Mass	Found At Fra...	Fragm... Mass E...	Ac... Ac...	Co... Ac...	Reporta...	Mass Error...	Frag... Mass...	RT Confi...	Isotope Confi...	Library Confi...	Library Hit	Library Score	Combi... Score	Isotope Ratio...
20	Blank osocze	Unknown	5/10/2022 11:45:32 PM	Fenantyl	Quantifiers	Fenantyl_group	N/A	3.63	N/A	N/A	N/A	N/A	☑	N/A	[M+H]+	N/A	C22H28N...	337.227	N/A	N/A	188.1436	N/A	N/A			☑							N/A	N/A	N/A
52	Druid osocze [0.001 ng/ml]	Standard	5/10/2022 11:57:27 PM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	3.503e2	3.71	0.09	29.3	☑	0.001	[M+H]+	110.67	C22H28N...	337.227	337.2444	50.3	188.1436	188.1416	-10.9			☑	●	▲	✓	●	●	Fentanyl (NIST) [Smart Confirmati...	28.2	27.888	Infinity
84	Druid osocze [0.001 ng/ml]	Standard	5/11/2022 12:09:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	4.428e2	3.75	0.13	36.1	☑	0.001	[M+H]+	128.77	C22H28N...	337.227	337.2417	42.2	188.1436	188.1416	-10.4	!		☑	●	▲	▲	●	●	Fentanyl (NIST) [Smart Confirmati...	12.7	20.462	Infinity
116	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:23:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	1.610e3	3.78	0.15	101.8	☑	0.004	[M+H]+	80.41	C22H28N...	337.227	337.2352	23.0	188.1436	188.1427	-4.9	!		☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	87.1	63.129	Infinity
148	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:35:17 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	2.109e3	3.74	0.12	1004.7	☑	0.005	[M+H]+	101.86	C22H28N...	337.227	337.2353	23.3	188.1436	188.1418	-9.5			☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	83.1	56.613	Infinity
180	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 12:47:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	4.093e3	3.71	0.08	1932.7	☑	0.010	[M+H]+	102.02	C22H28N...	337.227	337.2301	7.7	188.1436	188.1438	1.3			☑	▲	✓	✓	●	✓	Fentanyl (NIST) [Smart Confirmati...	99.8	77.800	23.4
212	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 1:01:13 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	3.940e3	3.72	0.10	397.3	☑	0.009	[M+H]+	89.69	C22H28N...	337.227	337.2320	13.6	188.1436	188.1435	-0.7	!		☑	●	✓	▲	●	▲	Fentanyl (NIST) [Smart Confirmati...	66.3	57.102	24.9
244	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:39:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	2.054e4	3.73	0.10	10094.7	☑	0.049	[M+H]+	98.67	C22H28N...	337.227	337.2276	0.6	188.1436	188.1434	-1.3			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.2	96.027	3.2
276	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:51:04 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	1.832e4	3.73	0.11	1438.2	☑	0.041	[M+H]+	81.33	C22H28N...	337.227	337.2274	-0.1	188.1436	188.1433	-1.3	!		☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.9	95.964	8.4
308	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:02:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.883e4	3.75	0.12	3439.6	☑	0.104	[M+H]+	103.81	C22H28N...	337.227	337.2272	-0.8	188.1436	188.1435	-0.4			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	100.0	96.560	3.7
340	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:16:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.513e4	3.72	0.09	3046.3	☑	0.102	[M+H]+	101.58	C22H28N...	337.227	337.2278	1.0	188.1436	188.1428	-4.3			☑	✓	✓	▲	▲	✓	Fentanyl (NIST) [Smart Confirmati...	98.7	89.128	14.3
372	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:47:22 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.850e5	3.70	0.07	14316.7	☑	0.541	[M+H]+	108.22	C22H28N...	337.227	337.2271	-1.0	188.1436	188.1436	0.1			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.0	96.783	1.6
404	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:59:14 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.785e5	3.71	0.09	101809.8	☑	0.465	[M+H]+	92.96	C22H28N...	337.227	337.2272	-0.6	188.1436	188.1438	0.9			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.4	96.668	2.1

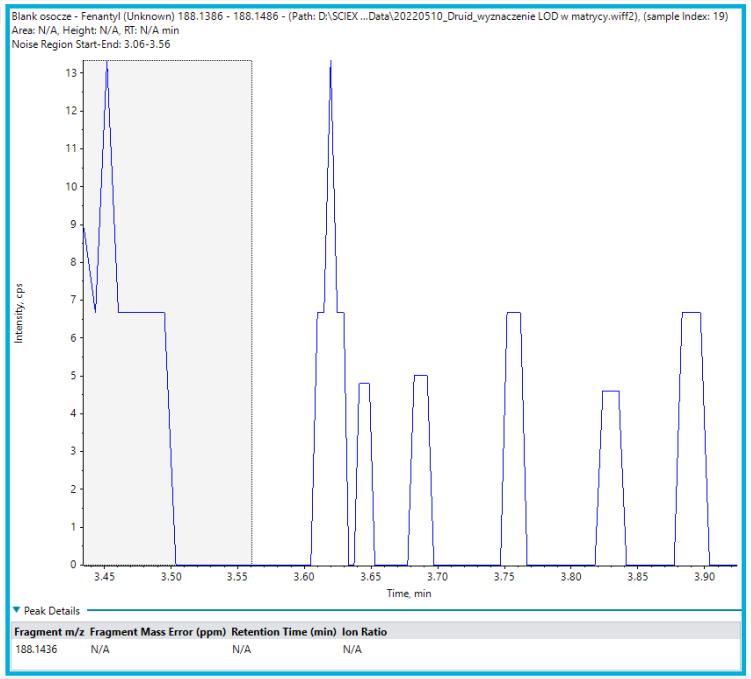
Manual Integration | Apply

▼ Retention Time (RT)
- Expected RT: 3.625 min
- RT Half Window: 30 sec
- Peak selection by: Expected RT

▼ Advanced Integration
Pre-Processing
- Smoothing: Low
- Noise filter / Moving average

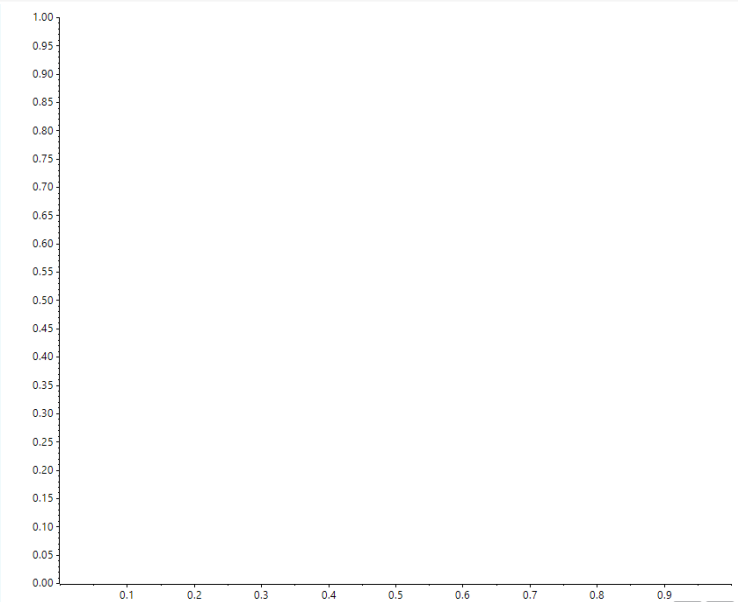
Integration
- Process by group
- Interference resolution: 50 %
- Peak baseline: Local / Linear
- Saturation correction
- Threshold: 3.5e6

Filtering
- Minimum peak height: 200.00
- Minimum signal/noise: 3.00



▼ Peak Details

Fragment m/z	Fragment Mass Error (ppm)	Retention Time (min)	Ion Ratio
188.1436	N/A	N/A	N/A



▼ Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MSMS (ppm)	Hit Count

▼ Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE (eV)

Blank serum

LabExperts
professionals in laboratory

Target confirmation
Example I - DRUID

13 rows | Filters: 0 | Qualify for Rules Filters

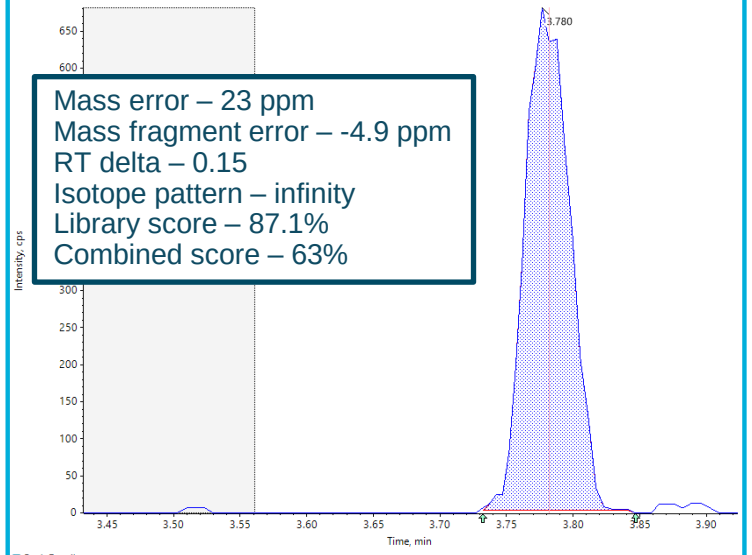
Index	Sample Name	Sample T...	Acquisition Date & Time	Component Name	Component Type	Component Group Name	Actual Concentration	Expected RT	Area	Retent... Time	Retenti... Time D...	Signal / Noise	U...	Calculated Concentrat...	Adduct / Charge	Accuracy	Formula	Precursor Mass	Found At Mass	Mass Error (...	Fragment Mass	Found At Fra...	Fragm... Mass E...	Ac... Ac...	Co... Ac...	Reporta...	Mass Error...	Frag... Mass...	RT Confi...	Isotope Confi...	Library Confi...	Library Hit	Library Score	Combi... Score	Isotope Ratio...
20	Blank osocze	Unknown	5/10/2022 11:45:32 PM	Fenantyl	Quantifiers	Fenantyl_group	N/A	3.63	N/A	N/A	N/A	N/A	☑	N/A	[M+H]+	N/A	C22H28N...	337.227	N/A	N/A	188.1436	N/A	N/A			☑							N/A	N/A	N/A
52	Druid osocze [0.001 ng/ml]	Standard	5/10/2022 11:57:27 PM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	3.503e2	3.71	0.09	29.3	☑	0.001	[M+H]+	110.67	C22H28N...	337.227	337.2444	50.3	188.1436	188.1416	-10.9			☑	●	▲	✓	●	●	Fentanyl (NIST) [Smart Confirmati...	28.2	27.888	Infinity
84	Druid osocze [0.001 ng/ml]	Standard	5/11/2022 12:09:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	4.428e2	3.75	0.13	36.1	☑	0.001	[M+H]+	128.77	C22H28N...	337.227	337.2417	42.2	188.1436	188.1416	-10.4	!		☑	●	▲	▲	●	●	Fentanyl (NIST) [Smart Confirmati...	12.7	20.462	Infinity
116	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:23:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	1.610e3	3.78	0.15	101.8	☑	0.004	[M+H]+	80.41	C22H28N...	337.227	337.2352	23.0	188.1436	188.1427	-4.9	!		☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	87.1	63.129	Infinity
148	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:35:17 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	2.109e3	3.74	0.12	1004.7	☑	0.005	[M+H]+	101.86	C22H28N...	337.227	337.2353	23.3	188.1436	188.1418	-9.5			☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	83.1	56.613	Infinity
180	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 12:47:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	4.093e3	3.71	0.08	1932.7	☑	0.010	[M+H]+	102.02	C22H28N...	337.227	337.2301	7.7	188.1436	188.1438	1.3			☑	▲	✓	✓	●	✓	Fentanyl (NIST) [Smart Confirmati...	99.8	77.800	23.4
212	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 1:01:13 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	3.940e3	3.72	0.10	397.3	☑	0.009	[M+H]+	89.69	C22H28N...	337.227	337.2320	13.6	188.1436	188.1435	-0.7	!		☑	●	✓	▲	●	▲	Fentanyl (NIST) [Smart Confirmati...	66.3	57.102	24.9
244	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:39:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	2.054e4	3.73	0.10	10094.7	☑	0.049	[M+H]+	98.67	C22H28N...	337.227	337.2276	0.6	188.1436	188.1434	-1.3			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.2	96.027	3.2
276	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:51:04 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	1.832e4	3.73	0.11	1438.2	☑	0.041	[M+H]+	81.33	C22H28N...	337.227	337.2274	-0.1	188.1436	188.1433	-1.3	!		☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.9	95.964	8.4
308	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:02:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.883e4	3.75	0.12	3439.6	☑	0.104	[M+H]+	103.81	C22H28N...	337.227	337.2272	-0.8	188.1436	188.1435	-0.4			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	100.0	96.560	3.7
340	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:16:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.513e4	3.72	0.09	3046.3	☑	0.102	[M+H]+	101.58	C22H28N...	337.227	337.2278	1.0	188.1436	188.1428	-4.3			☑	✓	✓	▲	▲	✓	Fentanyl (NIST) [Smart Confirmati...	98.7	89.128	14.3
372	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:47:22 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.850e5	3.70	0.07	14316.7	☑	0.541	[M+H]+	108.22	C22H28N...	337.227	337.2271	-1.0	188.1436	188.1436	0.1			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.0	96.783	1.6
404	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:59:14 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.785e5	3.71	0.09	101809.8	☑	0.465	[M+H]+	92.96	C22H28N...	337.227	337.2272	-0.6	188.1436	188.1438	0.9			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.4	96.668	2.1

Manual Integration | Apply

Retention Time (RT)
- Expected RT: 3.625 min
- RT Half Window: 30 sec
- Peak selection by: Expected RT

Advanced Integration
Pre-Processing
- Smoothing: Low
- Noise filter / Moving average
Integration
- Process by group
- Interference resolution: 50 %
- Peak baseline: Local / Linear
- Saturation correction
- Threshold: 3.5e6
Filtering
- Minimum peak height: 200.00
- Minimum signal/noise: 3.00

Druid osocze [0.005 ng/ml] - Fenantyl (Standard) 188.1386 - 188.1486 - (P...\Data\20220510_Druid_wyznaczenie LOD w matrycy.wiff2), (sample Index: 22)
Area: 1.610e3, Height: 6.786e2, RT: 3.78 min
Noise Region Start-End: 3.06-3.56

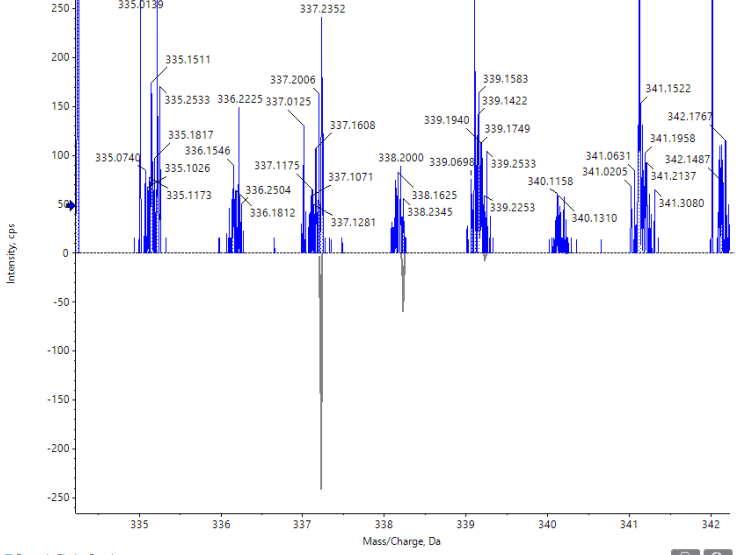


Mass error – 23 ppm
Mass fragment error – -4.9 ppm
RT delta – 0.15
Isotope pattern – infinity
Library score – 87.1%
Combined score – 63%

Peak Details

Fragment m/z	Fragment Mass Error (ppm)	Retention Time (min)	Ion Ratio
188.1436	-4.9	3.78	N/A

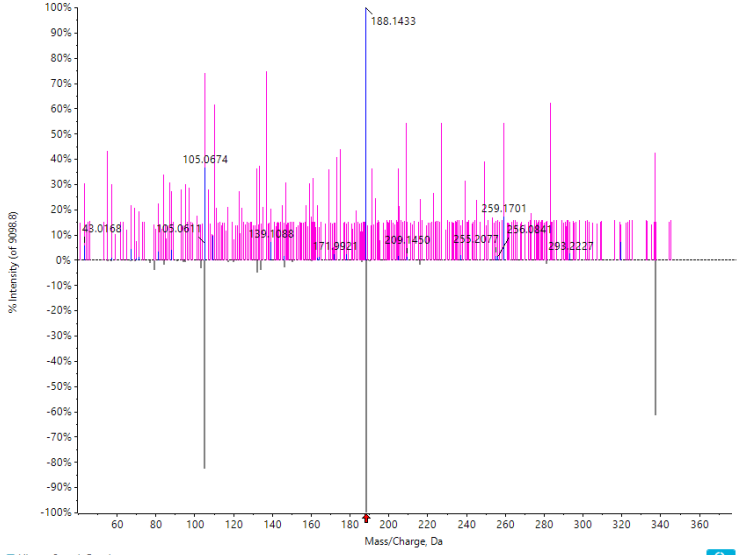
Spectrum from 20220510_Druid_wyznaczenie LOD w matrycy.wiff2 (s...periment 1, +sMRMhr TOF MS (100 - 840) from 3.774 to 3.784 min
[C22H28N2O+H]+



Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MSMS (ppm)	Hit Count

Deconvoluted, Spectrum from 20220510_Druid_wyznaczenie LOD w mat...40 - 350) from 3.896 to 3.923 min Precursor: 337.2 Da, CE: 36.0]
Spectrum from 20220510_Druid_wyznaczenie LOD w matrycy.wiff2 (sample 2...SMS (40 - 350) from 3.842 to 3.952 min Precursor: 337.2 Da, CE: 36.0]
Library Spectrum: Fentanyl (NIST) (437387) , CE=54.8502807617188±5.14971923828125



Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE (eV)
Fentanyl (NIST) [Smart Confirmation]	437387	C22H28N2O	336.22015	92.9	97.5	87.1	55

0.005 ng/ml

LabExperts
professionals in laboratory

Target confirmation
Example I - DRUID

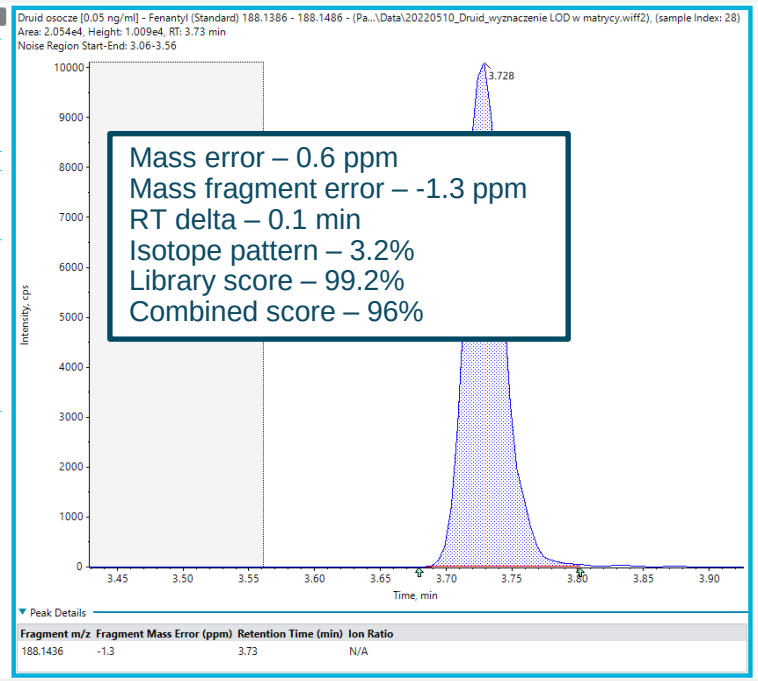
13 rows | Filters: 0 | Qualify for Rules Filters

Index	Sample Name	Sample T...	Acquisition Date & Time	Component Name	Component Type	Component Group Name	Actual Concentration	Expected RT	Area	Retent... Time	Retenti... Time D...	Signal / Noise	U...	Calculated Concentrat...	Adduct / Charge	Accuracy	Formula	Precursor Mass	Found At Mass	Mass Error (...	Fragment Mass	Found At Fra...	Fragm... Mass E...	Ac... Ac...	Co... Ac...	Reporta...	Mass Error...	Frag... Mass...	RT Confi...	Isotope Confi...	Library Confi...	Library Hit	Library Score	Combi... Score	Isotope Ratio...
20	Blank osocze	Unknown	5/10/2022 11:45:32 PM	Fenantyl	Quantifiers	Fenantyl_group	N/A	3.63	N/A	N/A	N/A	N/A	☑	N/A	[M+H]+	N/A	C22H28N...	337.227	N/A	N/A	188.1436	N/A	N/A			☑							N/A	N/A	N/A
52	Druid osocze [0.001 ng/ml]	Standard	5/10/2022 11:57:27 PM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	3.503e2	3.71	0.09	29.3	☑	0.001	[M+H]+	110.67	C22H28N...	337.227	337.2444	50.3	188.1436	188.1416	-10.9			☑	●	▲	✓	●	●	Fentanyl (NIST) [Smart Confirmati...	28.2	27.888	Infinity
84	Druid osocze [0.001 ng/ml]	Standard	5/11/2022 12:09:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	4.428e2	3.75	0.13	36.1	☑	0.001	[M+H]+	128.77	C22H28N...	337.227	337.2417	42.2	188.1436	188.1416	-10.4	!		☑	●	▲	▲	●	●	Fentanyl (NIST) [Smart Confirmati...	12.7	20.462	Infinity
116	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:23:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	1.610e3	3.78	0.15	101.8	☑	0.004	[M+H]+	80.41	C22H28N...	337.227	337.2352	23.0	188.1436	188.1427	-4.9	!		☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	87.1	63.129	Infinity
148	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:35:17 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	2.109e3	3.74	0.12	1004.7	☑	0.005	[M+H]+	101.86	C22H28N...	337.227	337.2353	23.3	188.1436	188.1418	-9.5			☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	83.1	56.613	Infinity
180	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 12:47:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	4.093e3	3.71	0.08	1932.7	☑	0.010	[M+H]+	102.02	C22H28N...	337.227	337.2301	7.7	188.1436	188.1438	1.3			☑	▲	✓	✓	●	✓	Fentanyl (NIST) [Smart Confirmati...	99.8	77.800	23.4
212	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 1:01:13 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	3.940e3	3.72	0.10	397.3	☑	0.009	[M+H]+	89.69	C22H28N...	337.227	337.2320	13.6	188.1436	188.1435	-0.7	!		☑	●	✓	▲	●	▲	Fentanyl (NIST) [Smart Confirmati...	66.3	57.102	24.9
244	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:39:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	2.054e4	3.73	0.10	10094.7	☑	0.049	[M+H]+	98.67	C22H28N...	337.227	337.2276	0.6	188.1436	188.1434	-1.3			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.2	96.027	3.2
276	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:51:04 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	1.832e4	3.73	0.11	1438.2	☑	0.041	[M+H]+	81.33	C22H28N...	337.227	337.2274	-0.1	188.1436	188.1433	-1.3	!		☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.9	95.964	8.4
308	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:02:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.883e4	3.75	0.12	3439.6	☑	0.104	[M+H]+	103.81	C22H28N...	337.227	337.2272	-0.8	188.1436	188.1435	-0.4			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	100.0	96.560	3.7
340	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:16:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.513e4	3.72	0.09	3046.3	☑	0.102	[M+H]+	101.58	C22H28N...	337.227	337.2278	1.0	188.1436	188.1428	-4.3			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	98.7	89.128	14.3
372	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:47:22 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.850e5	3.70	0.07	14316.7	☑	0.541	[M+H]+	108.22	C22H28N...	337.227	337.2271	-1.0	188.1436	188.1436	0.1			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.0	96.783	1.6
404	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:59:14 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.785e5	3.71	0.09	101809.8	☑	0.465	[M+H]+	92.96	C22H28N...	337.227	337.2272	-0.6	188.1436	188.1438	0.9			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.4	96.668	2.1

Manual Integration | Apply

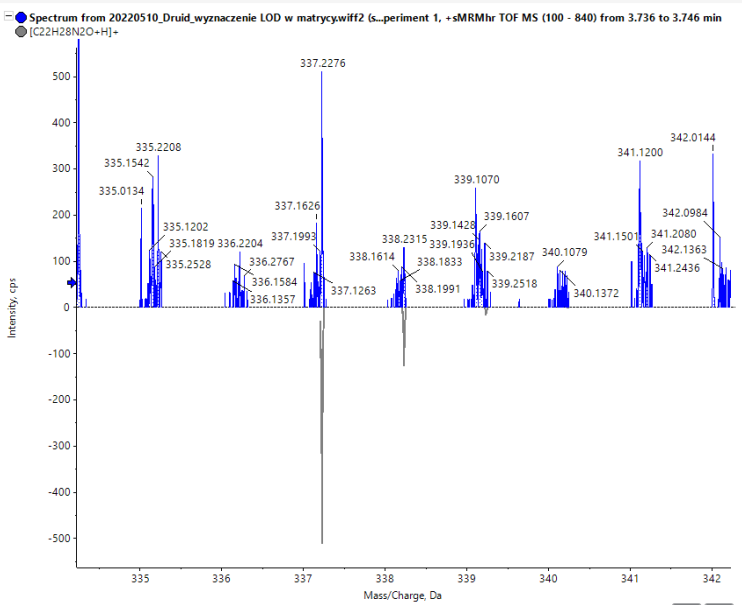
Retention Time (RT)
- Expected RT: 3.625 min
- RT Half Window: 30 sec
- Peak selection by: Expected RT

Advanced Integration
Pre-Processing
- Smoothing: Low
- Noise filter / Moving average
Integration
- Process by group
- Interference resolution: 50 %
- Peak baseline: Local / Linear
- Saturation correction
- Threshold: 3.5e6
Filtering
- Minimum peak height: 200.00
- Minimum signal/noise: 3.00



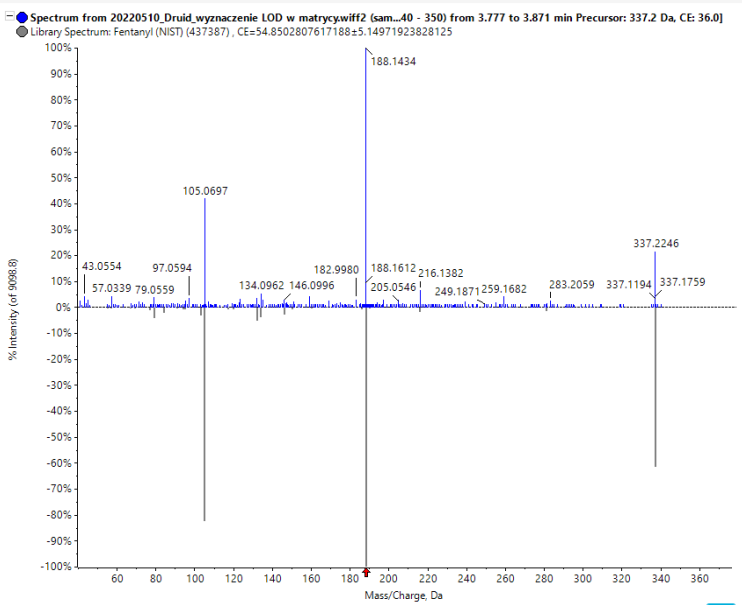
Peak Details

Fragment m/z	Fragment Mass Error (ppm)	Retention Time (min)	Ion Ratio
188.1436	-1.3	3.73	N/A



Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MSMS (ppm)	Hit Count



Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE (eV)
Fentanyl (NIST) [Smart Confirmation]	437387	C22H28N2O	336.22015	99.9	99.3	99.2	55

0.05 ng/ml

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professionals in laboratory

Target confirmation
Example I - DRUID

13 rows | Filters: 0 | Qualify for Rules Filters

Index	Sample Name	Sample T...	Acquisition Date & Time	Component Name	Component Type	Component Group Name	Actual Concentration	Expected RT	Area	Retent... Time	Retenti... Time D...	Signal / Noise	U...	Calculated Concentrat...	Adduct / Charge	Accuracy	Formula	Precursor Mass	Found At Mass	Mass Error (...	Fragment Mass	Found At Fra...	Fragm... Mass E...	Ac... Ac...	Co... Ac...	Reporta...	Mass Error...	Frag... Mass...	RT Confi...	Isotope Confi...	Library Confi...	Library Hit	Library Score	Combi... Score	Isotope Ratio...
20	Blank osocze	Unknown	5/10/2022 11:45:32 PM	Fenantyl	Quantifiers	Fenantyl_group	N/A	3.63	N/A	N/A	N/A	N/A	☑	N/A	[M+H]+	N/A	C22H28N...	337.227	N/A	N/A	188.1436	N/A	N/A			☑							N/A	N/A	N/A
52	Druid osocze [0.001 ng/ml]	Standard	5/10/2022 11:57:27 PM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	3.503e2	3.71	0.09	29.3	☑	0.001	[M+H]+	110.67	C22H28N...	337.227	337.2444	50.3	188.1436	188.1416	-10.9			☑	●	▲	✓	●	●	Fentanyl (NIST) [Smart Confirmati...	28.2	27.888	Infinity
84	Druid osocze [0.001 ng/ml]	Standard	5/11/2022 12:09:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.001	3.63	4.428e2	3.75	0.13	36.1	☑	0.001	[M+H]+	128.77	C22H28N...	337.227	337.2417	42.2	188.1436	188.1416	-10.4	!		☑	●	▲	▲	●	●	Fentanyl (NIST) [Smart Confirmati...	12.7	20.462	Infinity
116	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:23:23 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	1.610e3	3.78	0.15	101.8	☑	0.004	[M+H]+	80.41	C22H28N...	337.227	337.2352	23.0	188.1436	188.1427	-4.9	!		☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	87.1	63.129	Infinity
148	Druid osocze [0.005 ng/ml]	Standard	5/11/2022 12:35:17 AM	Fenantyl	Quantifiers	Fenantyl_group	0.005	3.63	2.109e3	3.74	0.12	1004.7	☑	0.005	[M+H]+	101.86	C22H28N...	337.227	337.2353	23.3	188.1436	188.1418	-9.5			☑	●	✓	▲	●	✓	Fentanyl (NIST) [Smart Confirmati...	83.1	56.613	Infinity
180	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 12:47:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	4.093e3	3.71	0.08	1932.7	☑	0.010	[M+H]+	102.02	C22H28N...	337.227	337.2301	7.7	188.1436	188.1438	1.3			☑	▲	✓	✓	●	✓	Fentanyl (NIST) [Smart Confirmati...	99.8	77.800	23.4
212	Druid osocze [0.01 ng/ml]	Standard	5/11/2022 1:01:13 AM	Fenantyl	Quantifiers	Fenantyl_group	0.010	3.63	3.940e3	3.72	0.10	397.3	☑	0.009	[M+H]+	89.69	C22H28N...	337.227	337.2320	13.6	188.1436	188.1435	-0.7	!		☑	●	✓	▲	●	▲	Fentanyl (NIST) [Smart Confirmati...	66.3	57.102	24.9
244	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:39:11 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	2.054e4	3.73	0.10	10094.7	☑	0.049	[M+H]+	98.67	C22H28N...	337.227	337.2276	0.6	188.1436	188.1434	-1.3			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.2	96.027	3.2
276	Druid osocze [0.05 ng/ml]	Standard	5/11/2022 1:51:04 AM	Fenantyl	Quantifiers	Fenantyl_group	0.050	3.63	1.832e4	3.73	0.11	1438.2	☑	0.041	[M+H]+	81.33	C22H28N...	337.227	337.2274	-0.1	188.1436	188.1433	-1.3	!		☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.9	95.964	8.4
308	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:02:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.883e4	3.75	0.12	3439.6	☑	0.104	[M+H]+	103.81	C22H28N...	337.227	337.2272	-0.8	188.1436	188.1435	-0.4			☑	✓	✓	▲	✓	✓	Fentanyl (NIST) [Smart Confirmati...	100.0	96.560	3.7
340	Druid osocze [0.1 ng/ml]	Standard	5/11/2022 2:16:59 AM	Fenantyl	Quantifiers	Fenantyl_group	0.100	3.63	4.513e4	3.72	0.09	3046.3	☑	0.102	[M+H]+	101.58	C22H28N...	337.227	337.2278	1.0	188.1436	188.1428	-4.3			☑	✓	✓	▲	▲	✓	Fentanyl (NIST) [Smart Confirmati...	98.7	89.128	14.3
372	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:47:22 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.850e5	3.70	0.07	14316.7	☑	0.541	[M+H]+	108.22	C22H28N...	337.227	337.2271	-1.0	188.1436	188.1436	0.1			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.0	96.783	1.6
404	CAL 5 [0.5ng/ml]	Standard	5/6/2022 3:59:14 PM	Fenantyl	Quantifiers	Fenantyl_group	0.500	3.63	1.785e5	3.71	0.09	101809.8	☑	0.465	[M+H]+	92.96	C22H28N...	337.227	337.2272	-0.6	188.1436	188.1438	0.9			☑	✓	✓	✓	✓	✓	Fentanyl (NIST) [Smart Confirmati...	99.4	96.668	2.1

Manual Integration | Apply

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Advanced Integration
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Integration
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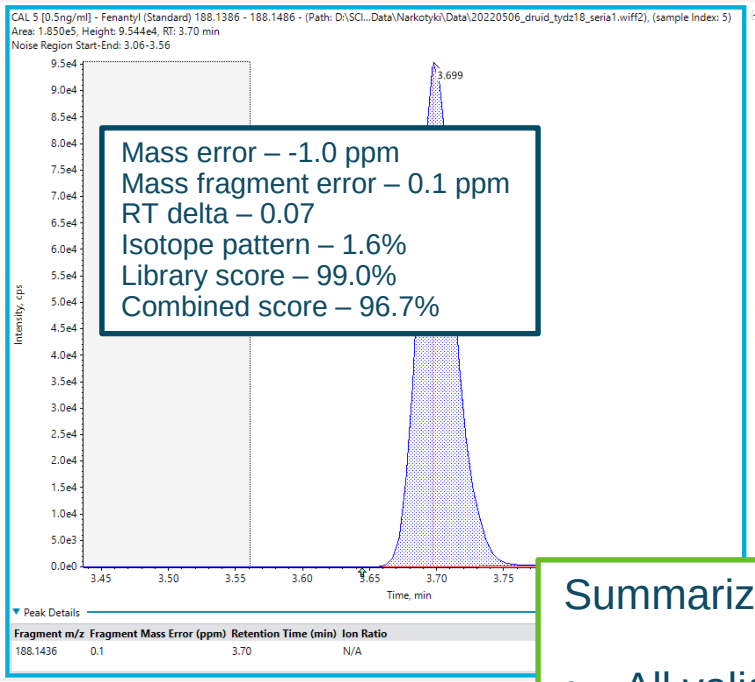
- Interference resolution: 50 %
- Peak baseline: Local / Linear
- Saturation correction
- Threshold: 3.5e6

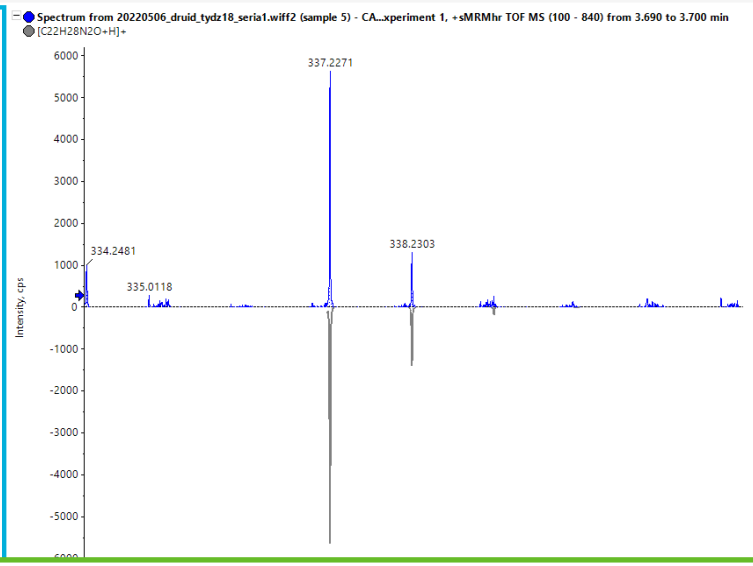
Filtering
- Minimum peak height: 200.00
- Minimum signal/noise: 3.00

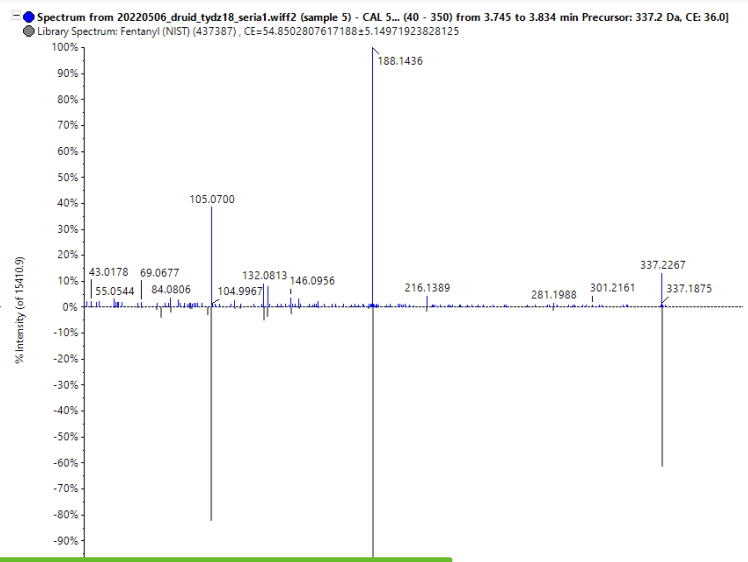


Mass error – -1.0 ppm
Mass fragment error – 0.1 ppm
RT delta – 0.07
Isotope pattern – 1.6%
Library score – 99.0%
Combined score – 96.7%

Peak Details

Fragment m/z	Fragment Mass Error (ppm)	Retention Time (min)	Ion Ratio
188.1436	0.1	3.70	N/A





Purity | CE (eV)
99.0 | 55

0.5 ng/ml

Summarizing:

- All validation criteria were met
- The results are finally clear, selective and misinterpretation resistant
- The sensitivity of the method is similar to high-end QqQ

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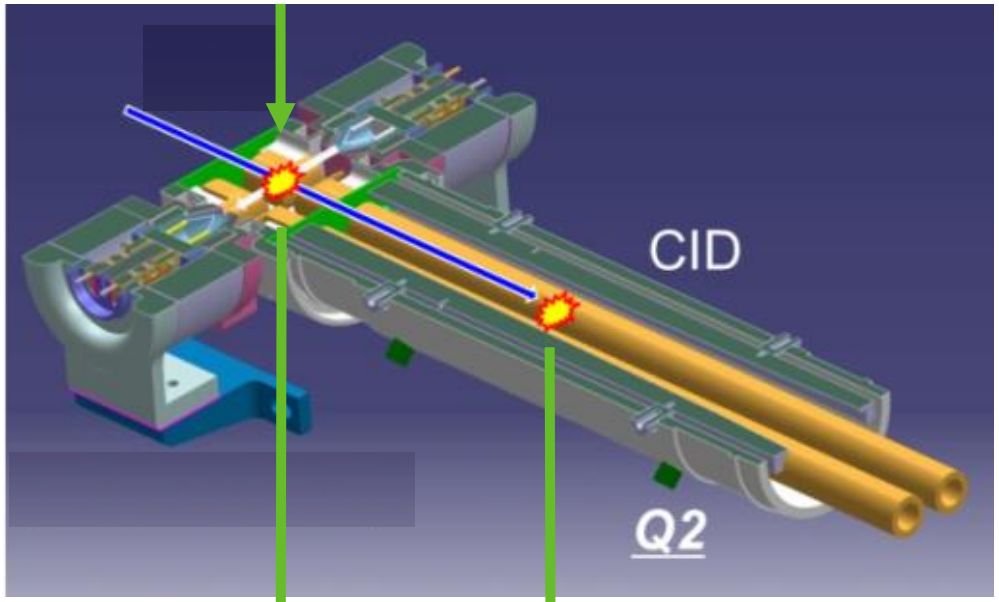
EAD Cell



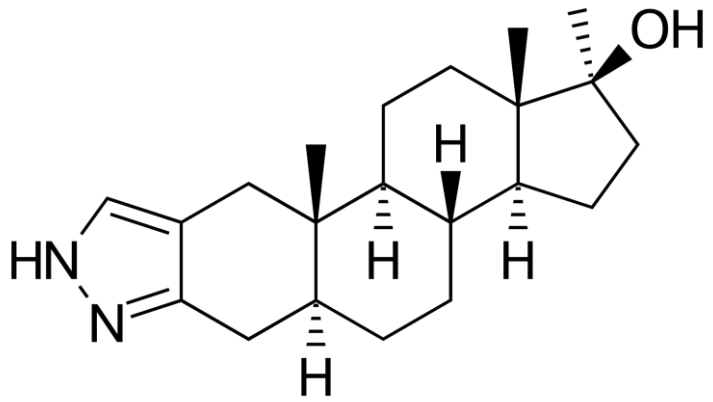
EAD MS/MS

CAD MS/MS

EAD & CAD MS/MS ?

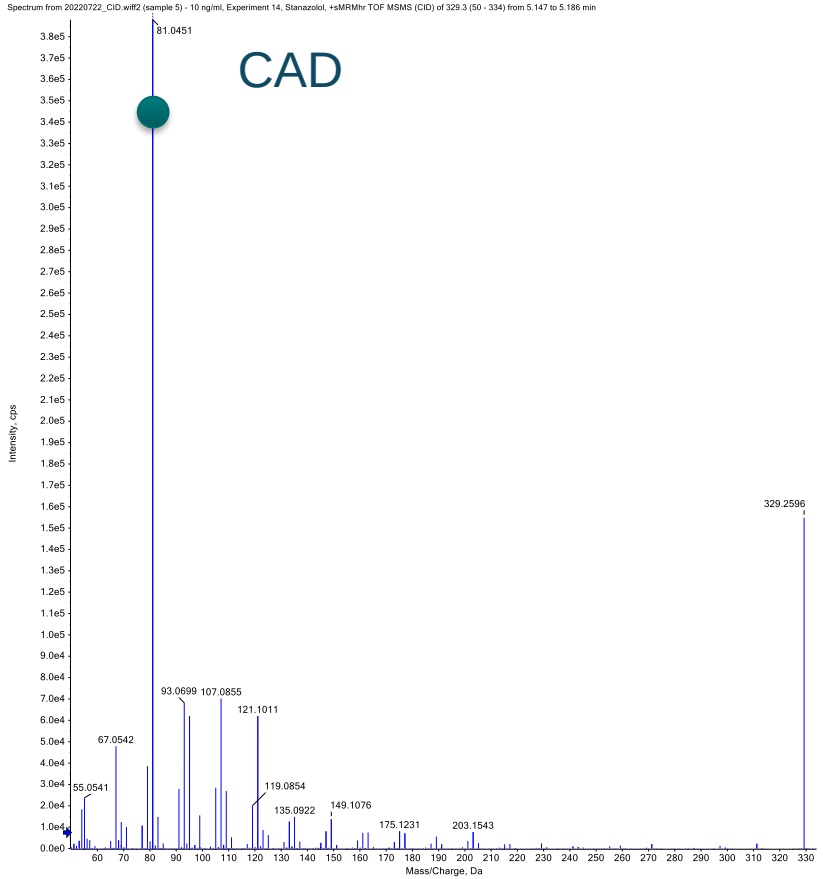
Steroids:

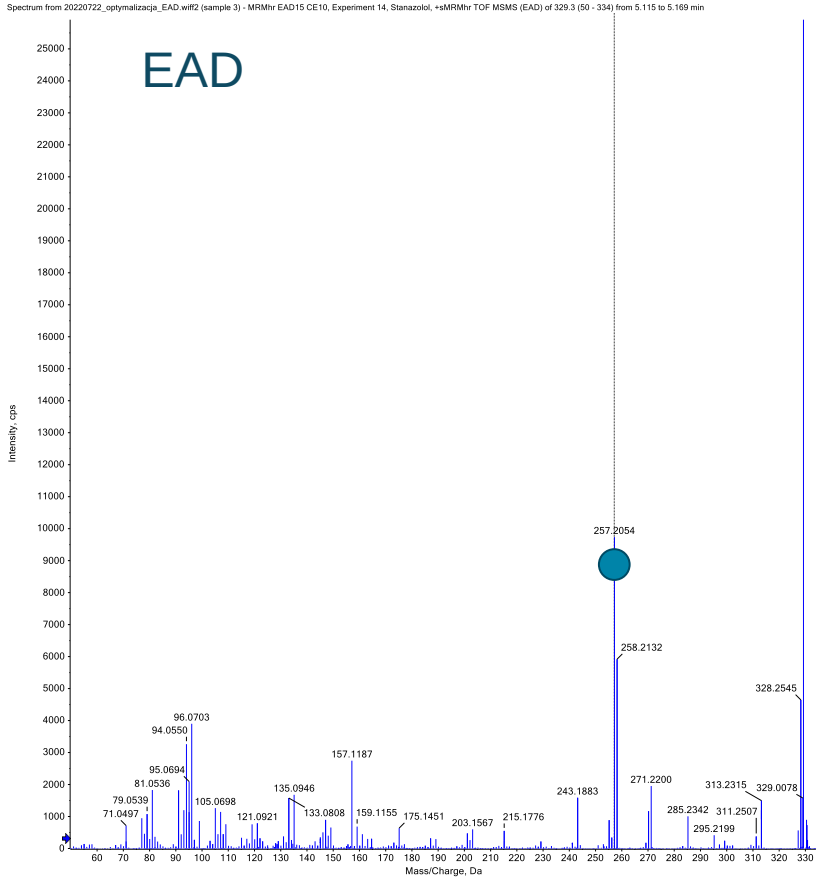
- Very similar and rich CAD fragmentation
- Sometimes the same chemical formulas
- Lots of interferences in matrix samples (urine)

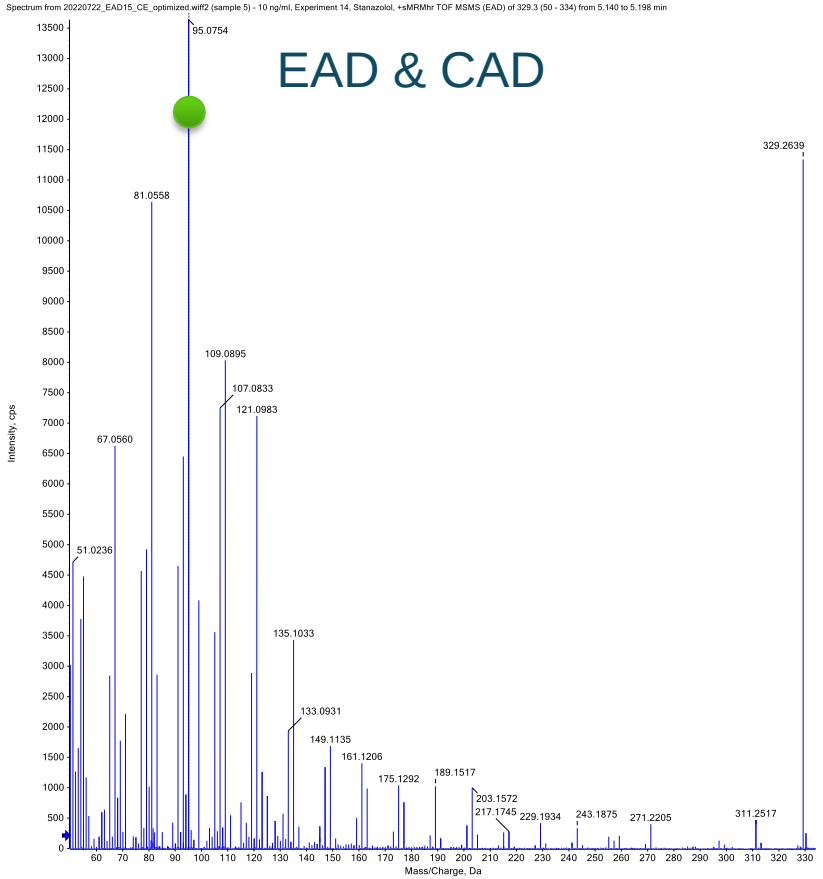


Stanazolol (as an example)

Target confirmation
Example II – anabolic steroids







In each case, the fragmentation energies were selected to obtain the best sensitivity for the best fragmentation ion

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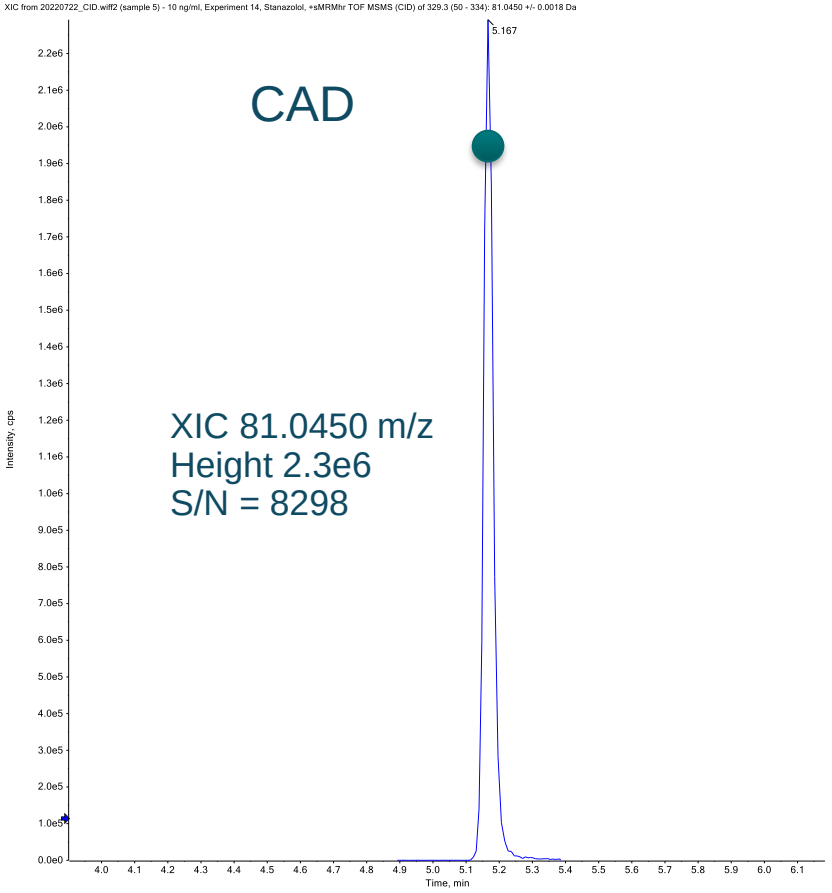
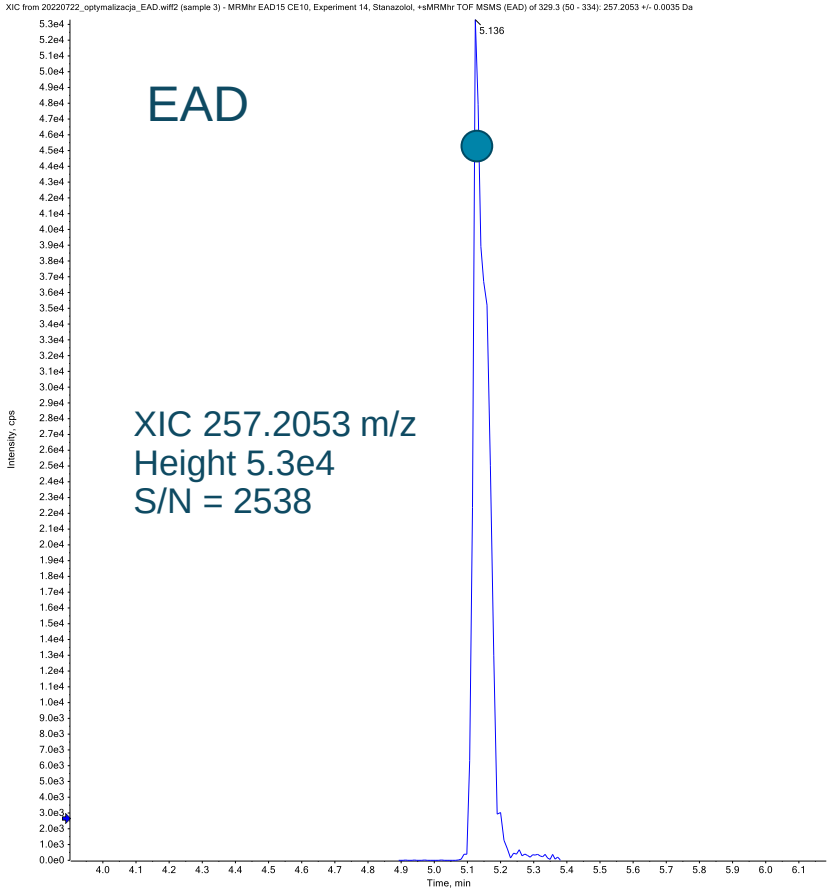
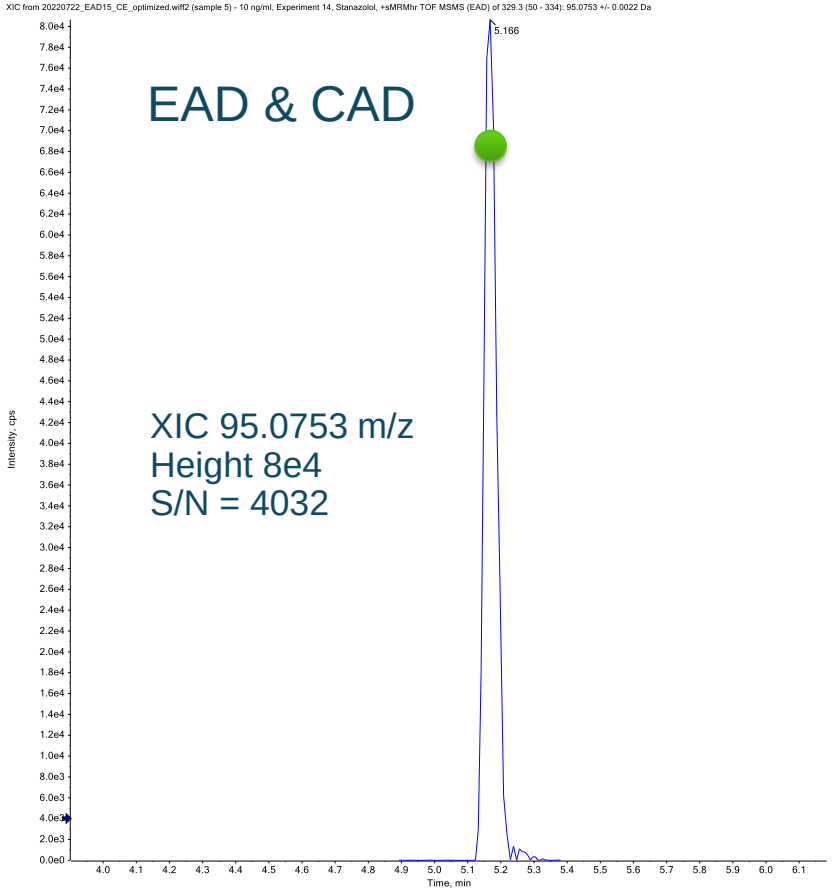
Target confirmation
Example II – anabolic steroids







10 ng/ml in matrix extract – despite approx. 100x higher intensity of in CAD fragmentation, S/N is only:

- about 3x smaller in EAD fragmentation,
- approx. 2x smaller in EAD & CAD mixed fragmentation

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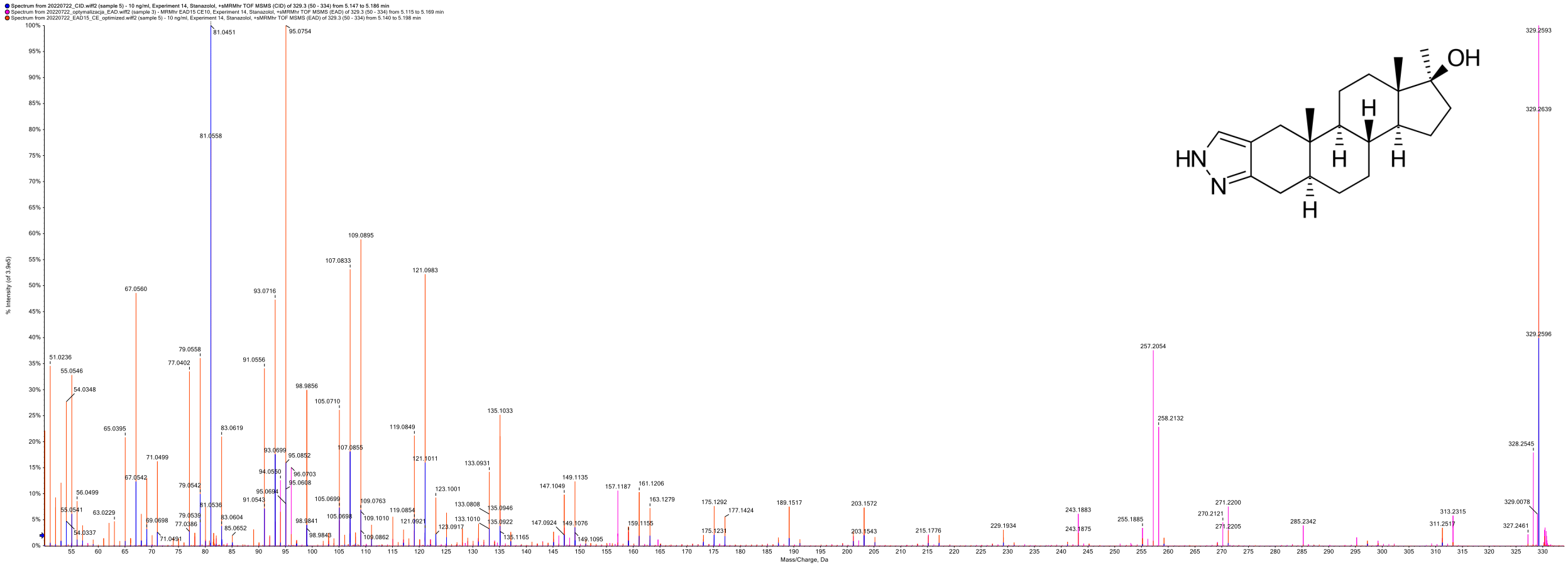
Target confirmation
Example II – anabolic steroids



CAD
EAD
EAD&CAD

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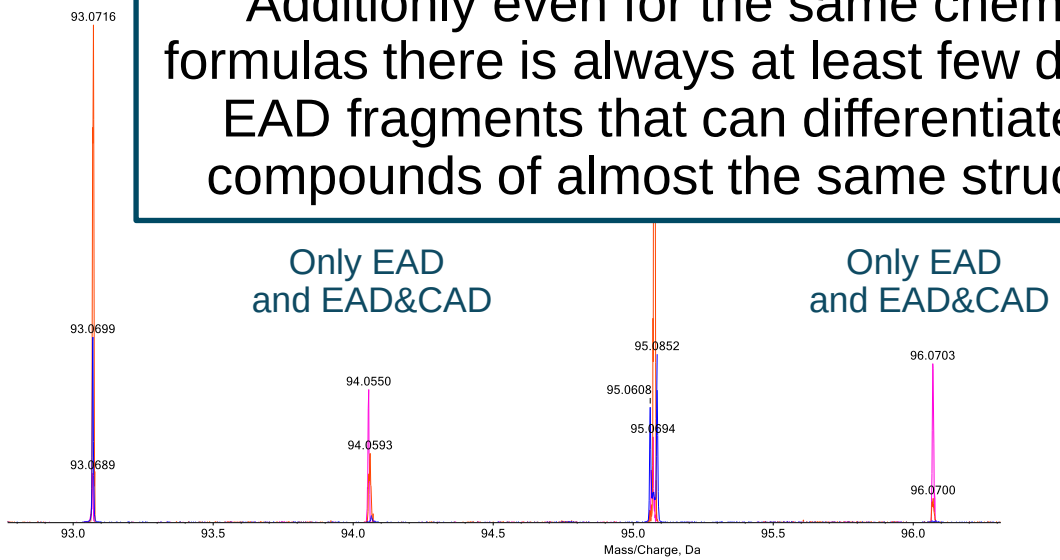
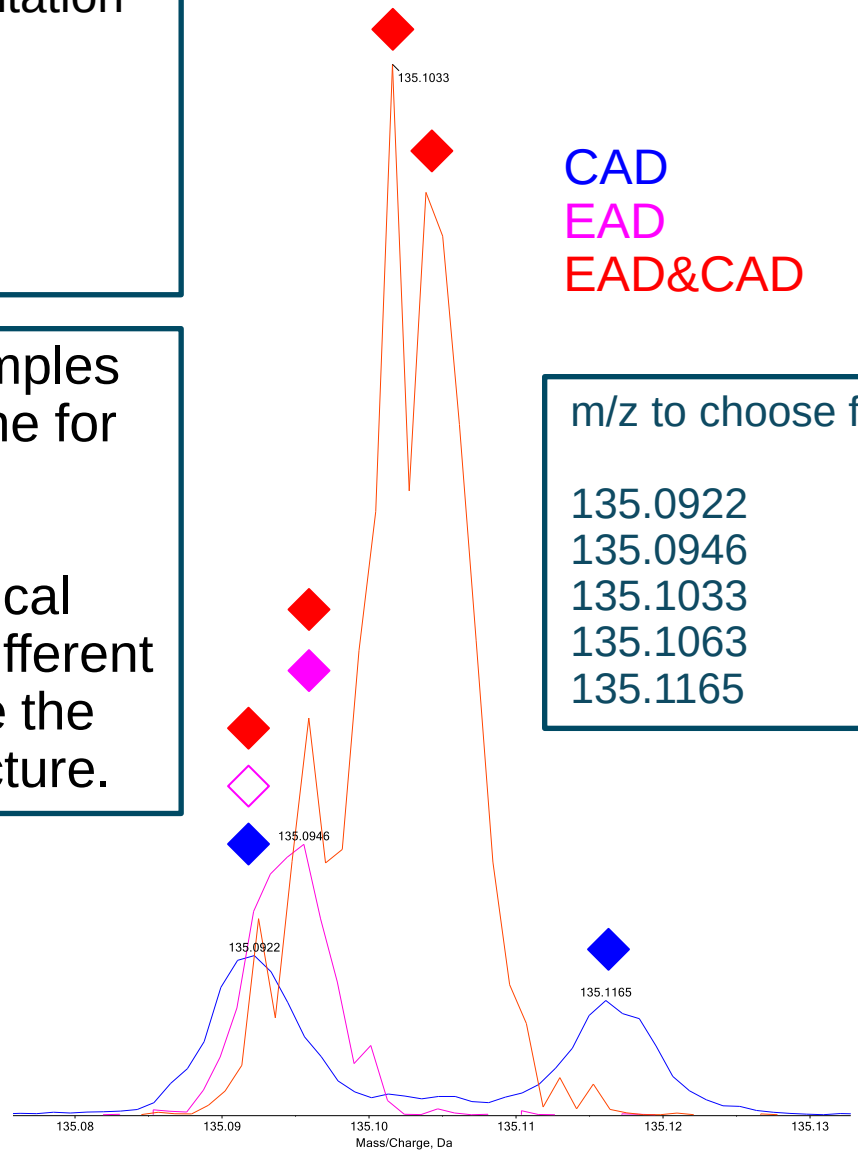
Target confirmation
Example II – anabolic steroids

The final method uses CAD, EAD or EAD&CAD fragmentation
The fragmenatation mode is compound dependant
There are 29 compounds in the method
New MS/MS spectra database was created
5-level confirmation of compound presence is applied
All validation criteria were met

The selectivity achieved in matrix samples was beyond anything that can be done for steroids on QqQ in MRM mode.

Additionly even for the same chemical formulas there is always at least few different EAD fragments that can differentiate the compounds of almost the same structure.





CAD
EAD
EAD&CAD

m/z to choose from:

135.0922
135.0946
135.1033
135.1063
135.1165

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SUMMARY

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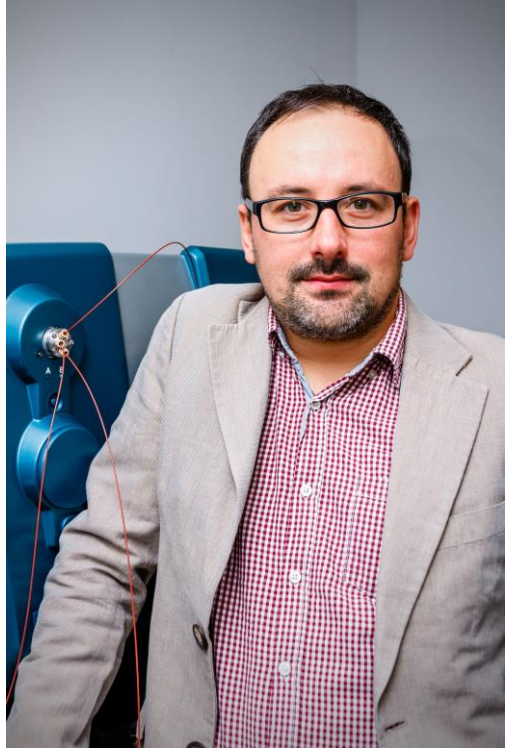
Summary

- In high resolution mass spectrometry mass defect is a foundation of successful mass spectrometry-based compound identification on both MS and MS/MS level (this doesn't exclude other techniques if needed),
- HR-MS/MS compound identification is nowadays strongly supported by software (traditional and more and more AI),
- By now understanding of fragmentation rules and experience is still important,
- DIA is becoming a major tool for simultaneous qualitative and quantitative analysis, however DDA is still and will be used for certain kinds of analysis,
- Targeted analysis and quantitation in HR gives unparallel confidence if compound presence confirmation is needed,
- Novel fragmentation tools such as EAD tool opened the door to another level of data complexity and selectivity

The future…

- AI-based self-learning software,
- Scanning speed → if we reach the point where DIA will have 1Da windows (or less) in the cycle time that corresponds with LC resolution DDA analysis will no longer be needed
- Scanning speed → for targeted analysis polarity switching time is still unacceptable in HR instruments
- Scanning speed → shorter methods

KATARZYNA KRUPCZYŃSKA-STOPA

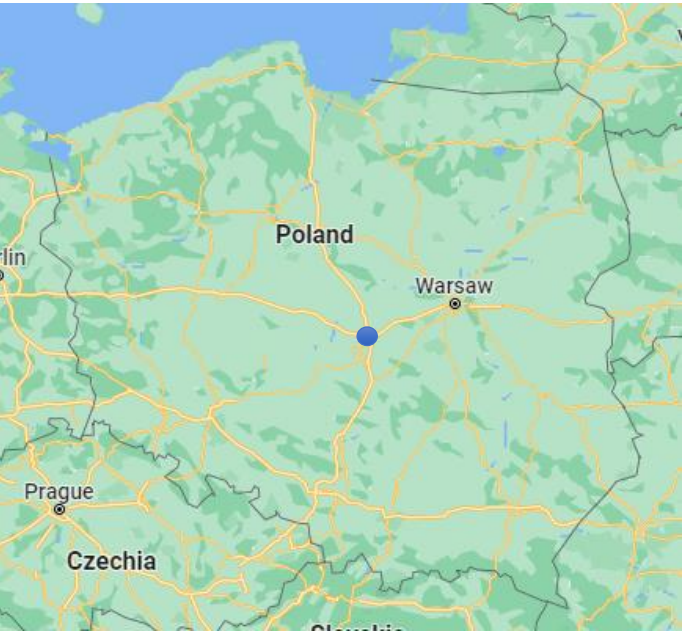


ADRIAN SOBOŃ

ANNA LENARTOWICZ

JULIA MIRONENKA

RAFAŁ SZEWCZYK



Areas of activity:

R&D, research projects
Routine/commercial analytics
Trainings

Summary of the lab team's experience:

Dozens of scientific publications (H index > 40)
Patents/Licenses (4)
Managers or contractors of scientific projects (> 20)
Implementation of analytical methods (> 70)
Trainings (> 150)

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Thank you
Q&A

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