BMSS & BSPR SUPER MEETING

WARWICK UK 04-06 SEPTEMBER 2024

Hands-on Automated MS Data Analysis using Lipid Data Analyzer (LDA)

Part II – Decision Rule Sets

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LDA – Data analysis workflow

MS data

mzXML, AB Sciex Agilent, Bruker, Thermo, (Waters)

Mass lists

Parameters
Fragmentation rules

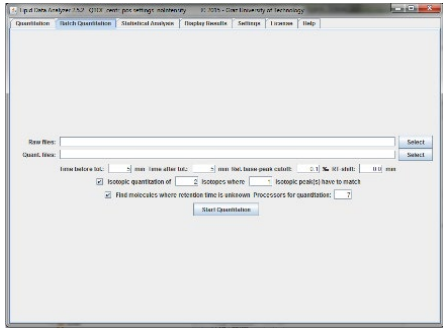
Quantitation

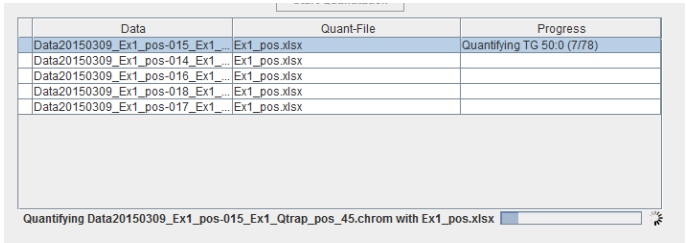
Manual correction

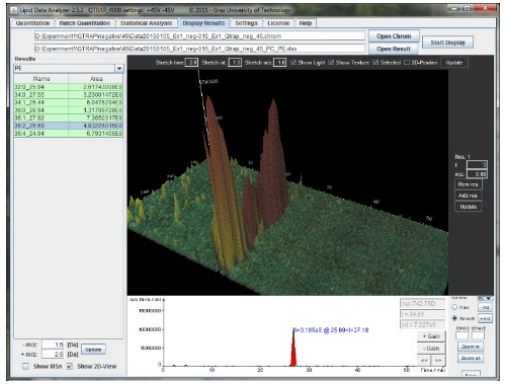
Statistical analysis

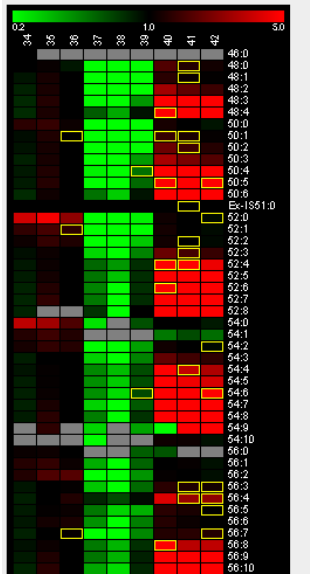
Results export

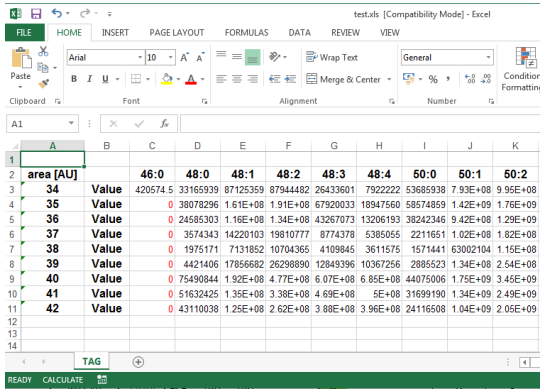
xlsx, txt, mzTab, png, svg











		46:0	48:0	48:1	48:2	48:3	48:4	50:0	50:1	50:2
area [AU]										
34	Value	420574.5	33165939	87125359	87944482	26433601	7922222	53685938	7.93E+08	9.95E+08
35	Value	0	38078296	1.61E+08	1.91E+08	67920033	18947560	58574859	1.42E+09	1.76E+09
36	Value	0	24585303	1.16E+08	1.34E+08	43267073	13206193	38242346	9.42E+08	1.29E+09
37	Value	0	3574343	14220103	19810777	8774378	5385055	2211651	1.02E+08	1.82E+08
38	Value	0	1975171	7131852	10704365	4109845	3611575	1571441	63002104	1.15E+08
39	Value	0	4421406	17856682	26298890	12849396	10367256	2885523	1.34E+08	2.54E+08
40	Value	0	75490844	1.92E+08	4.77E+08	6.07E+08	6.85E+08	44075006	1.75E+09	3.45E+09
41	Value	0	51632425	1.35E+08	3.38E+08	4.69E+08	5E+08	31699190	1.34E+09	2.49E+09
42	Value	0	43110038	1.25E+08	2.62E+08	3.88E+08	3.96E+08	24116508	1.04E+09	2.05E+09

Challenges MS^n – Introduction

MS^1	PI 38:4
	↓
Head group	PI 38:4 ✓
	↓
Chains	PI 20:4_18:0 ✓ ✓
	↓
Position	PI 18:0/20:4 ✓

Fragments (m/z) + Intensity

Decision rule sets - fragments

Fragment:

Name=PIhead Formula=C6H12O9P Charge=1 MSLevel=2 mandatory=false

Placeholders:

Name=NL-Ketene Formula=$PRECURSOR-$CHAIN+H2O Charge=1 MSLevel=2 mandatory=false

Reuse of definitions:

Name=NL-Ket-Head Formula=NL-Ketene-C6H12O6 Charge=1 MSLevel=2 mandatory=false

Decision rule sets - intensities

Relative intensities:

Equation=PIhead-H2O>2*PIhead mandatory=true

Placeholders:

Equation=PIhead-H2O>0.1*$BASEPEAK mandatory=true

Positions:

Equation=NL-Ketene[2]*0.7>NL-Ketene[1] mandatory=true

Decision rule sets – head group

```
[HEAD]
!FRAGMENTS
Name=PIhead          Formula=C6H12O9P    Charge=1    MSLevel=2    mandatory=false
Name=PIhead+Glyc     Formula=C9H14O9P    Charge=1    MSLevel=2    mandatory=false
Name=PIhead-H2O      Formula=C6H10O8P    Charge=1    MSLevel=2    mandatory=true
Name=PIhead-2H2O     Formula=C6H8O7P     Charge=1    MSLevel=2    mandatory=false
```

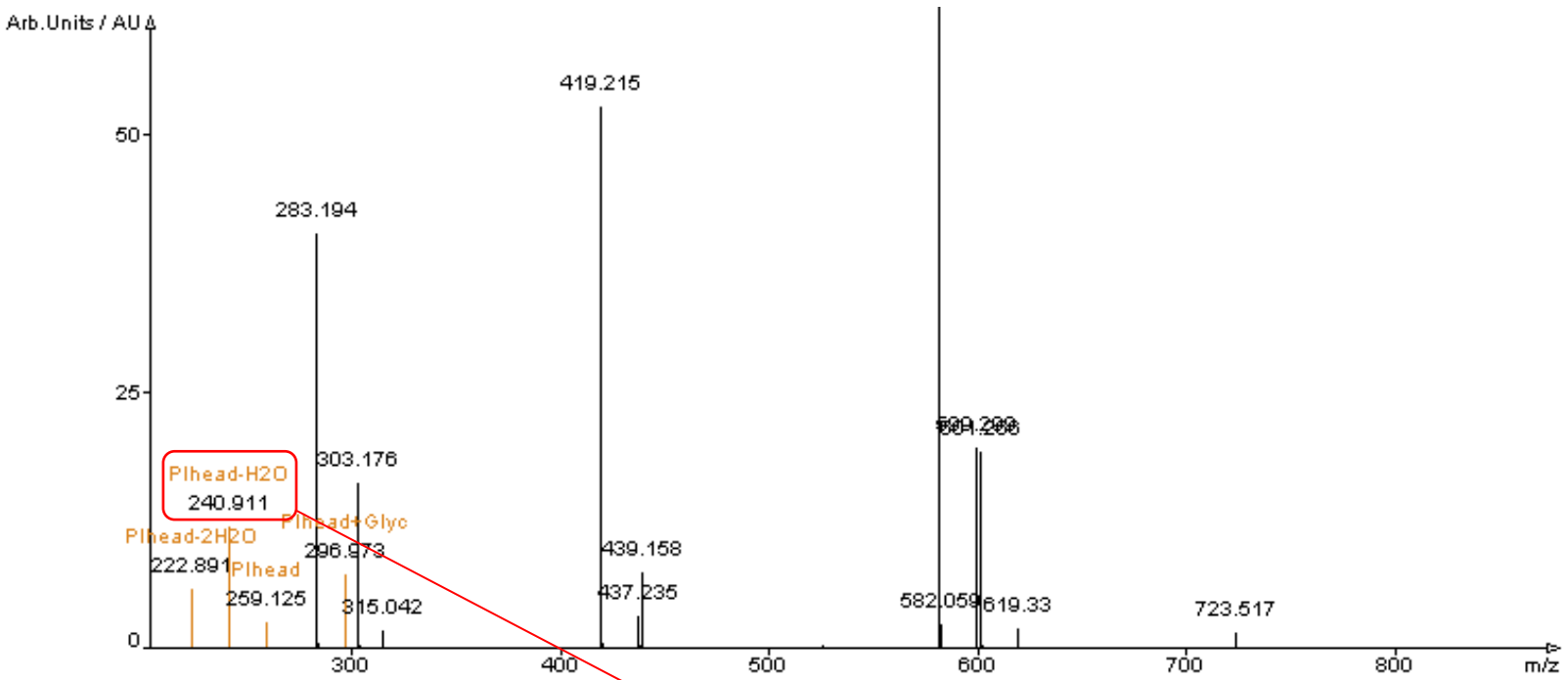
Mandatory fragments:

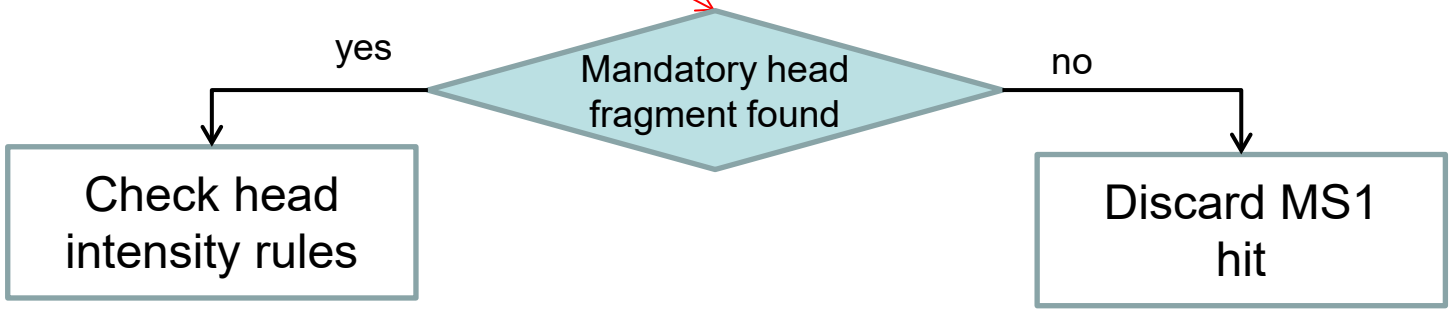
PIhead-H2O	m/z = 241.011

Optional fragments:

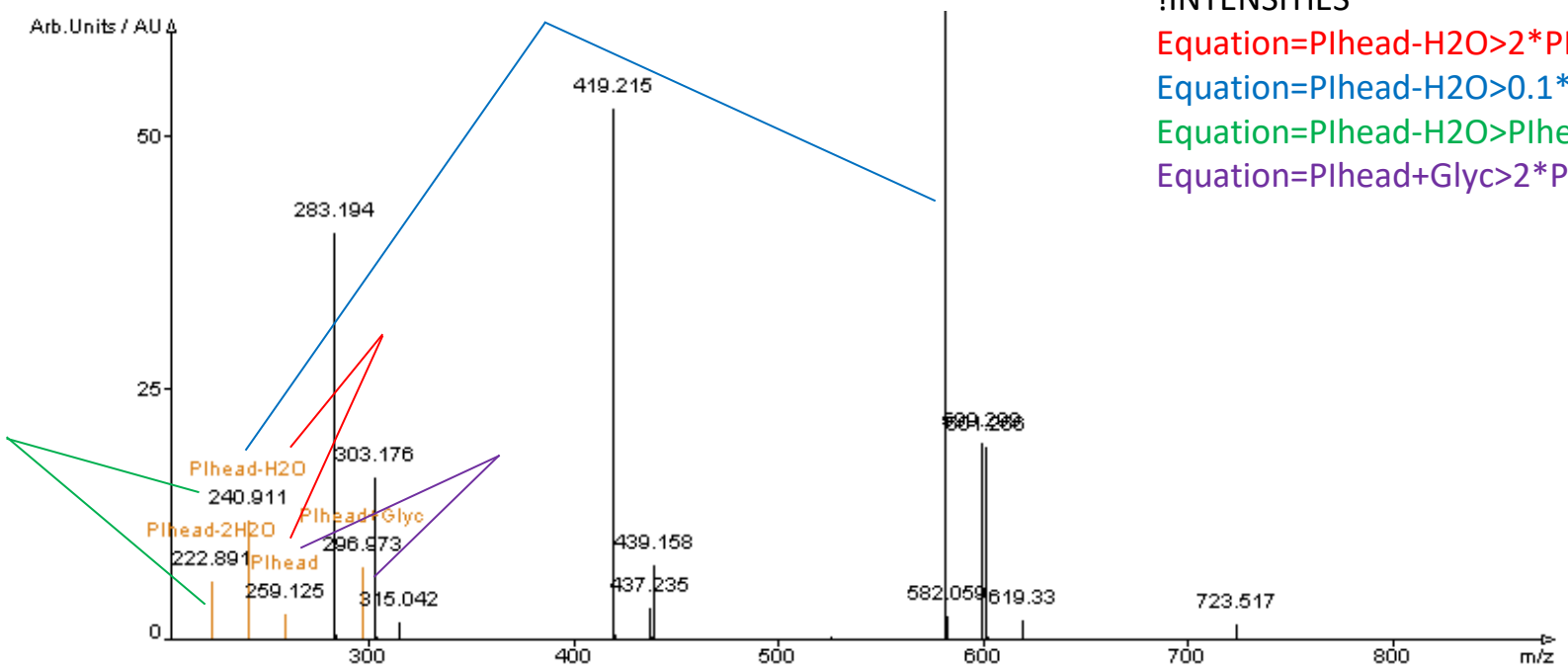
PIhead	m/z = 259.021
PIhead+Glyc	m/z = 297.037
Pihead-2H2O	m/z = 223.001

Decision rule sets – head group

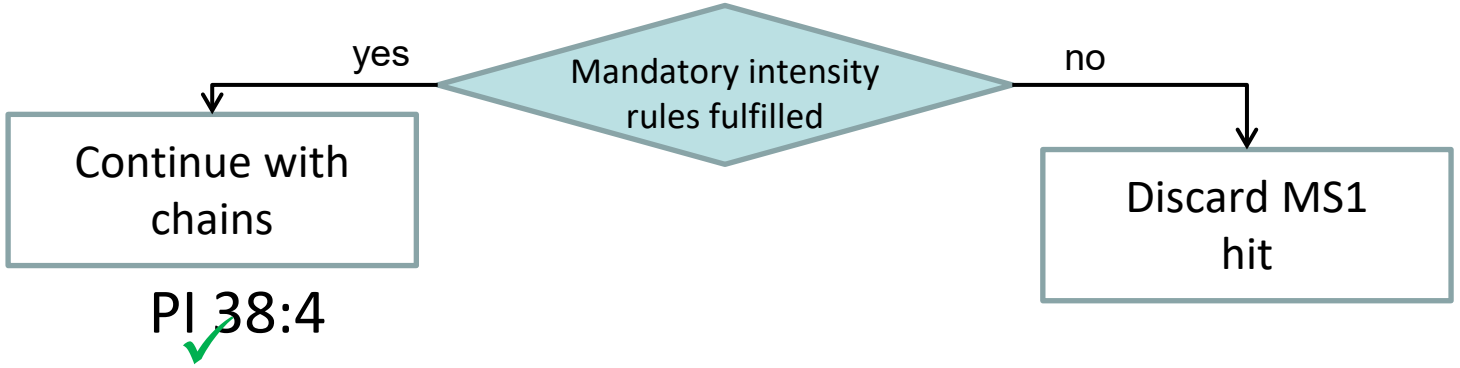




Decision rule sets – head group

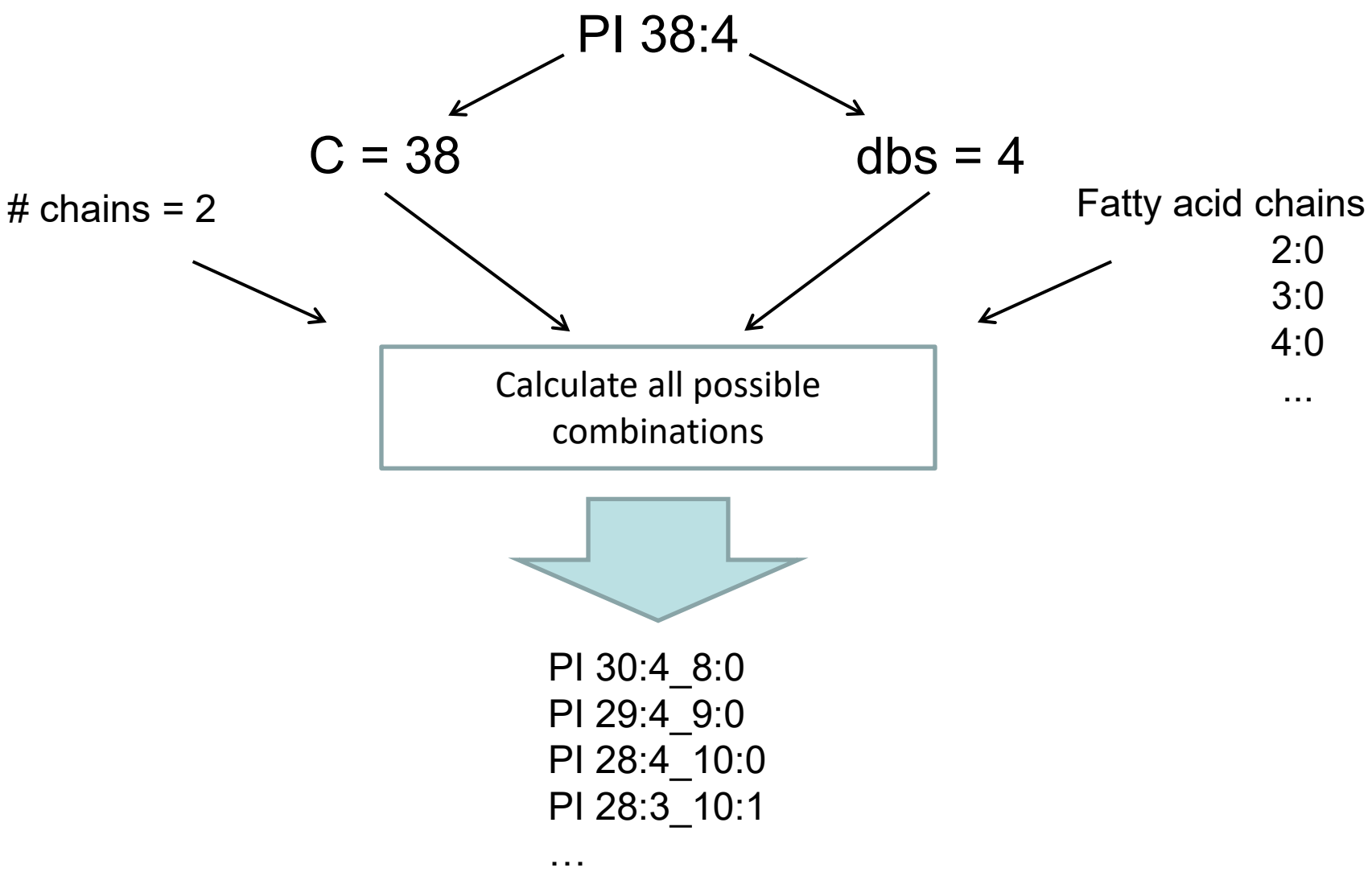



```
!INTENSITIES
Equation=PIhead-H2O>2*PIhead          mandatory=true
Equation=PIhead-H2O>0.1*$BASEPEAK     mandatory=true
Equation=PIhead-H2O>PIhead-2H2O       mandatory=true
Equation=PIhead+Glyc>2*PIhead         mandatory=false
```



PI 38:4 ✓

Decision rule sets - chains



Decision rule sets - chains

```
[CHAINS]
!FRAGMENTS
Name=Carboxy       Formula=$CHAIN-H                    Charge=1   MSLevel=2   mandatory=true
Name=NL-Carboxy    Formula=$PRECURSOR-$CHAIN           Charge=1   MSLevel=2   mandatory=true
Name=NL-Ketene     Formula=$PRECURSOR-$CHAIN+H2O       Charge=1   MSLevel=2   mandatory=false
Name=NL-Ket-Head   Formula=NL-Ketene-C6H12O6           Charge=1   MSLevel=2   mandatory=false
```

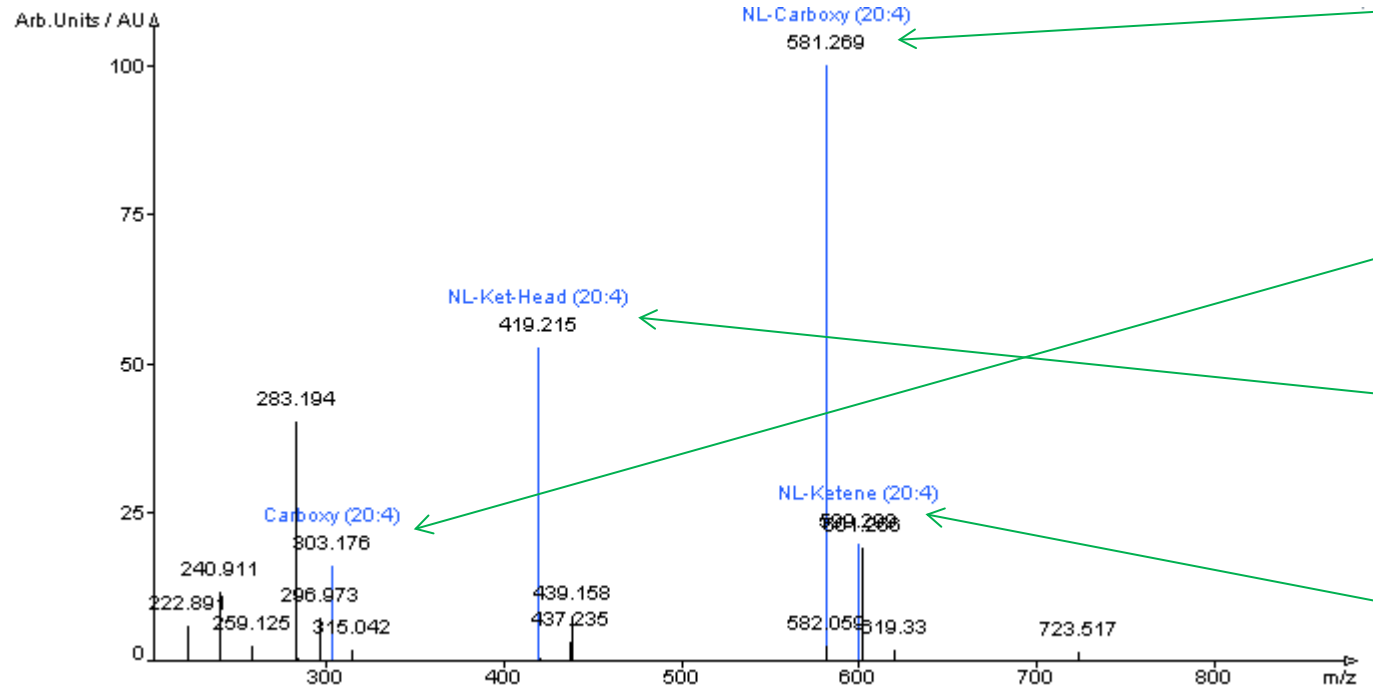
Mandatory fragments:

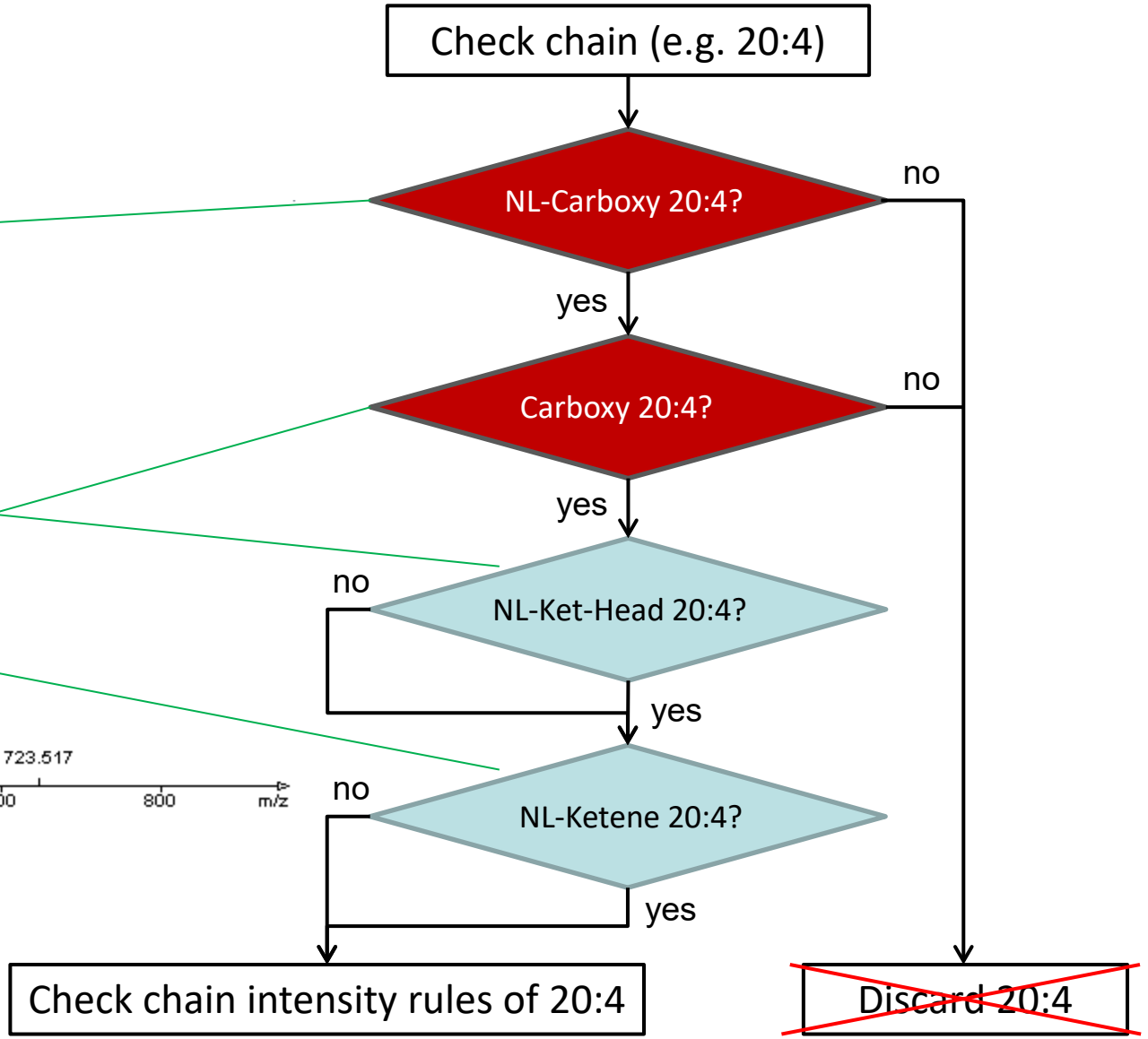
Carboxy(13:2)	m/z = 209.154
NL-Carboxy(13:2)	m/z = 675.388
Carboxy(24:1)	m/z = 365.341
…	

Optional fragments:

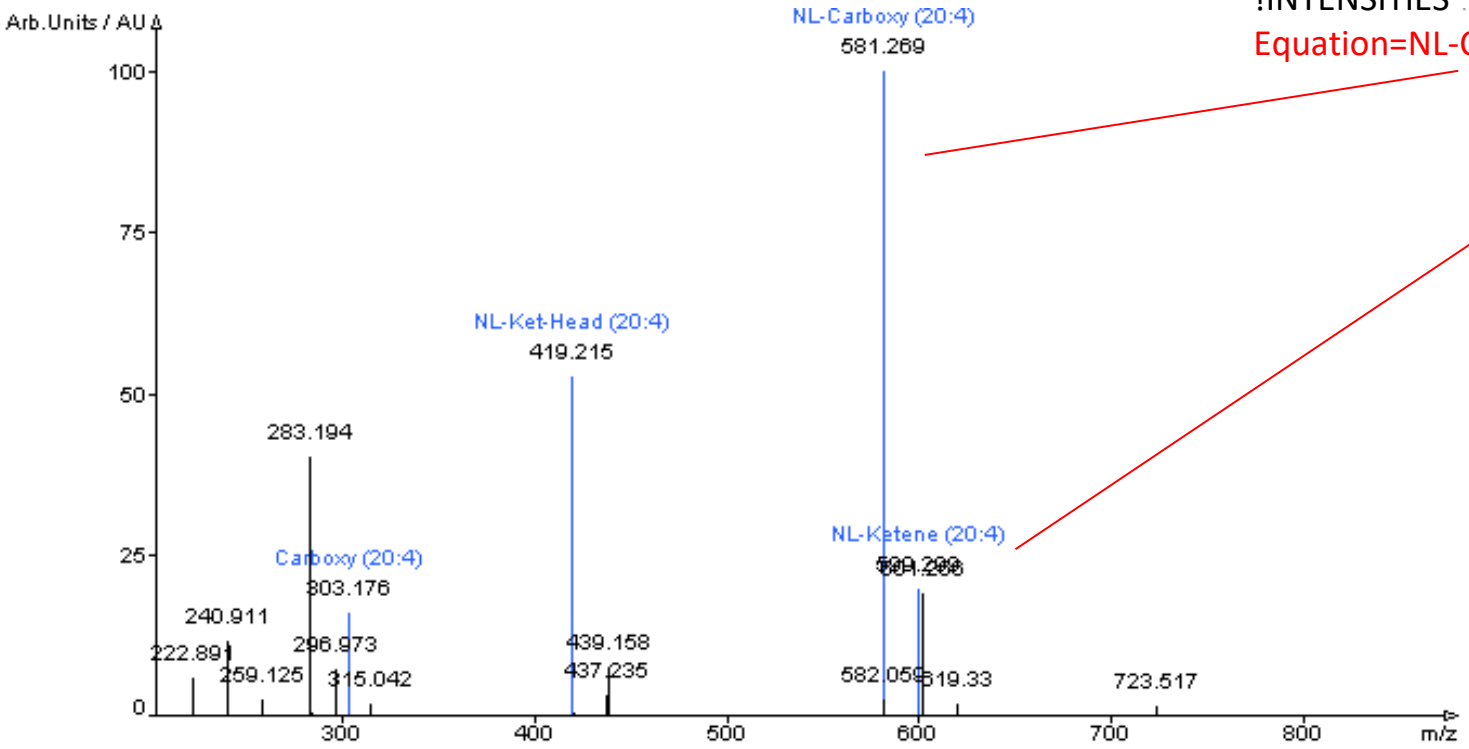
NL-Ket-Head(13:2)	m/z = 513.335
NL-Ketene(13:2)	m/z = 693.398
NL-Ket-Head(24:1)	m/z = 357.147

Decision rule sets - chains



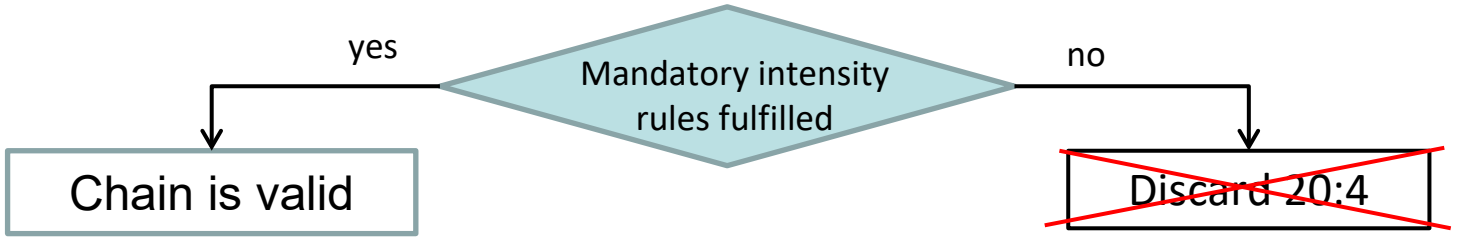


Decision rule sets - chains

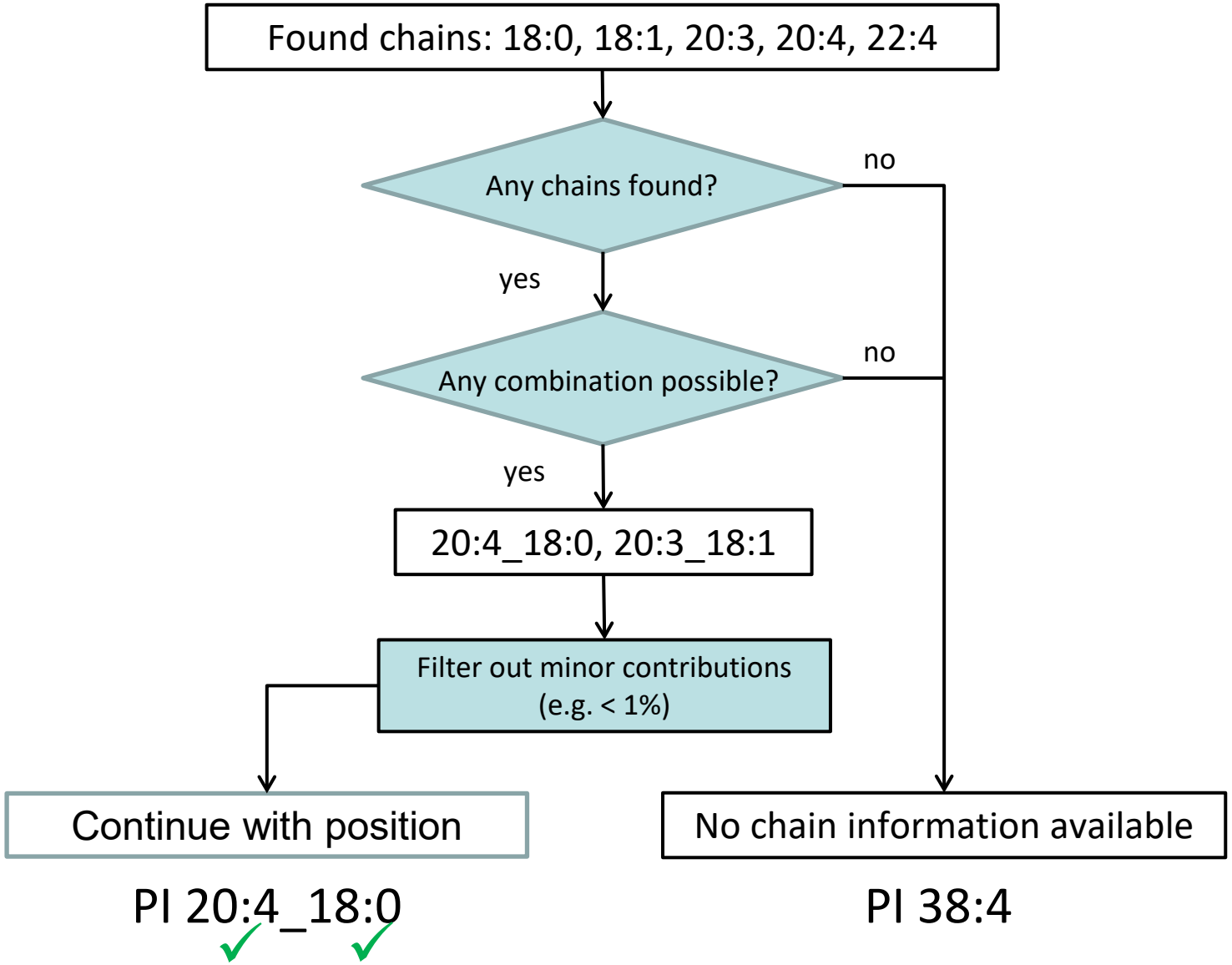


!INTENSITIES

Equation=NL-Carboxy>0.6*NL-Ketene mandatory=true

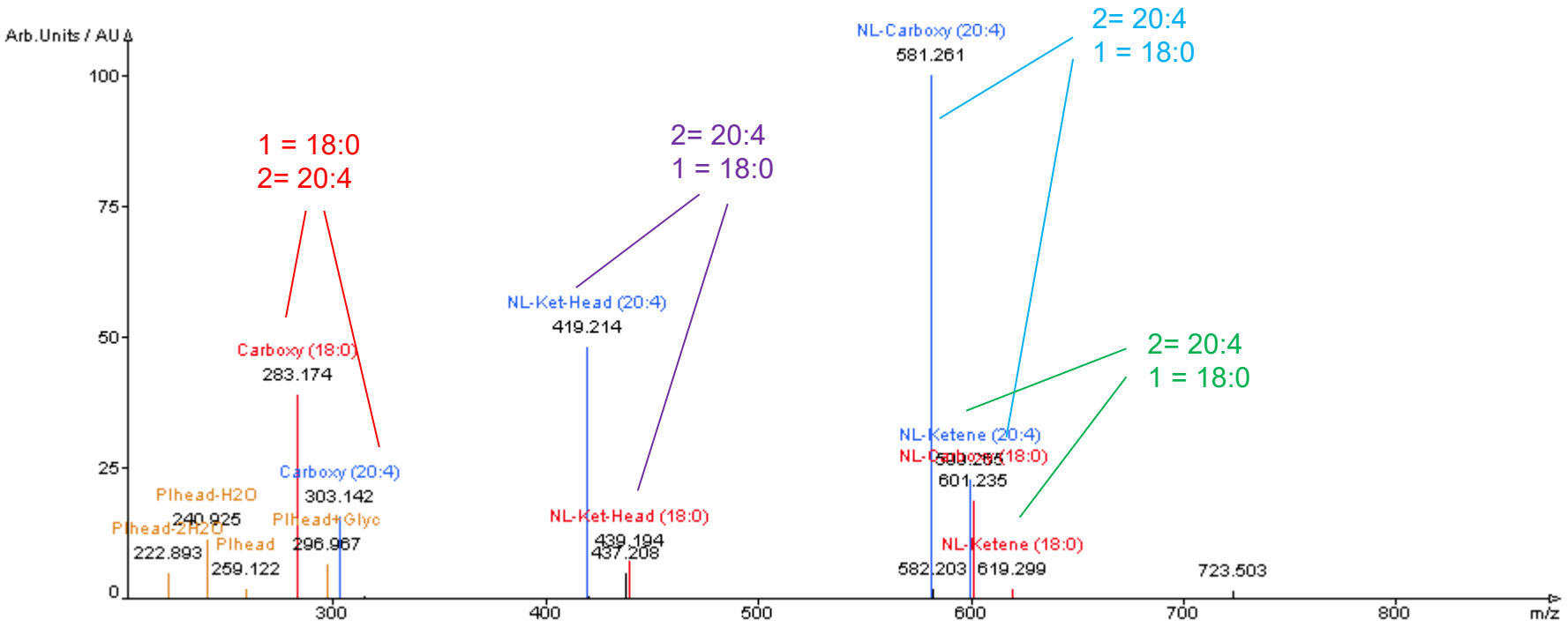


Decision rule sets - chains

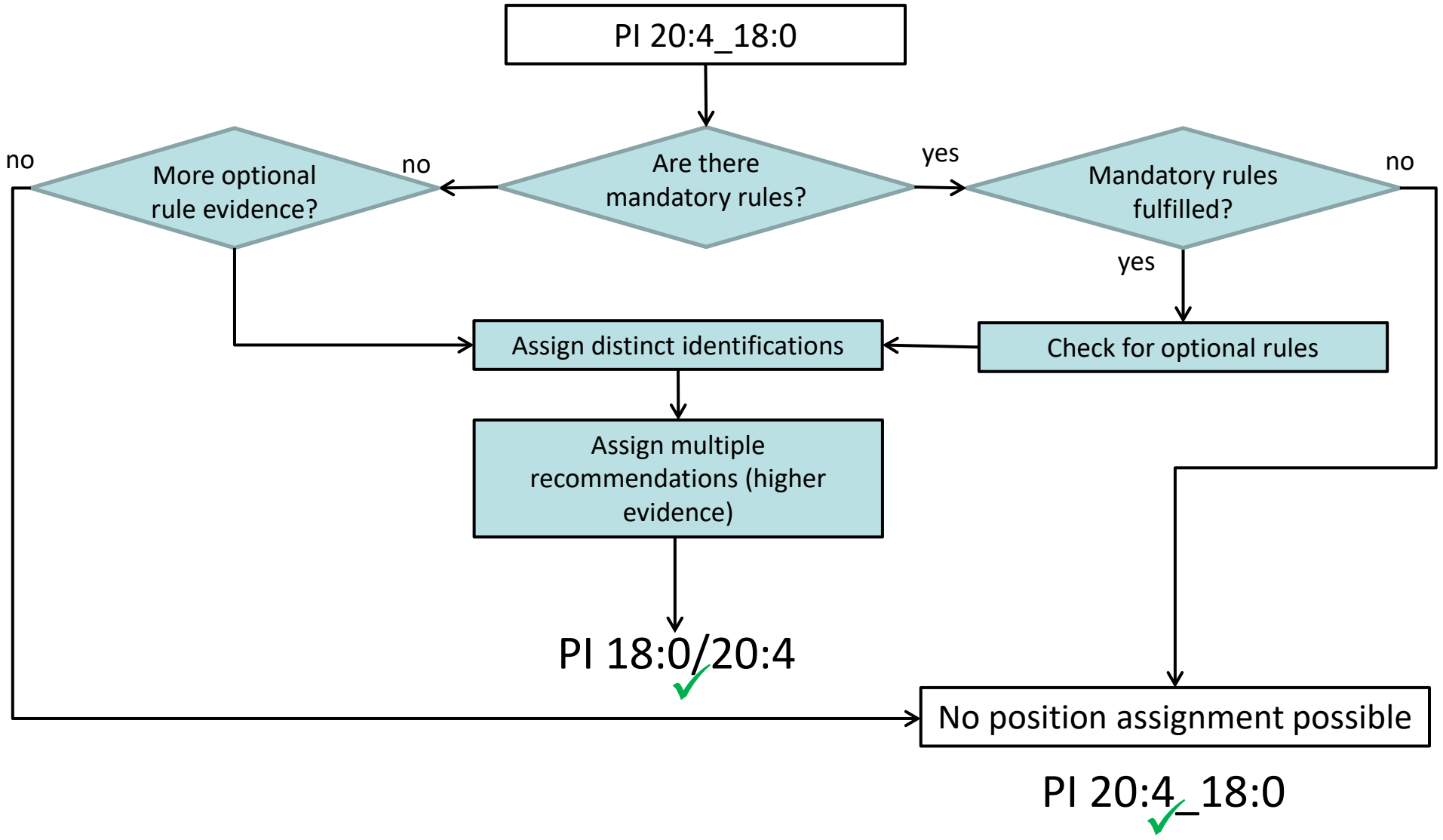


Decision rule sets - position

[POSITION]
!INTENSITIES
Equation=Carboxy[1]>Carboxy[2] mandatory=false
Equation=NL-Carboxy[2]*0.7>NL-Carboxy[1] mandatory=true
Equation=NL-Ketene[2]*0.7>NL-Ketene[1] mandatory=false
Equation=NL-Ket-Head[2]*0.9>NL-Ket-Head[1] mandatory=true



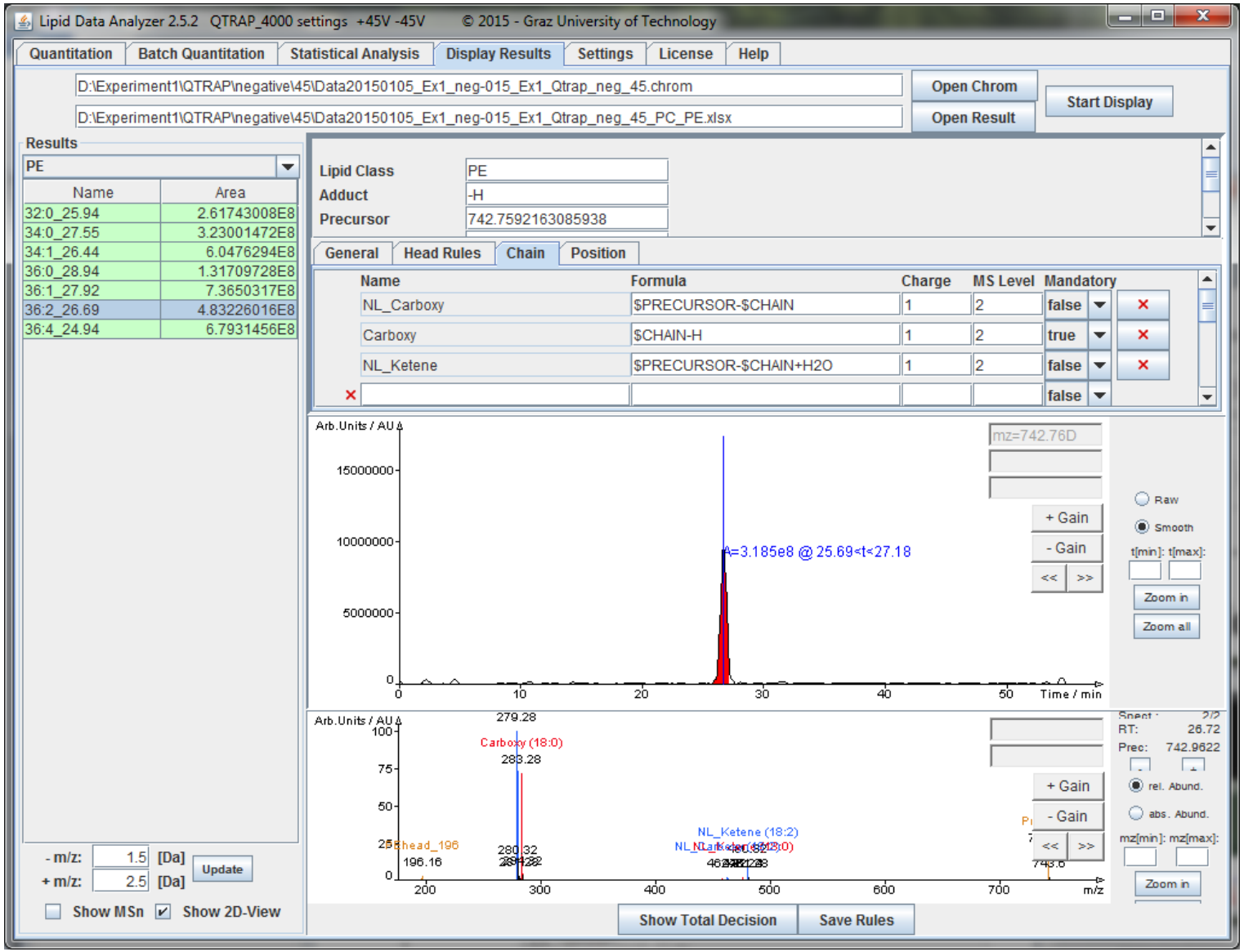
Decision rule sets - position



Decision rule sets - example for the lipid class PI

```
[GENERAL]
AmountOfChains=2
ChainLibrary=fattyAcidChains.xlsx
CAtomsFromName=(\d+):\d+
DoubleBondsFromName=\d+:(\d+)
BasePeakCutoff=0.01%

[HEAD]
!FRAGMENTS
Name=PIhead                 Formula=C6H12O9P      Charge=1    MSLevel=2    mandatory=false
Name=PIhead+Glyc Formula=C9H14O9P      Charge=1    MSLevel=2    mandatory=false
Name=PIhead-H2O Formula=C6H10O8P      Charge=1    MSLevel=2    mandatory=true
Name=PIhead-2H2O            Formula=C6H8O7P Charge=1    MSLevel=2    mandatory=false

!INTENSITIES
Equation=PIhead-H2O>2*PIhead                mandatory=true
Equation=PIhead-H2O>0.1*$BASEPEAKmandatory=true
Equation=PIhead-H2O>PIhead-2H2O     mandatory=true
Equation=PIhead+Glyc>2*PIhead               mandatory=false


[CHAINS]
!FRAGMENTS
Name=Carboxy                Formula=$CHAIN-H      Charge=1    MSLevel=2    mandatory=true
Name=NL-Carboxy  Formula=$PRECURSOR-$CHAIN        Charge=1    MSLevel=2    mandatory=true
Name=NL-Ketene              Formula=$PRECURSOR-$CHAIN+H2O Charge=1    MSLevel=2    mandatory=false
Name=NL-Ket-Head            Formula=NL-Ketene-C6H12O6     Charge=1    MSLevel=2    mandatory=false

!INTENSITIES
Equation=NL-Carboxy>0.6*NL-Ketene   mandatory=true


[POSITION]
!INTENSITIES
Equation=Carboxy[1]>Carboxy[2]              mandatory=false
Equation=NL-Carboxy[2]*0.7>NL-Carboxy[1]    mandatory=true
Equation=NL-Ketene[2]*0.7>NL-Ketene[1]      mandatory=false
Equation=NL-Ket-Head[2]*0.9>NL-Ket-Head[1]  mandatory=true
```

Decision rule sets - graphical user interface



Your task:

- Change the decision rules of PC $[M+H]^+$ and PE $[M+H]^+$
- Recalculate PC and PE for a single file
- What has changed? – Compare the previous to the new results

Suggested Changes:

- PC $[M+H]^+$ and PE $[M+H]^+$

  Name=PChead_184 Formula=C5H15NO4P Charge=1 MSLevel=2 mandatory=true

  Equation=1.5*PChead_184>NL_PEhead_141_WRONG mandatory=false

- PE $[M+H]^+$

  Equation=NL_PEhead_141*0.9>PEhead_Na_164_wrong mandatory=false