RMassBank

eawag
aquatic research

RMassBank: Automatic Recalibration and Processing of Tandem HR-MS Spectra for MassBank

Interactive Demonstration

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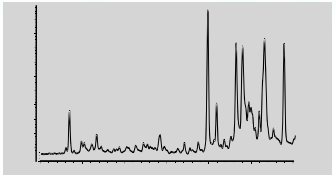
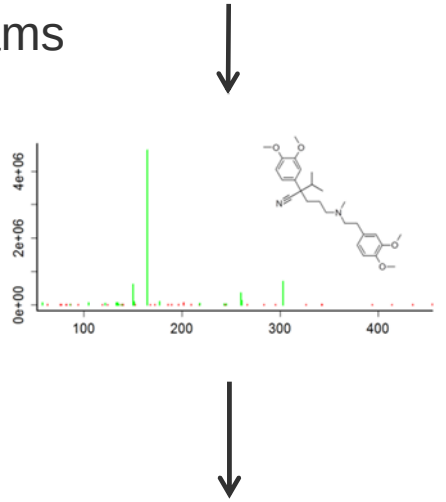
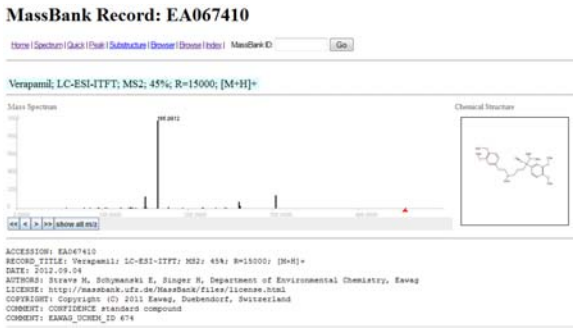
MARIE CURIE ACTIONS

Demonstration Overview



- Install (and update) RMassBank and associated programs
- Run RMassBank for trial data
- Get a basic understanding for the workflow
- Understand the manual checking required
  - “Fail peaks” – checking these in raw data
  - Automatic annotation with CTS – editing this data
- Generate records with RMassBank
- Upload these records to MassBank

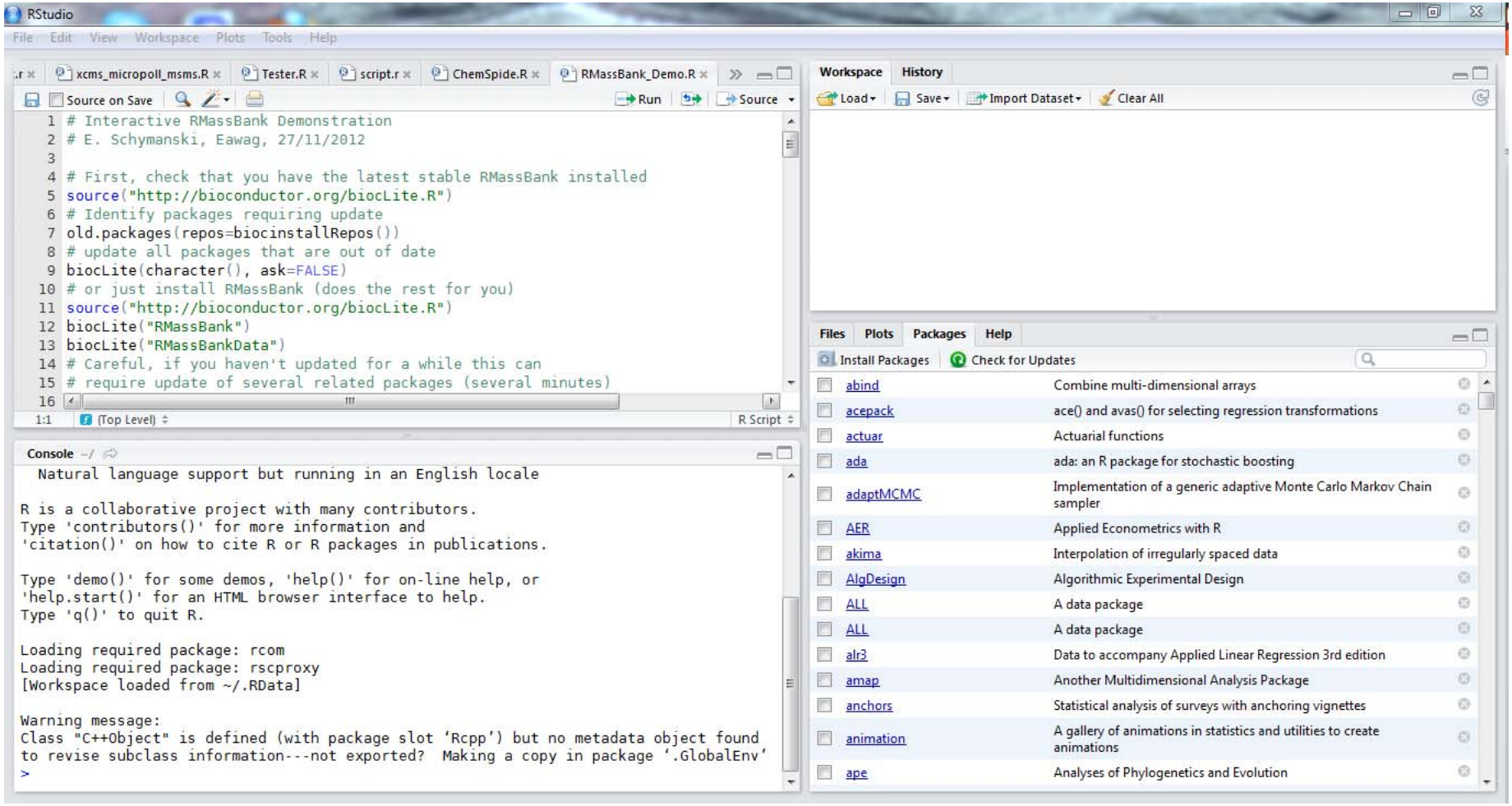




Starting RMassBank

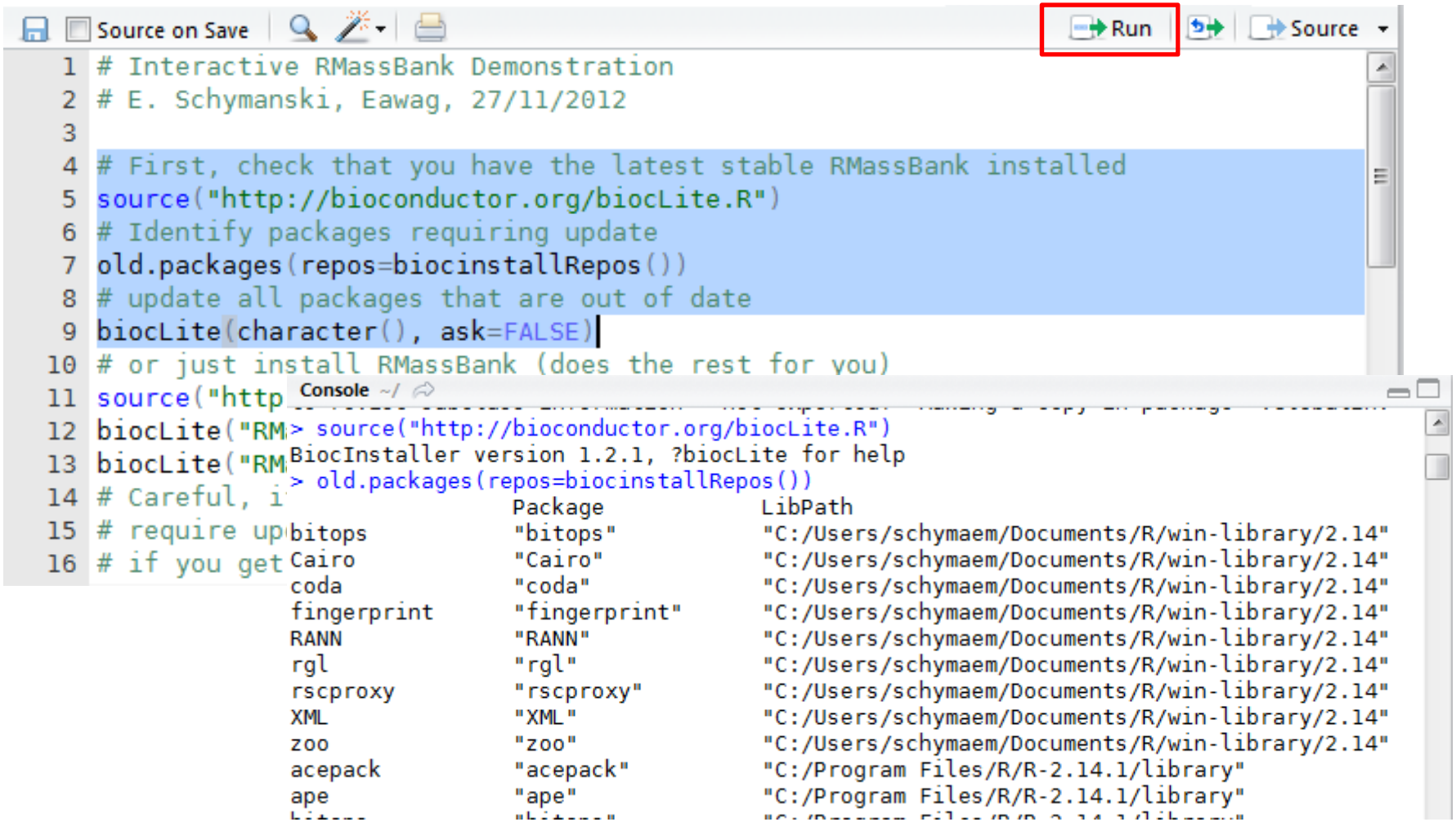
Open up RStudio (or R console)

- And open up “RMassBank_Demo.R”



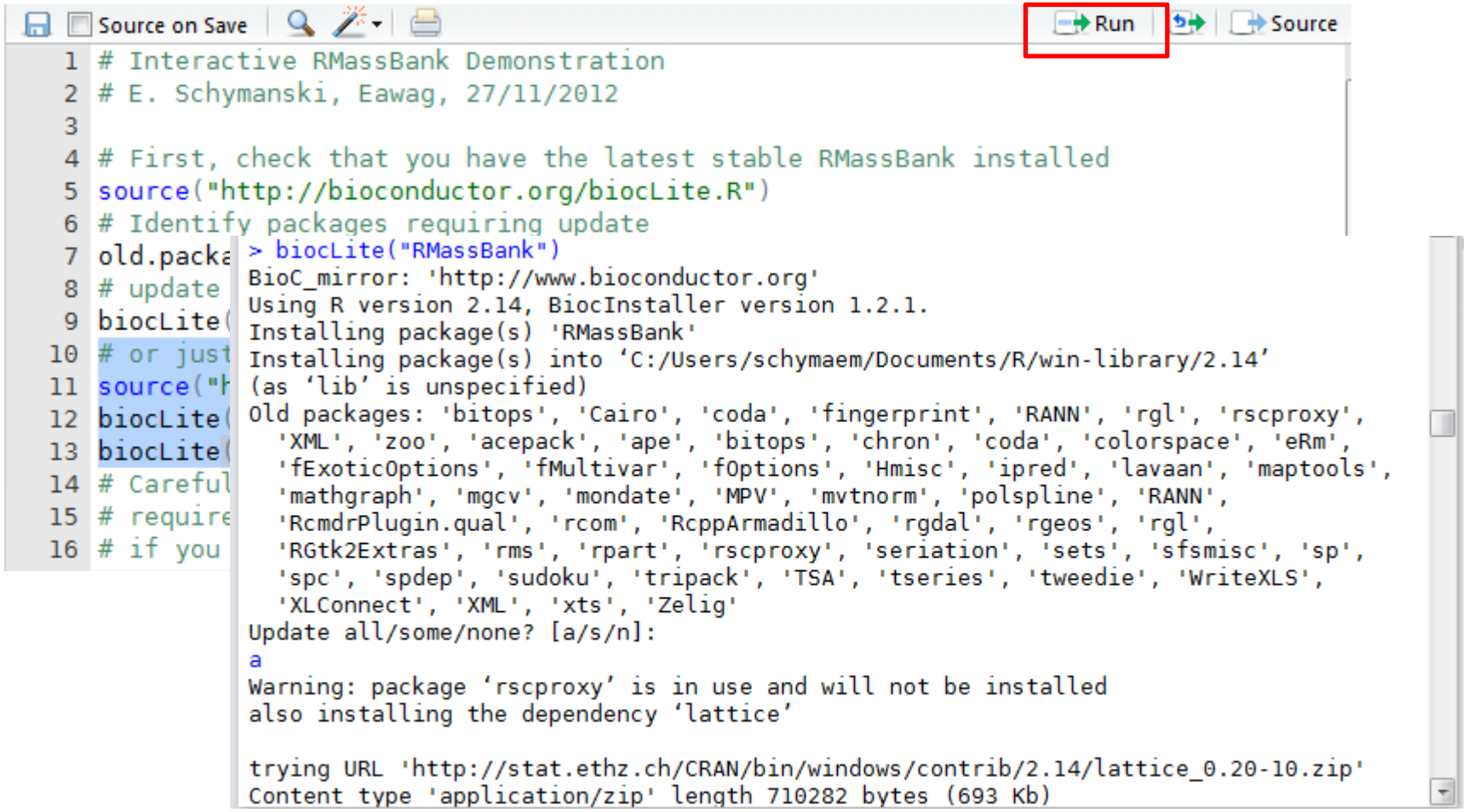
Installing / Updating RMassBank

To check if you need to update RMassBank:



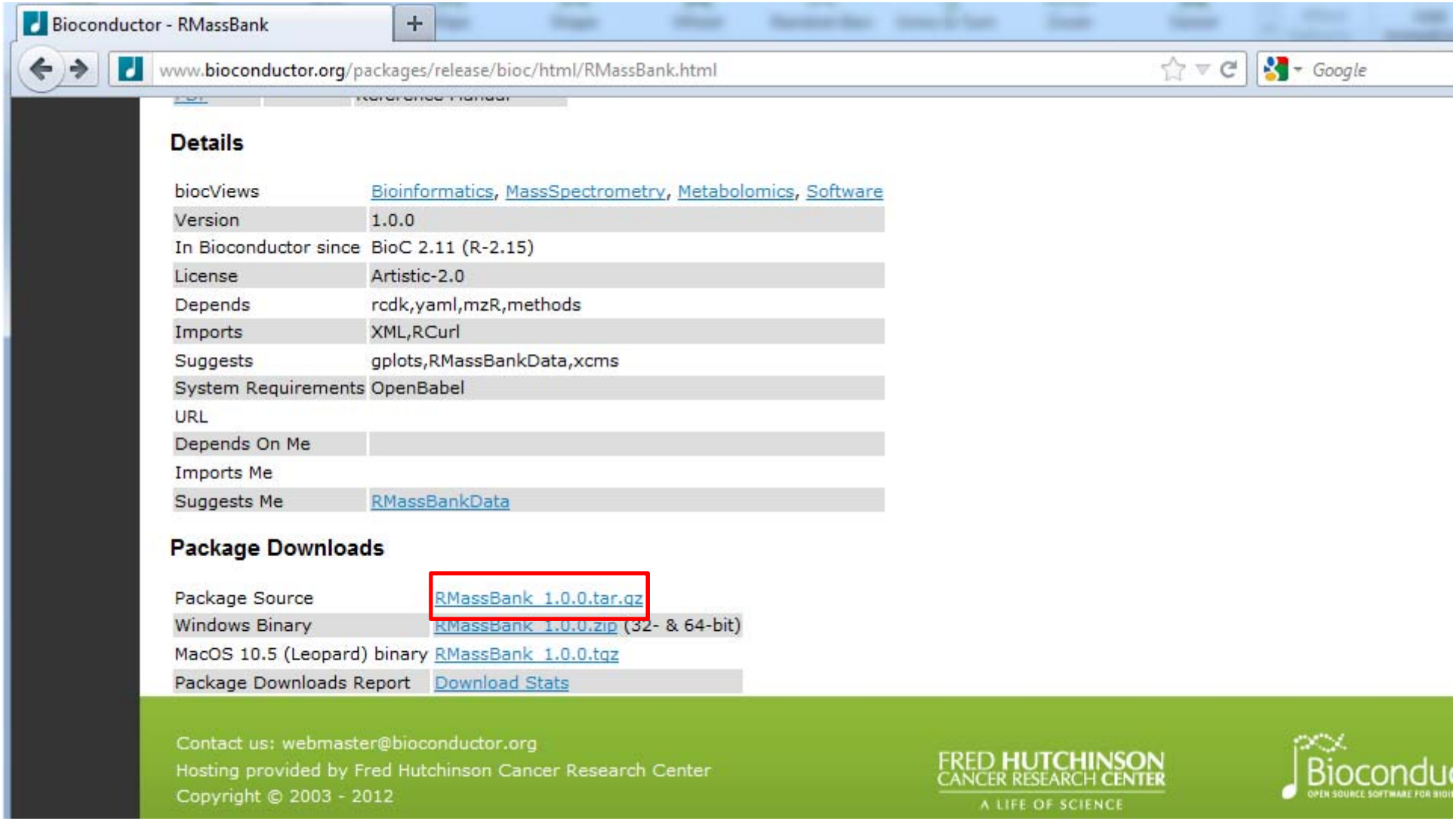
Installing / Updating RMassBank

To install RMassBank:



Installing from source – if all else fails

(this means hand-installing other packages, avoid if possible!)



Installing from source – if all else fails

(this means hand-installing other packages, avoid if possible!)

```
#If this doesn't work, try installing from source
# http://www.bioconductor.org/packages/release/bioc/html/RMassBank.html
# download Package
# http://www.biocon
install.packages("f
                 re
# http://www.biocon
# download package
# http://www.biocon
install.packages("f
                 re
```

```
> install.packages("C:/DATA/RMassBank/RMassBank_Versions/RMassBank_1.0.0.tar.gz",
repos=NULL, type="source")
Installing package(s) into 'C:/Users/schymaem/Documents/R/win-library/2.14'
(as 'lib' is unspecified)
* installing *source* package 'RMassBank' ...
** R
** inst
** preparing package for lazy loading
Warning: package 'rcdk' was built under R version 2.14.2
Warning: package 'rcdklibs' was built under R version 2.14.2
Warning: package 'fingerprint' was built under R version 2.14.2
Warning: package 'iterators' was built under R version 2.14.2
Warning: package 'yaml' was built under R version 2.14.2
Warning: package 'Rcpp' was built under R version 2.14.2
** help
*** installing help indices
** building package indices ...
*** tangling vignette sources ...
   'RMassBank.Rnw'
** testing if installed package can be loaded
Warning messages:
1: package 'rcdk' was built under R version 2.14.2
2: package 'rcdklibs' was built under R version 2.14.2
3: package 'fingerprint' was built under R version 2.14.2
4: package 'iterators' was built under R version 2.14.2
5: package 'yaml' was built under R version 2.14.2
6: package 'Rcpp' was built under R version 2.14.2

* DONE (RMassBank)
>
```

Loading RMassBank

```
30 # Once you have got through all that, you need to load the packages:
31 library("RMassBank")
32 library("RMassBankData")
33
```

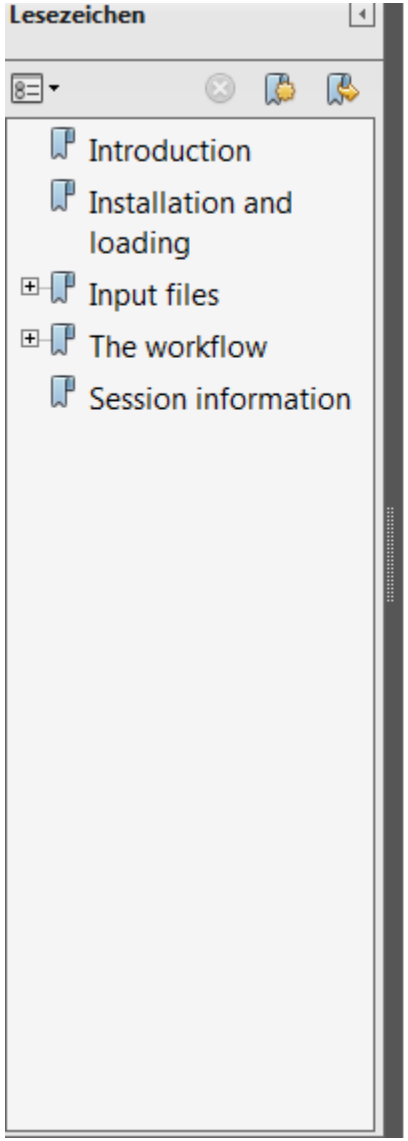
32:25 (Top Level) R Script

Console ~/

```
> # Once you have got through all that, you need to load the packages:
> library("RMassBank")
Loading required package: mzR
Loading required package: Rcpp
Loading required package: XML
Loading required package: RCurl
Loading required package: bitops
Loading required package: zoo

Attaching package: 'zoo'

The following object(s) are masked from 'package:base':

    as.Date, as.Date.numeric

Loading required package: iterators
Warning messages:
1: package 'Rcpp' was built under R version 2.14.2
2: package 'XML' was built under R version 2.14.2
3: package 'bitops' was built under R version 2.14.2
4: package 'zoo' was built under R version 2.14.2
5: package 'rcdk' was built under R version 2.14.2
6: package 'rcdklibs' was built under R version 2.14.2
7: package 'fingerprint' was built under R version 2.14.2
8: package 'iterators' was built under R version 2.14.2
> library("RMassBankData")
Warning message:
package 'RMassBankData' was built under R version 2.14.2
>
```

Finding out more about RMassBank

vignette("RMassBank")



RMassBank: The workflow by example

Michael Stravs

October 2, 2012

We'll kind-of work through this today…

Before getting started:

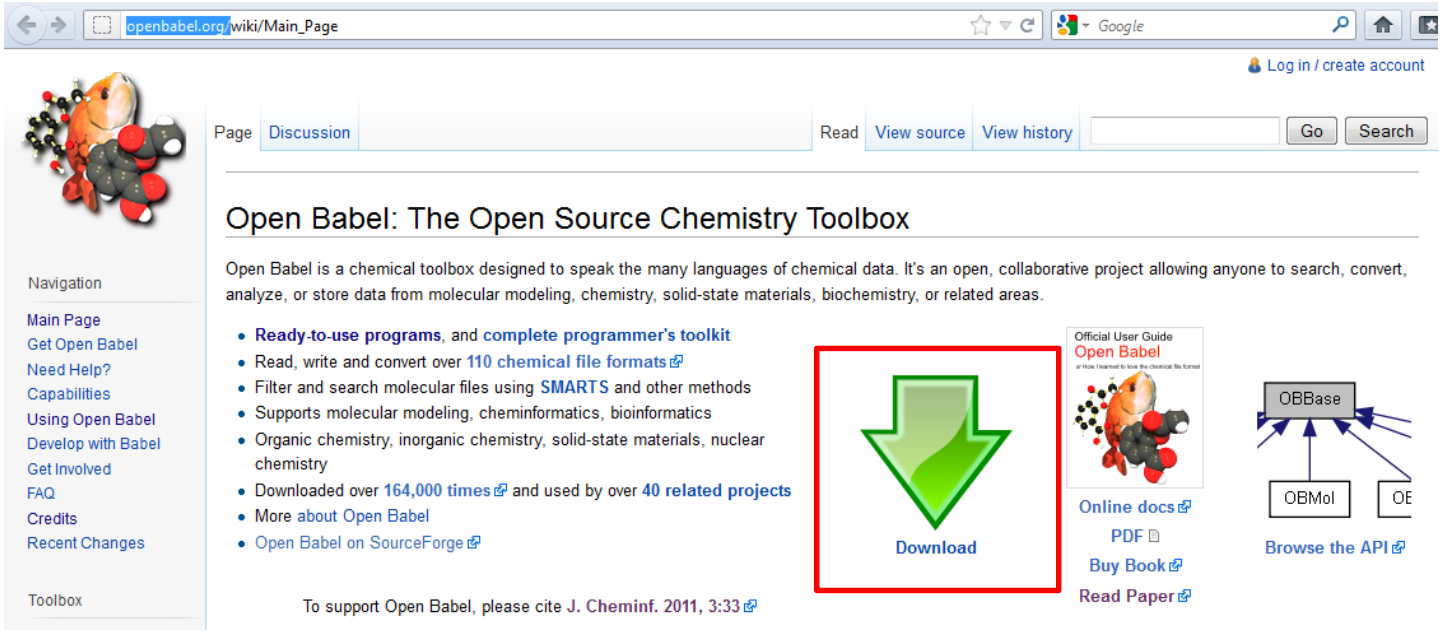
Install / Check OpenBabel

If you think you already have this, check e.g. (for Windows….)

- All Programs => OpenBabel 2.X.X
- C:\Program Files\OpenBabel-2.3.0

If you don't have this, please download (not *required* but it is *recommended*)

- http://openbabel.org/

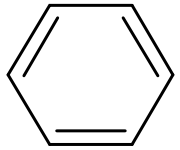


Before getting started:

Install / Check OpenBabel

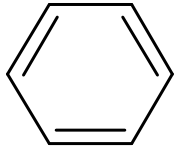
Why OpenBabel?

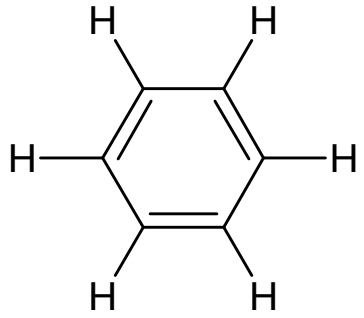
- Convert SMILES (c1ccccc1) to structure
- C:\Program Files\OpenBabel-2.3.0



NOTE:

- By default, RMassBank will work without OpenBabel and has a back-up solution using CACTUS
- But, structures are less visually appealing





Before getting started:

Install / Check Proteowizard

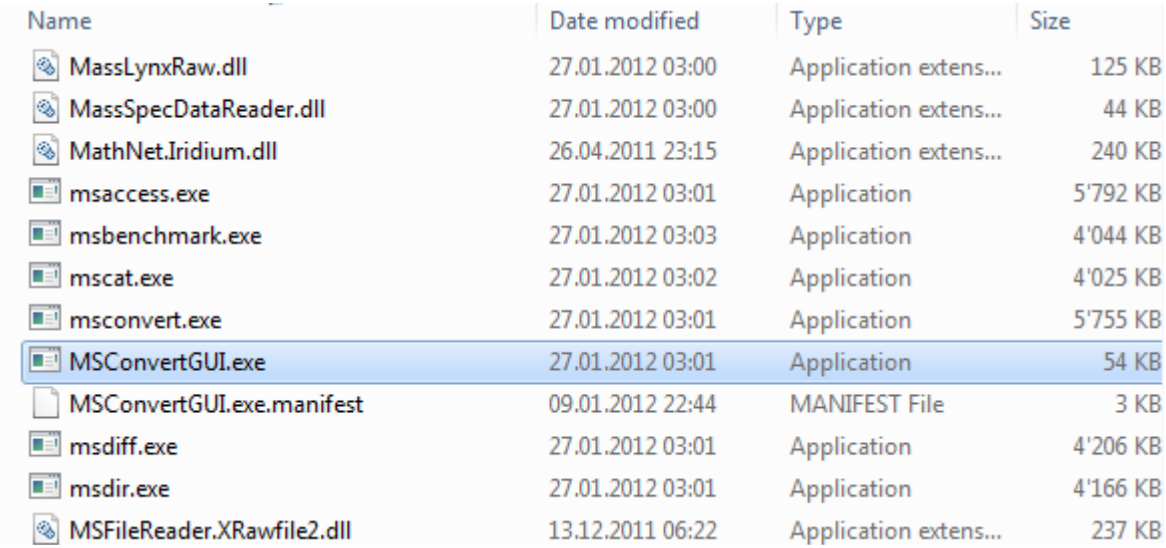
Why Proteowizard?

- Convert raw data files to an open format (we chose mzML)
- Is one of most established and supported converters

NOTE: If you will only ever use RMassBank once (i.e. today)
Don't bother installing! The test data is already converted!

Check to see if you have it

- E.g. for me: C:\DATA\Program_Info\pwiz
- You want "MSConvertGUI.exe"

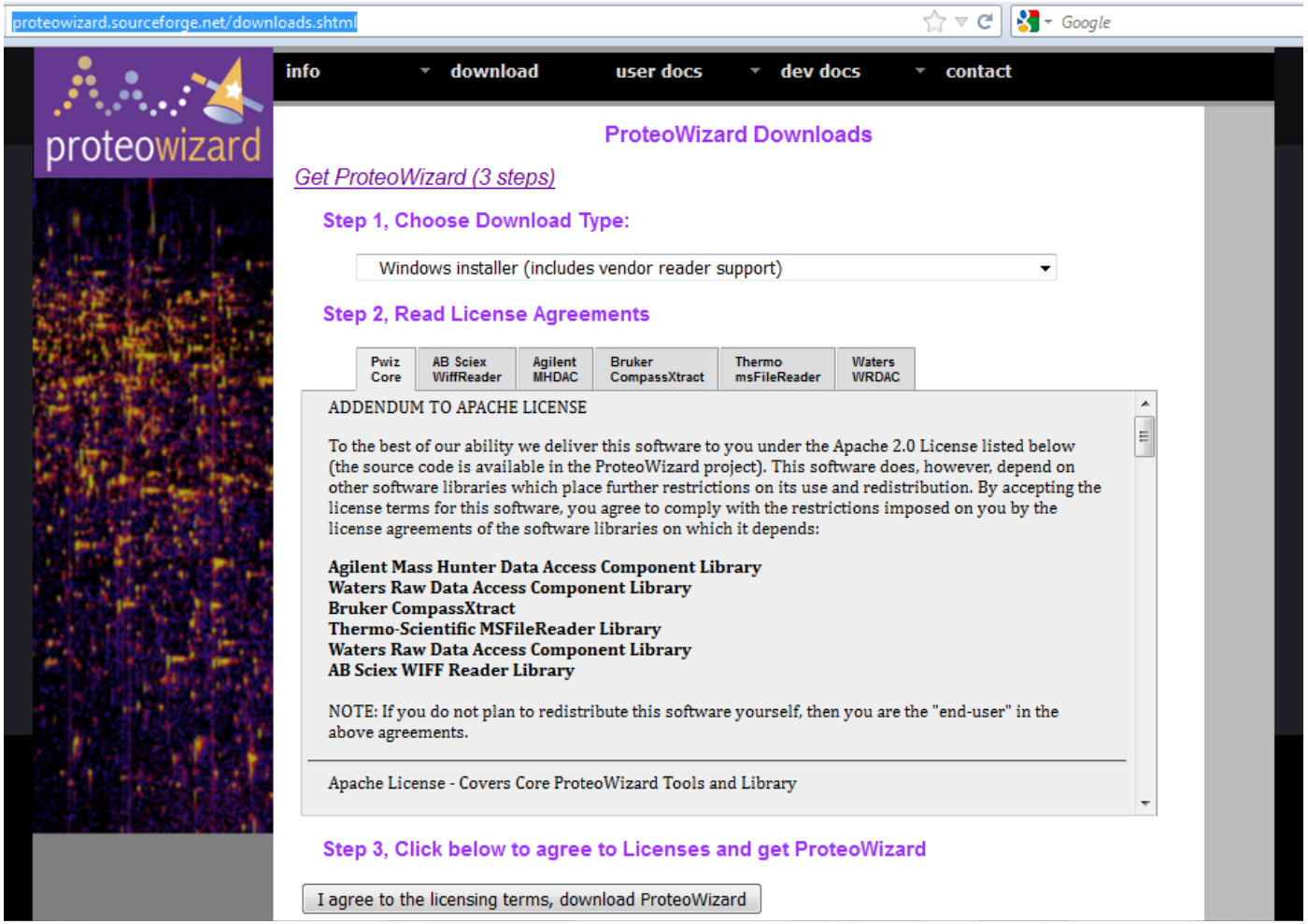


Name	Date modified	Type	Size
MassLynxRaw.dll	27.01.2012 03:00	Application extens...	125 KB
MassSpecDataReader.dll	27.01.2012 03:00	Application extens...	44 KB
MathNet.Iridium.dll	26.04.2011 23:15	Application extens...	240 KB
msaccess.exe	27.01.2012 03:01	Application	5'792 KB
msbenchmark.exe	27.01.2012 03:03	Application	4'044 KB
mscat.exe	27.01.2012 03:02	Application	4'025 KB
msconvert.exe	27.01.2012 03:01	Application	5'755 KB
MSConvertGUI.exe	27.01.2012 03:01	Application	54 KB
MSConvertGUI.exe.manifest	09.01.2012 22:44	MANIFEST File	3 KB
msdiff.exe	27.01.2012 03:01	Application	4'206 KB
msdir.exe	27.01.2012 03:01	Application	4'166 KB
MSFileReader.XRawfile2.dll	13.12.2011 06:22	Application extens...	237 KB

Before getting started:

Install / Check Proteowizard

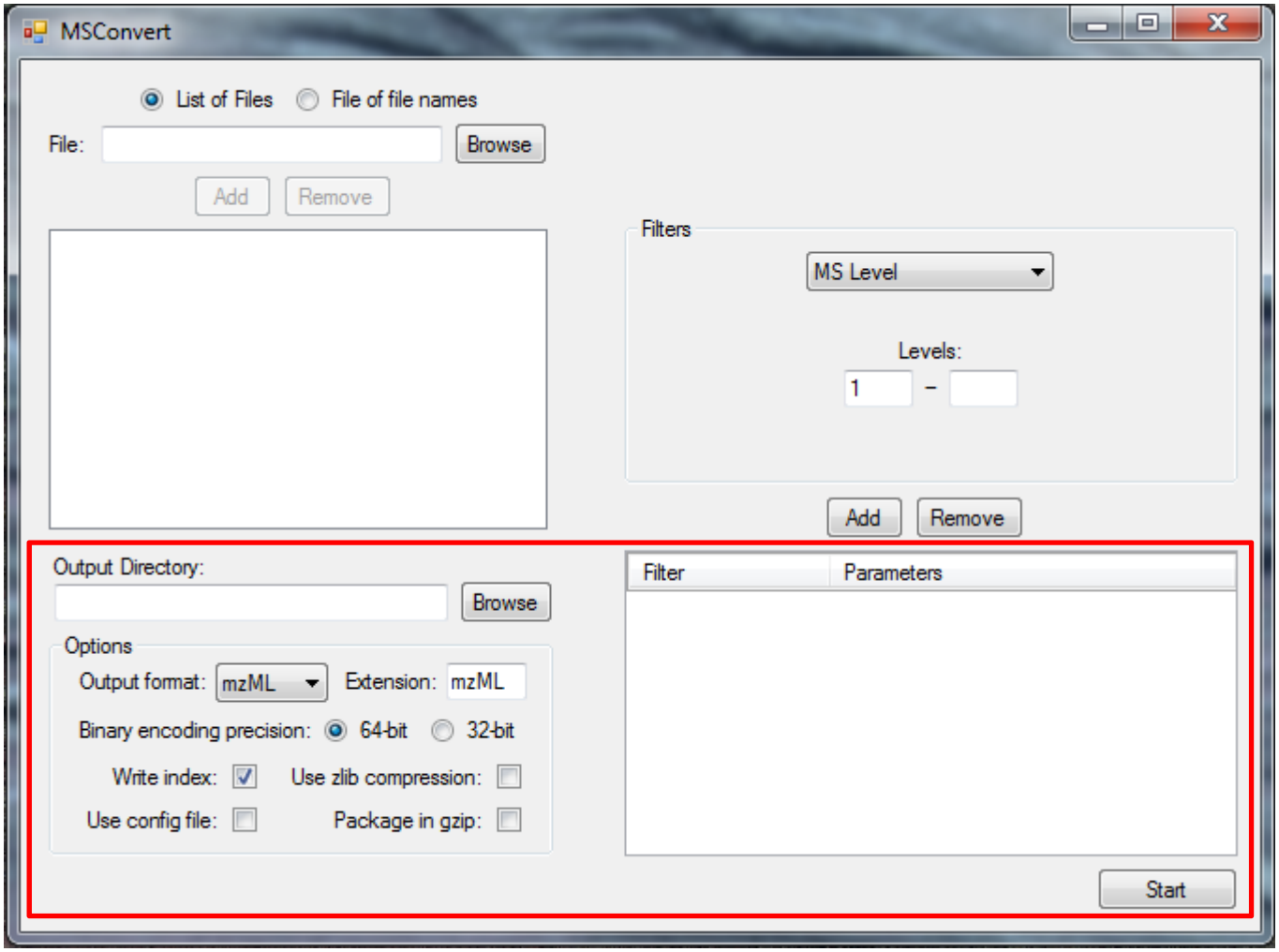
No Proteowizard (pwiz)?: http://proteowizard.sourceforge.net/downloads.shtml



Before getting started:

Install / Check Proteowizard

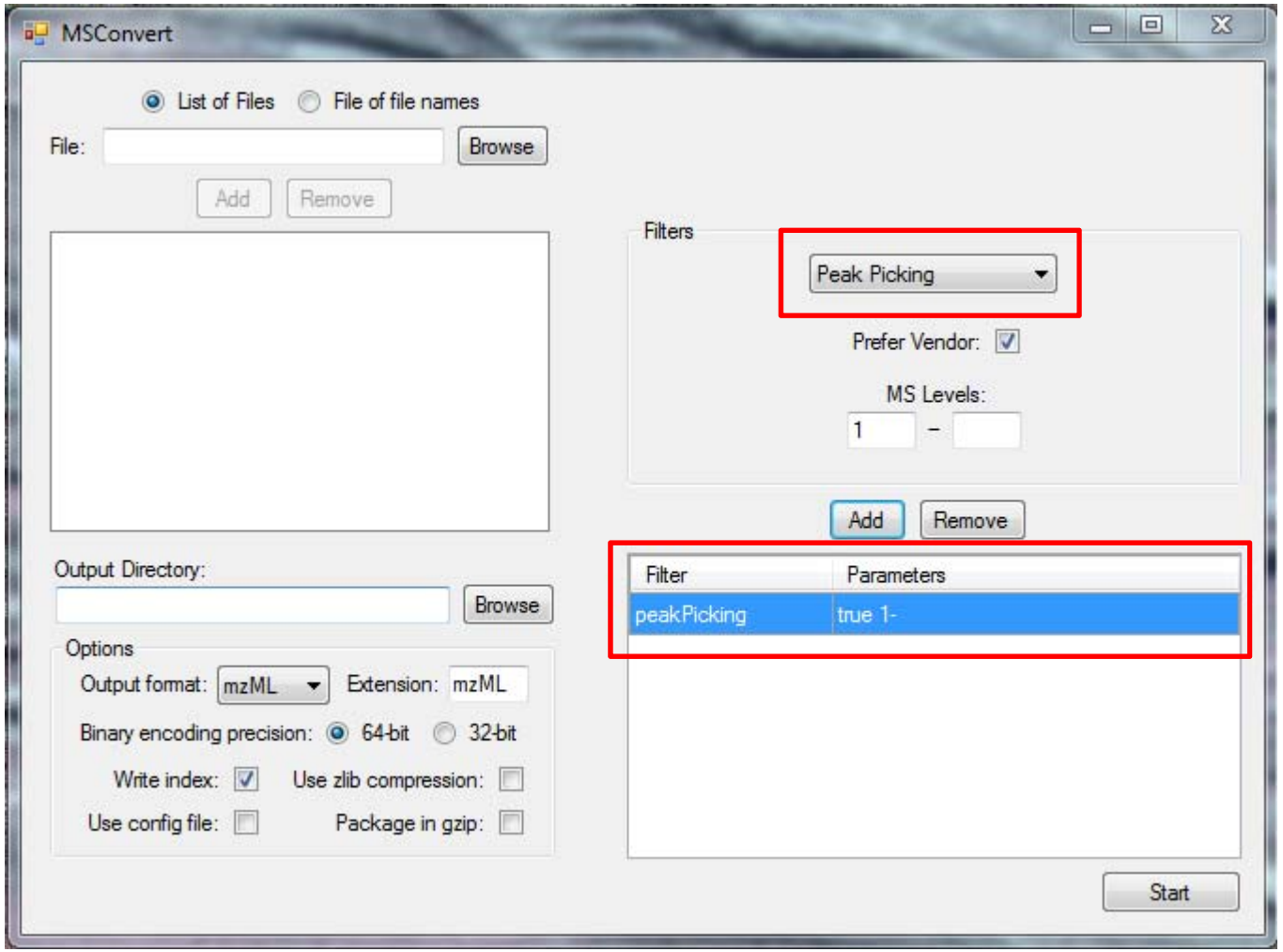
To use profile data (RMassBank deprofiler):



Before getting started:

Install / Check Proteowizard

To use centroid data (Pwiz deprofiler – example data prepared this way):



Before getting started

File names…

The files are used to identify a compound in the workflow

- Currently, we require a format like:

  xxxxxxxxxxxx**_1234_**xxx.**mzML**
- _1234_ is the compound ID which is also in the compound list
- mzML is the format currently read by RMassBank

- Advanced / alternative formats possible
  - Will require adjustment to the RMassBank code
  - Check out functions msms_workflow and findMsMsHR

Before getting started:

The compound list

Go back to the R / RStudio window

- Choose a working directory (change this!!! and create a new one if you like)
- Copy the compound list into this directory:

```
39 # Save a copy of the compound list
40 # First, set a working directory (Imake sure this exists)
41 setwd("C:/DATA/RMassBank/RMassBank_Demo")
42 # then copy the file there
43 file.copy(system.file("list/NarcoticsDataset.csv",
44                       package="RMassBankData"), "./Compoundlist.csv")
45
```

```
> setwd("C:/DATA/RMassBank/RMassBank_Demo")
> # then copy the file there
> file.copy(system.file("list/NarcoticsDataset.csv",
+                       package="RMassBankData"), "./Compoundlist.csv")
[1] TRUE
>
```

Before getting started:

The compound list

Open up the compound list (Excel, OpenOffice):

- Ignore any error messages Excel gives you (it is CSV, not SYLK!!!)
- Red fields are required: ID for file name, SMILES,
- Optional: Name (SMILES back-up) and RT for MS and MS/MS retrieval window

	A	B	C	D	E	F	G
1	ID	Name_de	Name_En	Name	SMILES	RT	CAS
2	2817	Kokain	Cocaine	Cocaine	[C@H]1([C@@H]2N([C@@H](C[C@@H]1OC(	5.15	50-36-2
3	2818	1-(3-Chlor	1-(3-Chlor	1-(3-Chlorophenyl)pipera	c1c(Cl)cccc1N1CCNCC1	5.25	6640-24-0
4	2819	1-(3-Triflu	1-(3-Triflu	1-(3-Trifluoromethylphen	c1c(C(F)(F)F)cccc1N1CCNCC1	6.05	15532-75-9
5	2820	1-Benzylp	1-Benzylp	1-Benzylpiperazine	C1CN(CCN1)Cc1ccccc1	1.64	2759-28-6
6	2821	Amitriptyl	Amitriptyl	Amitriptyline	C1(\c2c(CCc3c1cccc3)cccc2)=C\CCN(C)C	8.65	50-48-6
7	2822	Amphetar	Amphetar	Amphetamine	c1(ccccc1)CC(N)C	3.7	300-62-9
8	2823	Benzoylec	Benzoylec	Benzoylecgonine	O(C(=O)c1ccccc1)[C@@H]1[C@@H]([C@@H]	4.7	519-09-5
9	2824	Dextrome	Dextrome	Dextromethorphan	c12[C@]34[C@@H]([C@@H]([N@@](C)CC3)	6.7	125-71-3
10	2825	EDDP (2-E	EDDP (2-E	EDDP (2-Ethylidene-1,5-di	C1[C@@H](C)N(C)C(\C1(c1ccccc1)c1ccccc1)=(	6.65	30223-73-5
11	2826	Ketamin	Ketamine	Ketamine	c1([C@@]2(C(CCCC2)=O)NC)c(cccc1)Cl	4.7	6740-88-1
12	2827	Mephedrc	Mephedrc	Mephedrone (4-Methylm	Cc1ccc(cc1)C(=O)C(C)NC	4.45	1189805-46-6
13	2828	Methador	Methador	Methadone	CCC(=O)C(CC(C)N(C)C)(c1ccccc1)c2ccccc2	8.35	76-99-3
14	2829	Methamp	Methamp	Methamphetamine	c1cccc(c1)C[C@H](C)NC	3.85	537-46-2
15	2830	Naltrexon	Naltrexon	Naltrexone	c12[C@]34[C@@]5([C@H]([N@](CC6CC6)CC4	3.25	16590-41-3
16	2758	Ephedrin	Ephedrine	Ephedrine	c1([C@H]([C@@H](NC)C)O)ccccc1	3.03	299-42-3
17							

Before getting started:

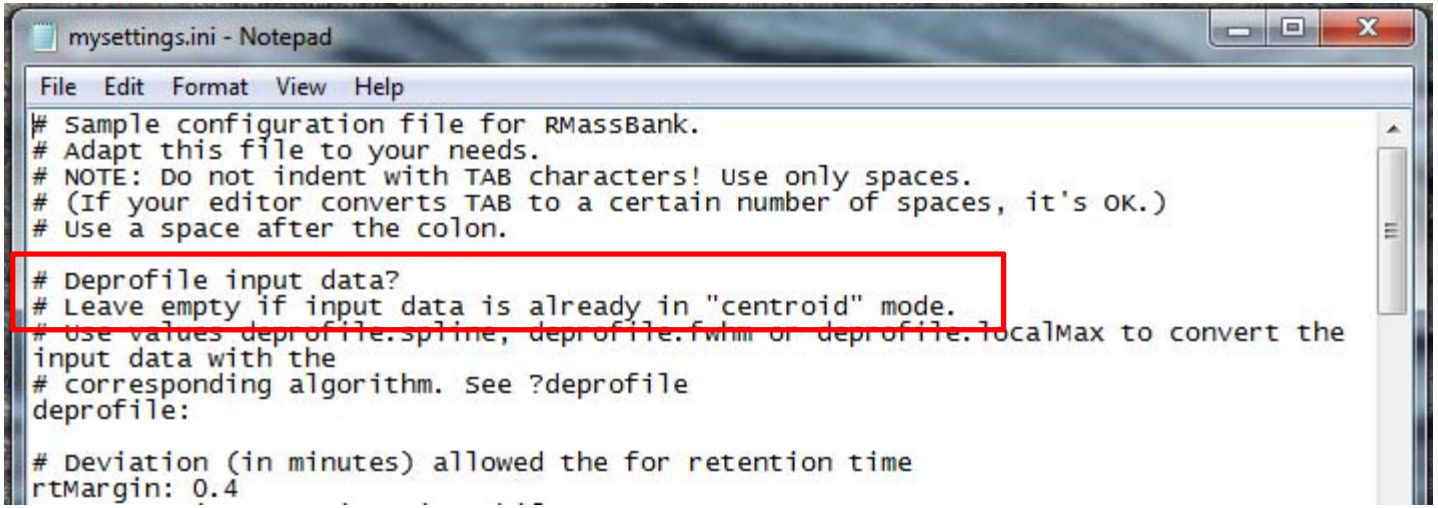
The settings file (lucky last bit!!)

A number of settings are necessary for successful generation:

- Data processing
- Correct annotation of spectra

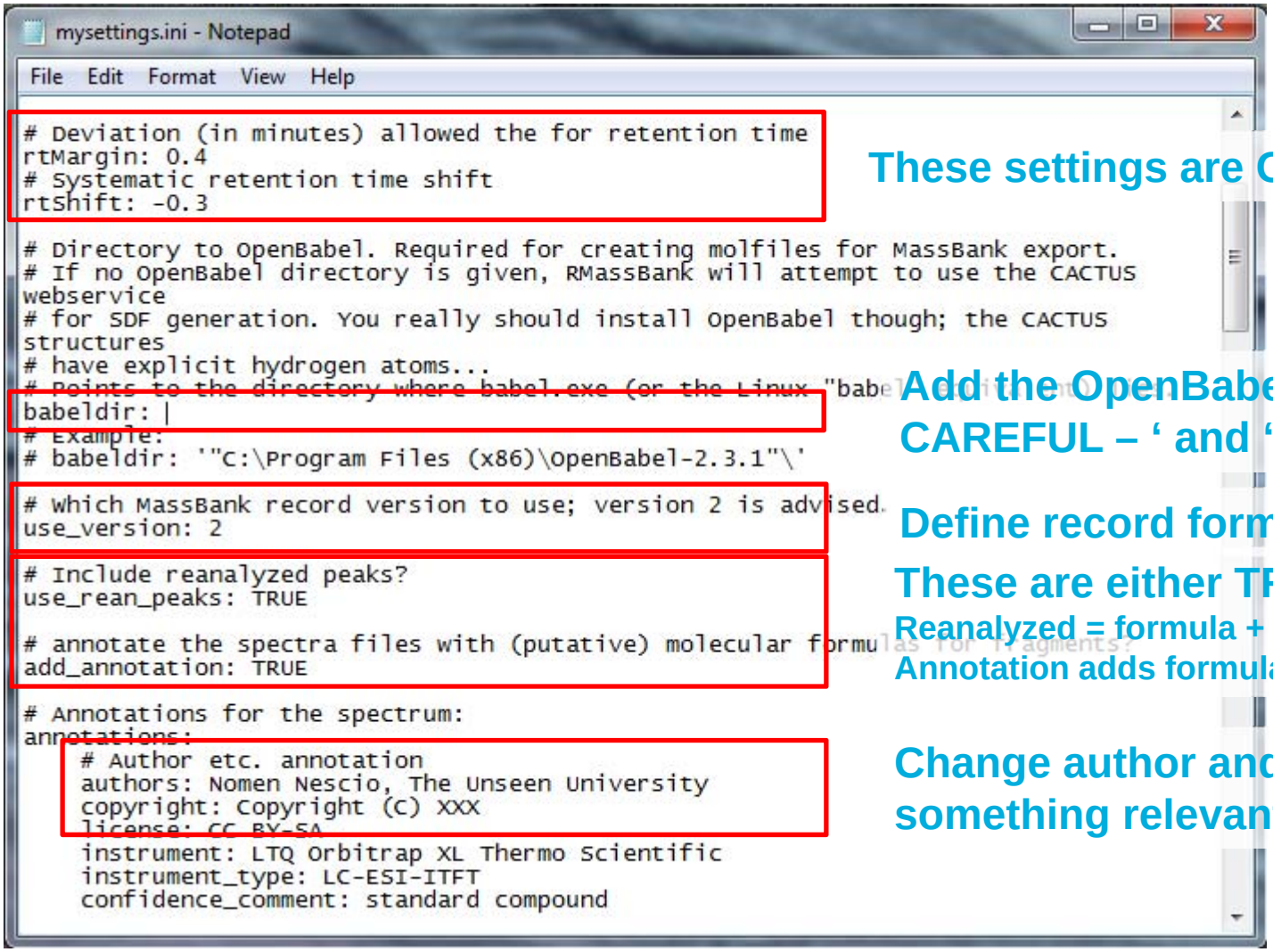
```
# Now, generate a settings file:
RmbSettingsTemplate("mysettings.ini")
```

- Open up in any text editor and edit…



Before getting started:

The settings file (lucky last bit!!)



These settings are OK for test data

Add the OpenBabel location here
CAREFUL – ‘ and “ as in example

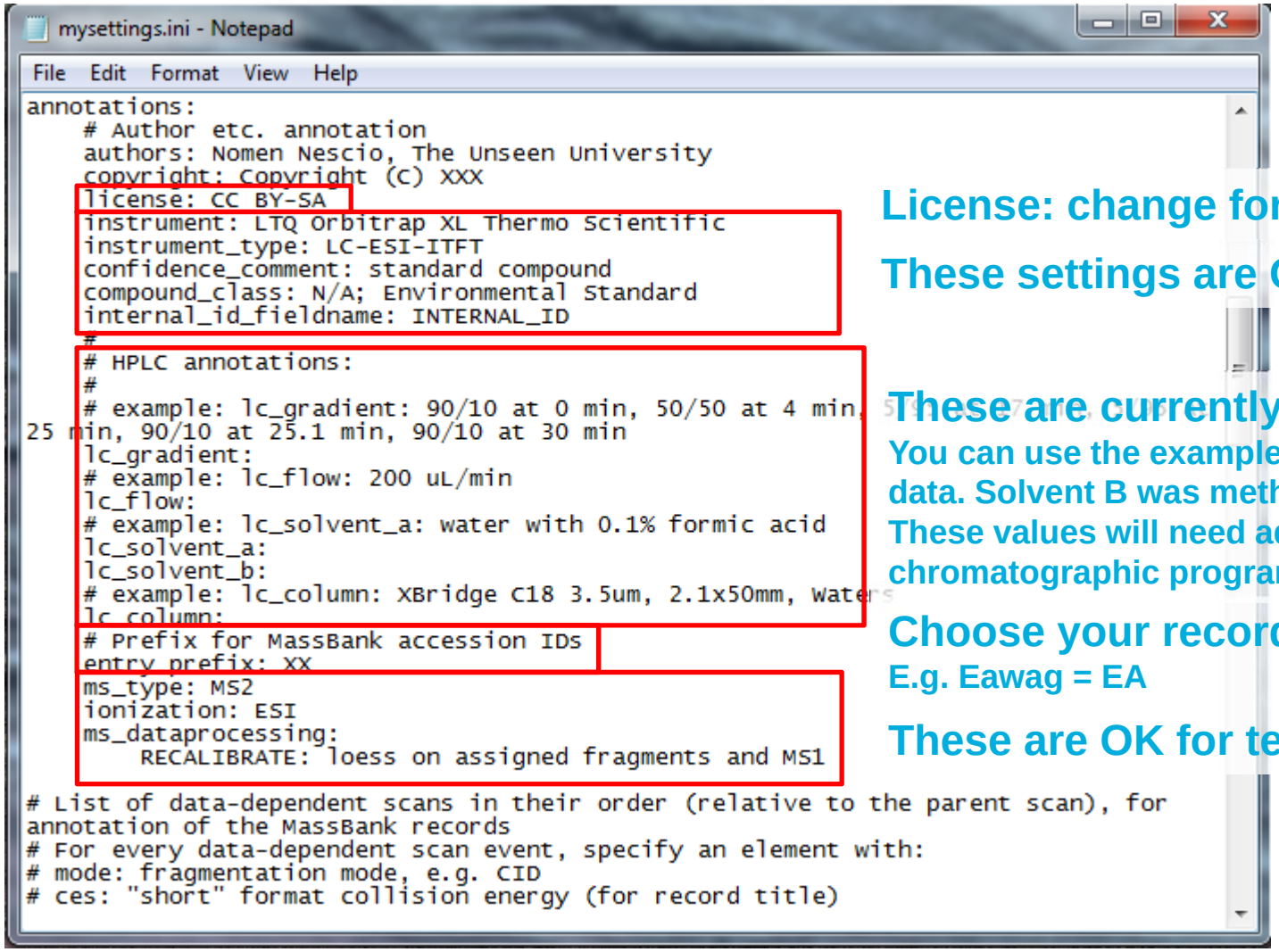
Define record format (keep 2)

These are either TRUE or FALSE
Reanalyzed = formula + N_2O
Annotation adds formula to records

Change author and copyright to something relevant for you…

Before getting started:

The settings file (lucky last bit!!)



License: change for own records?

These settings are OK for test data

These are currently empty

You can use the examples for the example data. Solvent B was methanol!

These values will need adjusting for own chromatographic program

Choose your record letters!

E.g. Eawag = EA

These are OK for test data

Before getting started:

The settings file (lucky last bit!!)

mysettings.ini - Notepad

File Edit Format View Help

```
# List of data-dependent scans in their order (relative to the parent scan), for
annotation of the MassBank records
# For every data-dependent scan event, specify an element with:
# mode: fragmentation mode, e.g. CID
# ces: "short" format collision energy (for record title)
# ce: "long" format collision energy (for annotation field)
# res: FT resolution
spectraList:
 # First scan: CID 35% NCE, resolution 7500
- mode: CID
  ces: 35%
  ce: 35 % (nominal)
  res: 7500
 # Second scan: HCD 15% NCE, resolution 7500
- mode: HCD
  ces: 15%
  ce: 15 % (nominal)
  res: 7500
 # Third scan, etc.
- mode: HCD
  ces: 30%
  ce: 30 % (nominal)
  res: 7500
- mode: HCD
  ces: 45%
  ce: 45 % (nominal)
  res: 7500
- mode: HCD
  ces: 60%
  ce: 60 % (nominal)
  res: 7500
- mode: HCD
  ces: 75%
  ce: 75 % (nominal)
```

These settings are OK for test data

However:
This will need adjustment for own experiments!

Before getting started:

The settings file (lucky last bit!!)

Finally: Save this before you go on!!!

mysettings.ini - Notepad

File Edit Format View Help

```
# Shifts of the starting points for RMassBank accession numbers.
# Change these if you measure different adducts
accessionNumberShifts:
    pH: 0 # [M+H]+: Accession numbers 1-14
    pM: 16 # [M]+: 17-30
    pNa: 32 # [M+Na]+: 33-46
    mH: 50 # [M-H]-: 51-64
    mFA: 66 # [M+FA]-: 67-80

# A list of known electronic noise peaks
electronicNoise:
- 189.825
- 201.725
- 196.875
# Exclusion width of electronic noise peaks (from unmatched peaks, prior to
# reanalysis)
electronicNoiseWidth: 0.3

# recalibration settings:
# recalibrate by: dppm or dmz
recalibrateBy: dppm

# recalibrate MS1:
# separately (separate)
# with common curve (common)
# do not recalibrate (none)
recalibrateMS1: common

# Custom recalibration function: You can overwrite the recal function by
# making any function which takes rcdata$recalfield ~ rcdata$mzFound.
# The settings define which recal function is used.
# Note: if recalibrateMS1 is "common", the setting "recalibrator: MS1" is
meaningless
# because the MS1 points will be recalibrated together with the MS2 points in
# the MS2 recalibration function.
recalibrator:
    MS1: recalibrate.loess
    MS2: recalibrate.loess
```

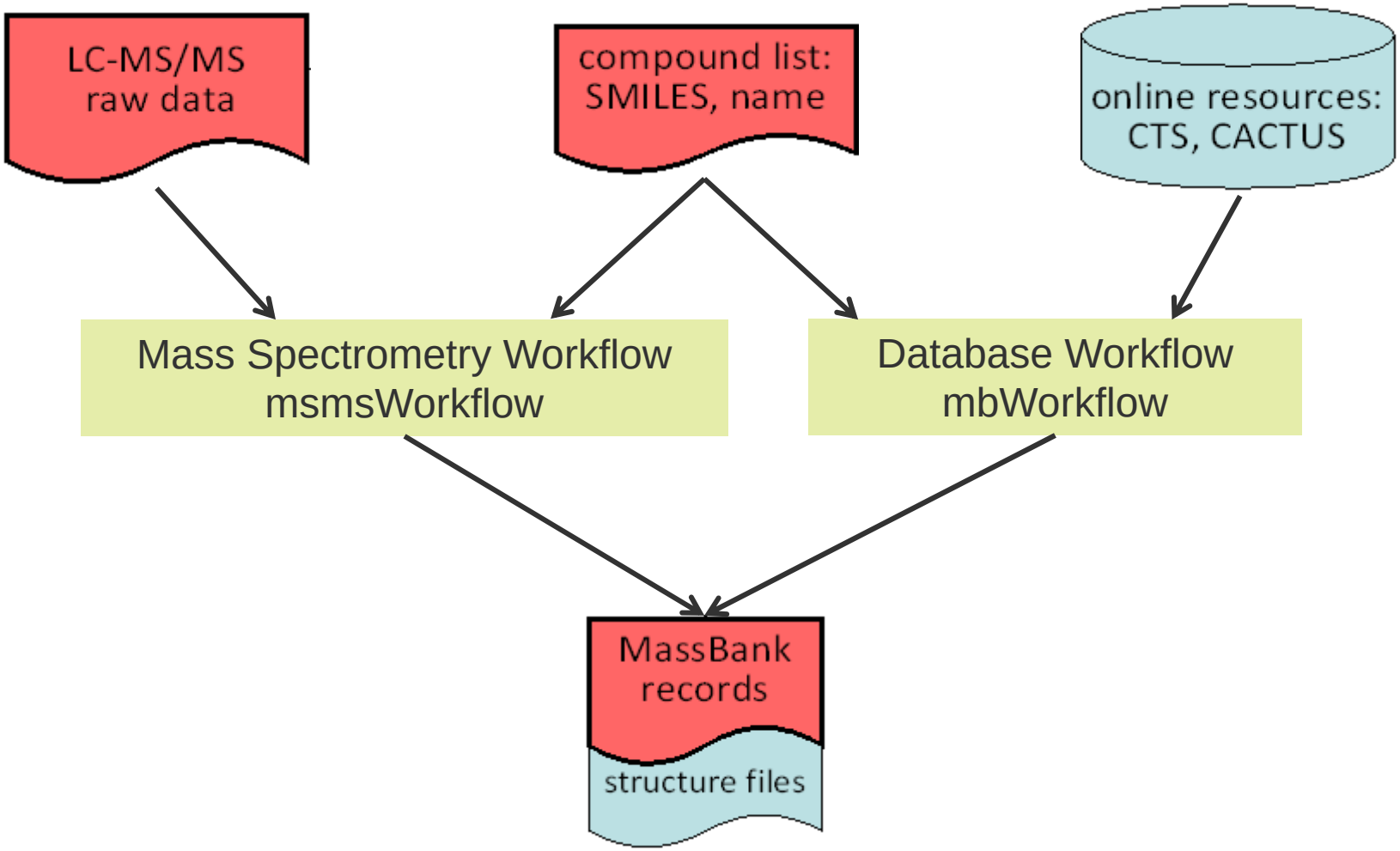
Fixed accession number: XXYYYYZZ;
XX = letters, YYYY = compound ID
ZZ = "shifts" – for us, these adducts

Remove consistent electronic noise
Add the middle mass of the noise (often broad peaks, or slightly varying)
Then add exclusion width –not too generous!

Recalibrate settings:
Mass accuracy unit
MS & MS/MS

These are OK for test data
Changes necessary if recalibration needs adjusting

RMassBank Workflow – Simple Form

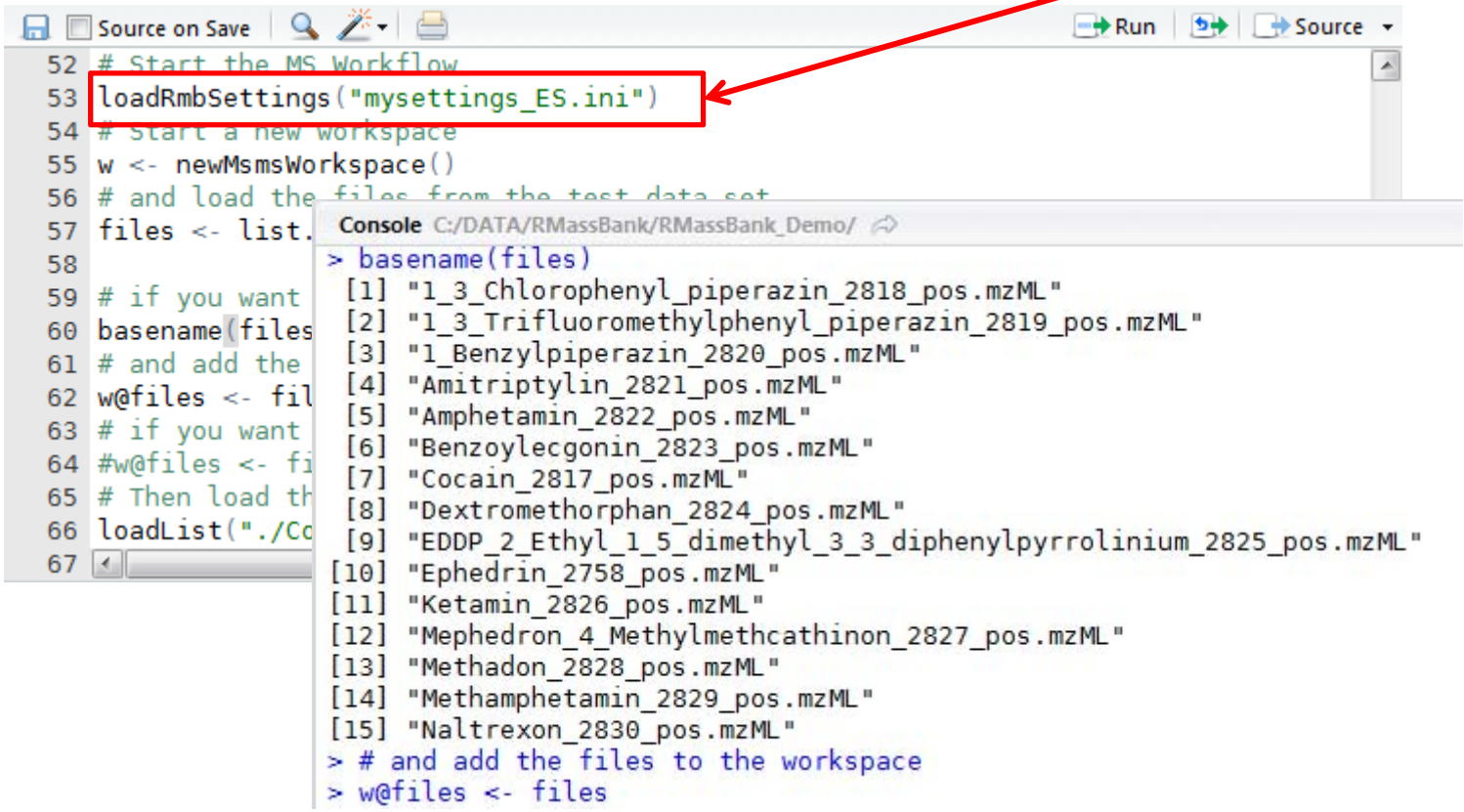


Mass Spectrometry Workflow

Now we can get started!

Go back to your R session and “RMassBank_Demo.R”

- Select and run this whole snippet – but change the file name first!



Mass Spectrometry Workflow

msmsWorkflow: 8 Steps in summary

- Step 1: Search all `files` for MS/MS spectra of compound
- Step 2: Molecular formula fitting for all peaks (large tolerance)
- Step 3: Analyzed spectra aggregated into a list
- Step 4: Recalibration curve is calculated; all spectra are recalibrated
- Step 5: Recalibrated spectra are reanalyzed (new formula fit post calibration)
- Step 6: Reanalyzed spectra aggregated. Electronic noise removed.
  - Back-up saved as `archiveName.RData`
- Step 7: Fail peaks are reanalyzed (formula + 2N, O)
  - Back-up saved as `archiveName_RA.RData`
- Step 8: Multiplicity Filtering: peaks occurring once only are removed.
  - Back-up saved as `archiveName_RF.RData`
  - High Intensity Fail Peaks: `archiveName_Failpeaks.csv`

Mass Spectrometry Workflow

8 steps in RMassBank code…

- Steps 1:4 – up to recalibration

```
# Start the workflow with steps 1 to 4:
w <- msmsWorkflow(w, mode="pH", steps=c(1:4), archivename = "pH_narcotics")
```

  - Warning: this can take a while….
  - Progress:

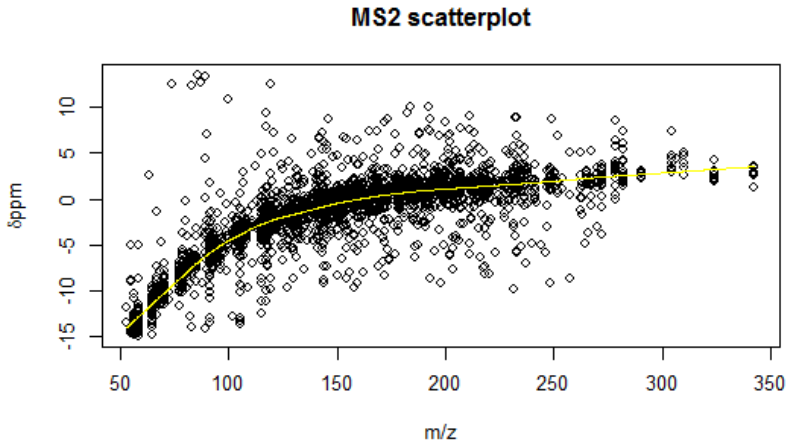
```
> # Start the workflow with steps 1 to 4:
> w <- msmsWorkflow(w, mode="pH", steps=c(1:4), archivename = "pH_narcotics")
msmsWorkflow: Step 1
  |
|   0%3: >C:/Users/schymaem/Documents/R/win-
library/2.14/RMassBankData/spectra/1_3_Chlorophenyl_piperazin_2818_pos.mzML
  |
|   7%3: >C:/Users/schymaem/Documents/R/win-
library/2.14/RMassBankData/spectra/1_3_Trifluoromethylphenyl_piperazin_2819_pos.mzML
  |
|  13%3: >C:/Users/schymaem/Documents/R/win-
library/2.14/RMassBankData/spectra/1_Benzylpiperazin_2820_pos.mzML
  |
|===============                                                  |  20%3:
>C:/Users/schymaem/Documents/R/win-library/2.14/RMassBankData/spectra/Amitriptylin_282
  |
```

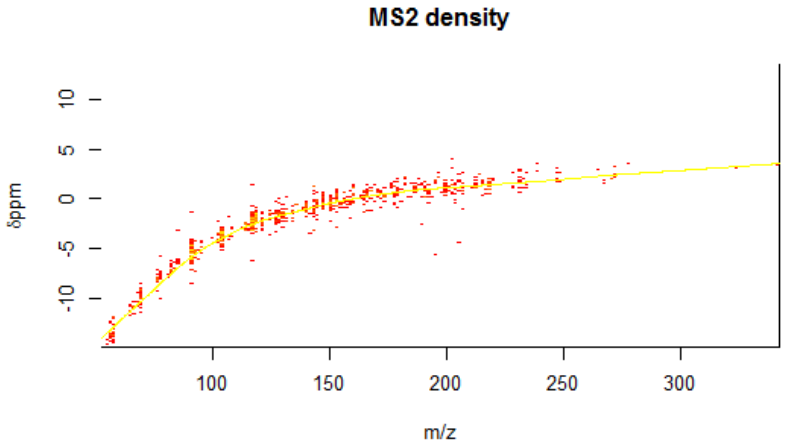
  - P.S. don’t worry if lots of “3”s appear, this is (relatively) meaningless

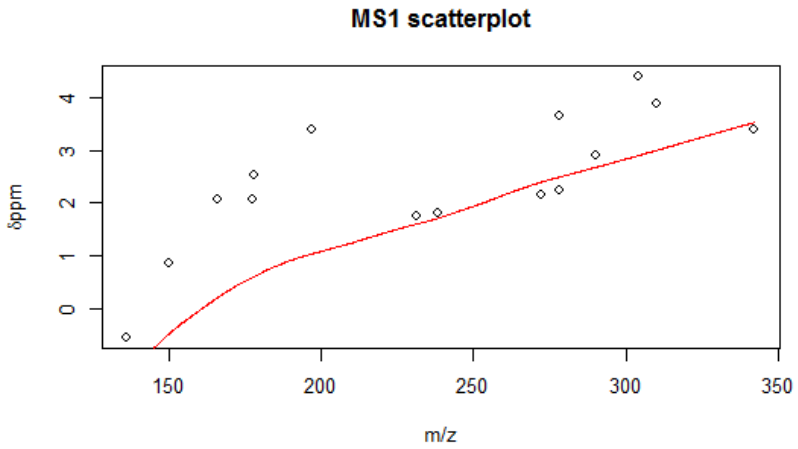
Mass Spectrometry Workflow

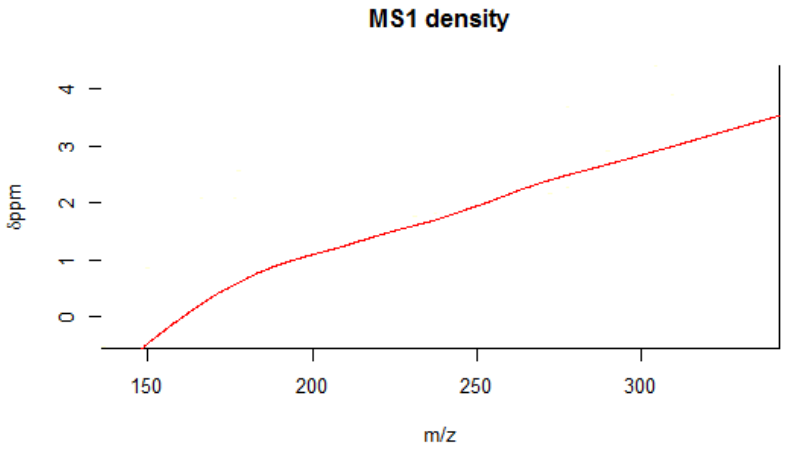
Steps 1:4 of RMassBank code…

- Recalibration plot at the end









Mass Spectrometry Workflow

Steps 5:8 in RMassBank code…

- Steps 5:8 – complete the MS workflow

```
# Keep going with steps 5-8:
w <- msmsWorkflow(w, mode="pH", steps=c(5:8), archivename = "pH_narcotics")
```

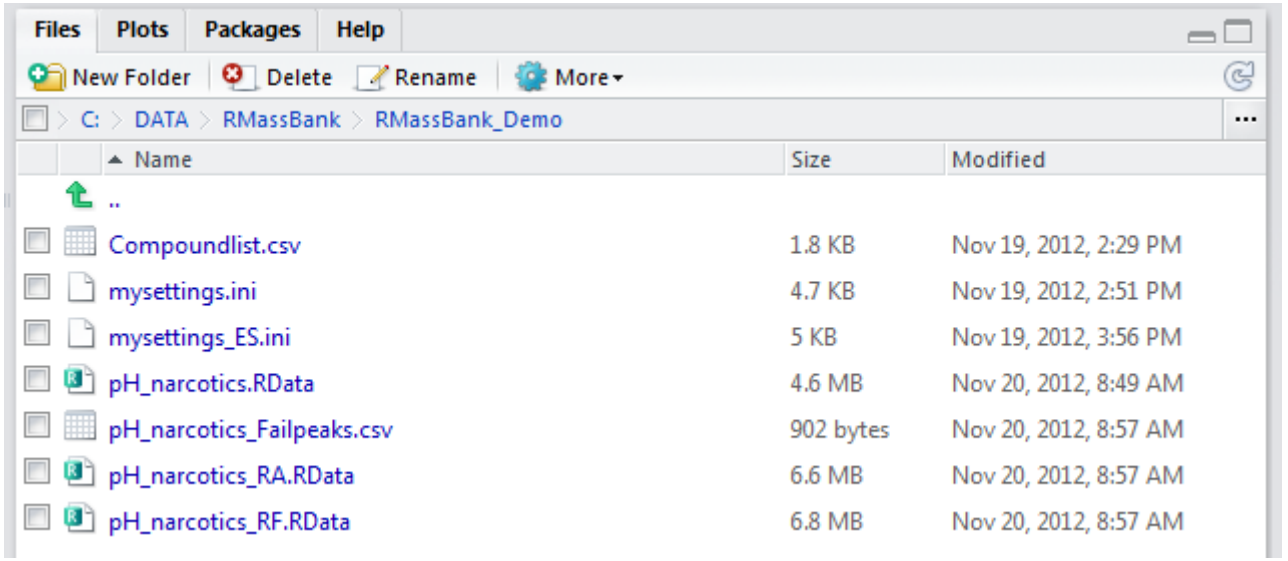
  - Warning: this can also take a while….
  - Progress:

```
> # Keep going with steps 5-8:
> w <- msmsWorkflow(w, mode="pH", steps=c(5:8), archivename = "pH_narcotics")
msmsWorkflow: Step 5
  |
|   0%  |
|   7%  |
|==========                                                       |  13%  |
|===============                                                  |  20%  |
|====================                                             |  27%  |
|=========================                                        |  33%  |
|==============================                                   |  40%  |
|===================================                              |  47%  |
|========================================                         |  53%  |
|=============================================                    |  60%
```

  - Now we need to look at the “Fail Peaks”

Mass Spectrometry Workflow

File listing should now look something like:



- Open up the fail peaks (outside R) and have a look

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	OK	name	cpdID	scan	mzFound	formula	mzCalc	dppm	dbe	mz	int	formulaC	parentSca	aMax	mzCenter
2		407	2817	558	182.1191	NA	NA	NA	NA	182.1193	117786.4	0	556	9902212	304.1543
3		2231	2821	963	233.1351	NA	NA	NA	NA	233.1355	255822.6	0	961	16383558	278.1903
4		3491	2824	723	215.1468	NA	NA	NA	NA	215.1471	242559.4	0	721	15875343	272.2009
5		3988	2825	725	249.1548	NA	NA	NA	NA	249.1553	516037.1	0	723	23457888	278.1903
6		4110	2825	730	193.0993	NA	NA	NA	NA	193.0995	138038.3	0	723	5673618	278.1903
7		5543	2828	938	265.1612	NA	NA	NA	NA	265.1618	146493.5	0	933	4158767	310.2165
8		6503	2830	352	211.0738	C12H9N3O	211.074	-0.9633	13	211.0741	24055.95	1	346	195149.4	342.17

Mass Spectrometry Workflow

Fail Peaks

Compound ID

MS/MS Scan

m/z & I of MS/MS peak

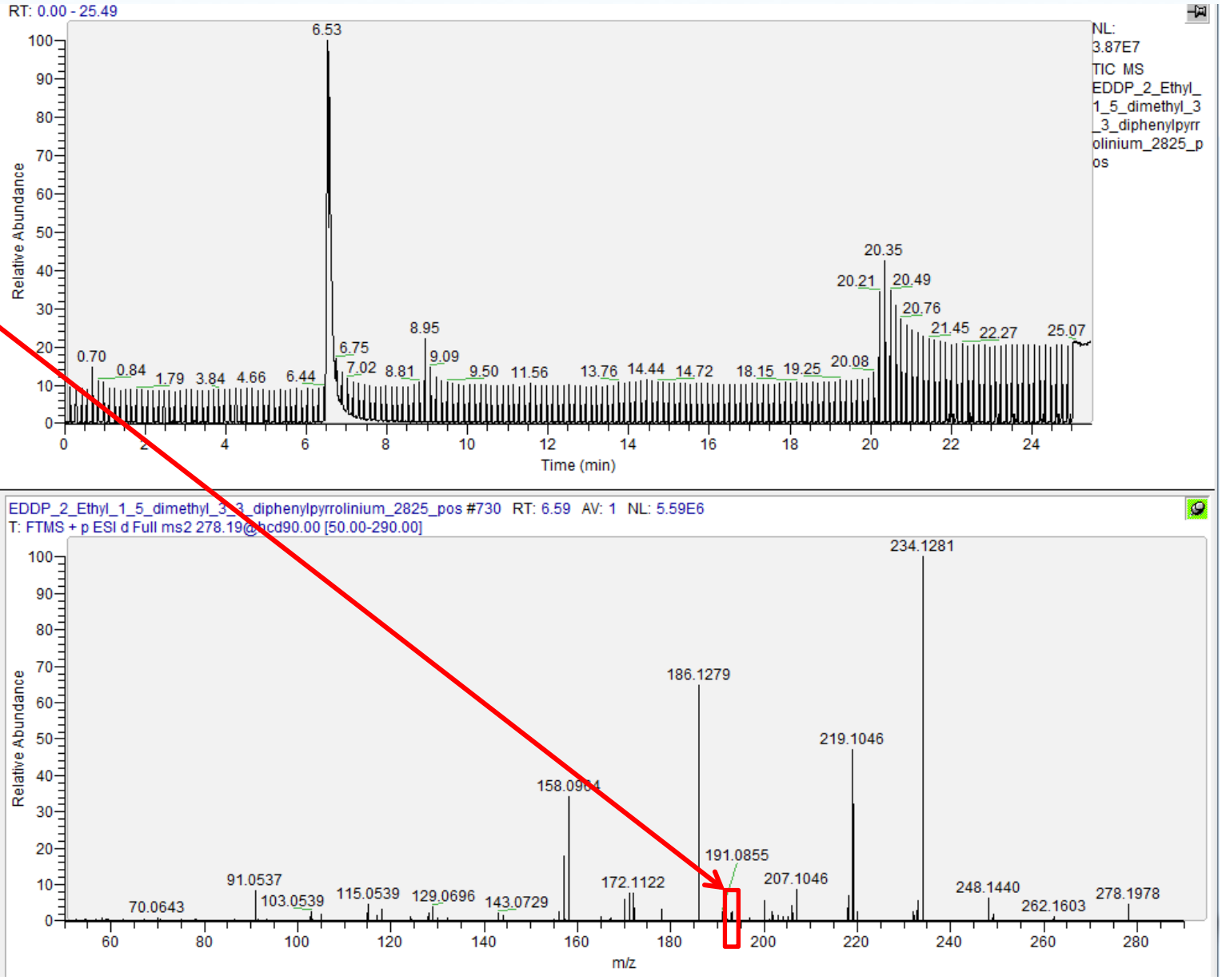
I, m/z of precursor

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	OK	name	cpdID	scan	mzFound	formula	mzCalc	dppm	dbe	mz	int	formulaCo	parentSca	aMax	mzCenter
2		407	2817	558	182.1191	NA	NA	NA	NA	182.1193	117786.4	0	556	9902212	304.1543
3		2231	2821	963	233.1351	NA	NA	NA	NA	233.1355	255822.6	0	961	16383558	278.1903
4		3491	2824	723	215.1468	NA	NA	NA	NA	215.1471	242559.4	0	721	15875343	272.2009
5		3988	2825	725	249.1548	NA	NA	NA	NA	249.1553	516037.1	0	723	23457888	278.1903
6		4110	2825	730	193.0993	NA	NA	NA	NA	193.0995	138038.3	0	723	5673618	278.1903
7		5543	2828	938	265.1612	NA	NA	NA	NA	265.1618	146493.5	0	933	4158767	310.2165
8		6503	2830	352	211.0738	C12H9N3O	211.074	-0.9633	13	211.0741	24055.95	1	346	195149.4	342.17

No formula calculated; not replicated (only present once) and low I compared with parent => “true” fail peaks
Look at raw data of “2825”

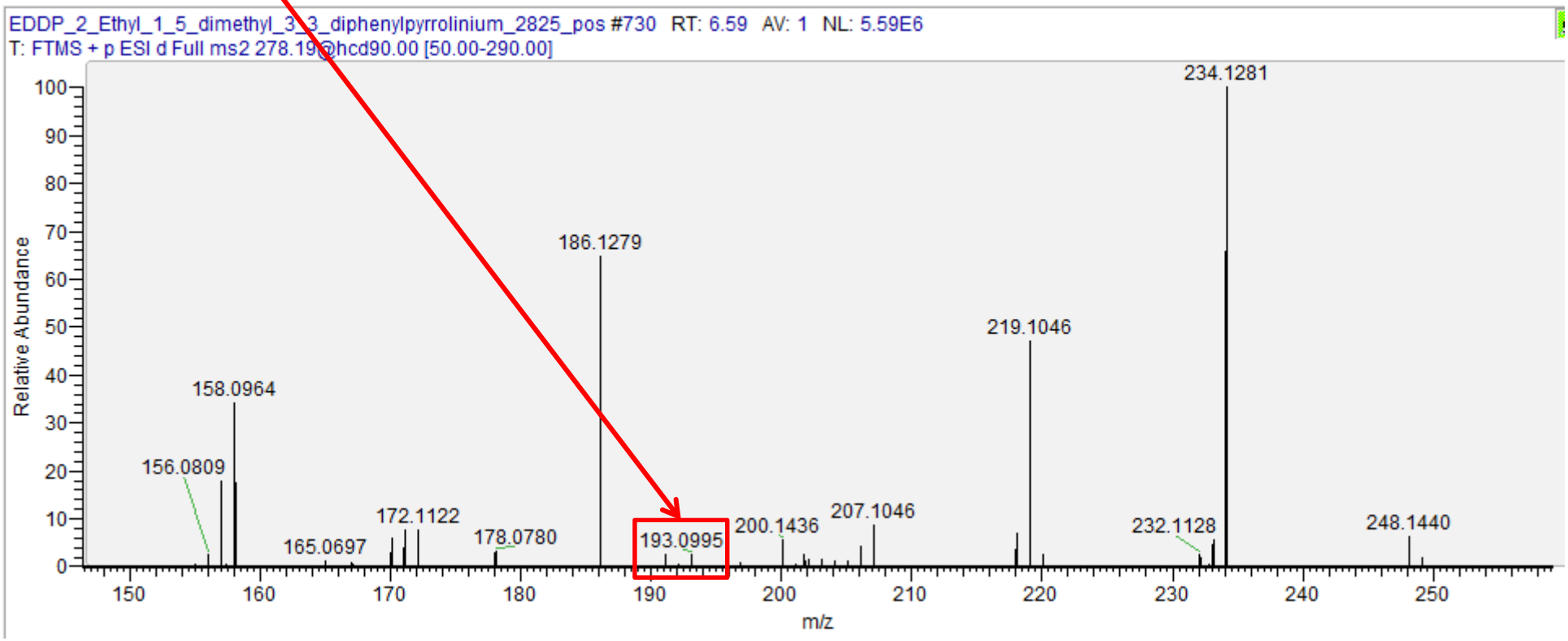
Mass Spectrometry Workflow

Fail Peak



Mass Spectrometry Workflow

Fail Peaks



"Fail peak" is very minor part of spectrum, already within noise levels

Mass Spectrometry Workflow

Fail Peaks

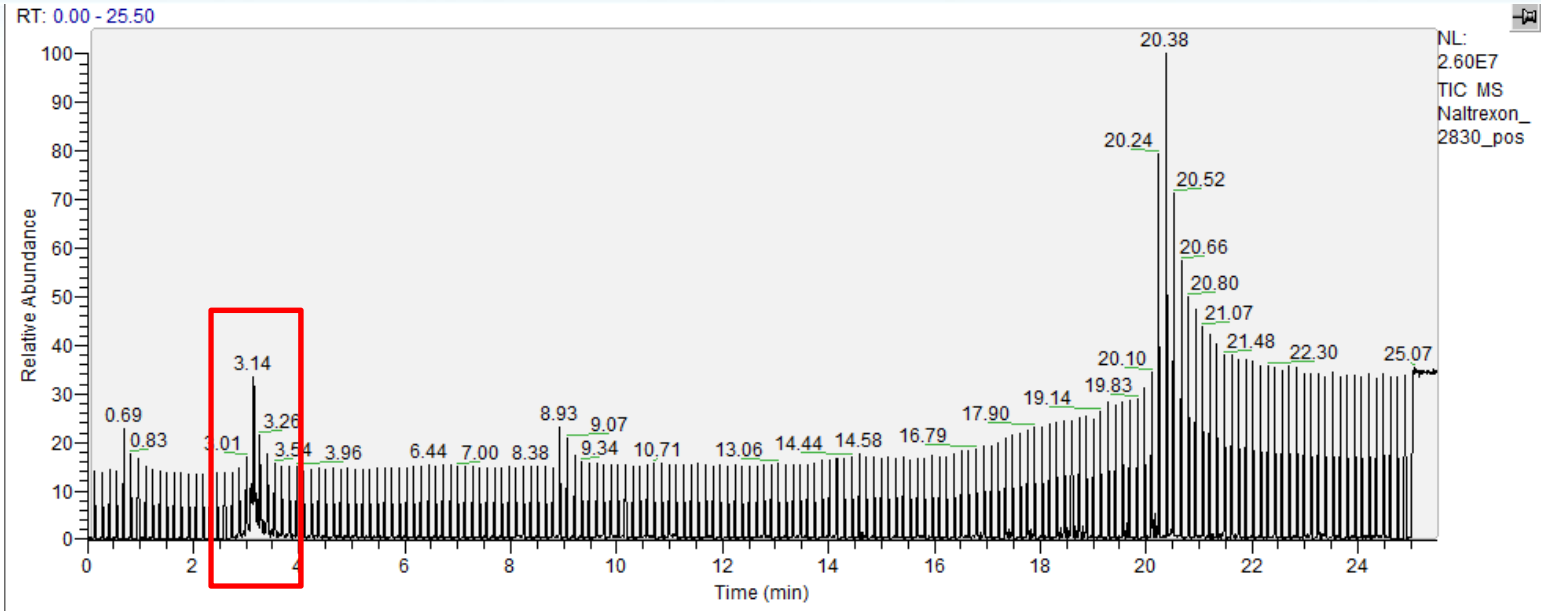
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	OK	name	cpdID	scan	mzFound	formula	mzCalc	dppm	dbe	mz	int	formulaCc	parentSca	aMax	mzCenter
2		407	2817	558	182.1191	NA	NA	NA	NA	182.1193	117786.4	0	556	9902212	304.1543
3		2231	2821	963	233.1351	NA	NA	NA	NA	233.1355	255822.6	0	961	16383558	278.1903
4		3491	2824	723	215.1468	NA	NA	NA	NA	215.1471	242559.4	0	721	15875343	272.2009
5		3988	2825	725	249.1548	NA	NA	NA	NA	249.1553	516037.1	0	723	23457888	278.1903
6		4110	2825	730	193.0993	NA	NA	NA	NA	193.0995	138038.3	0	723	5673618	278.1903
7		5543	2828	938	265.1612	NA	NA	NA	NA	265.1618	146495.5	0	933	4158767	310.2165
8		6503	2830	352	211.0738	C12H9N3O	211.074	-0.9633	13	211.0741	24055.95	1	346	195149.4	342.17

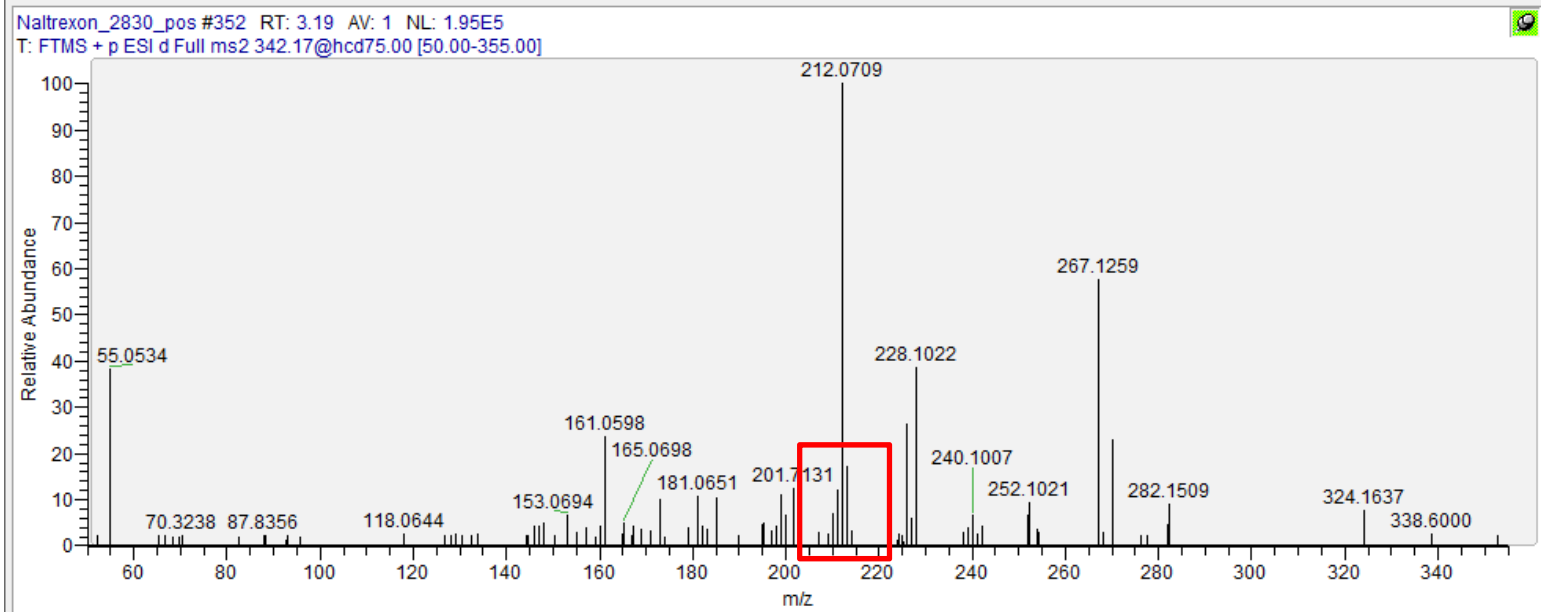
Formula calculated, but also low intensity compared with precursor
Go to raw data…

Mass Spectrometry Workflow

Fail Peak

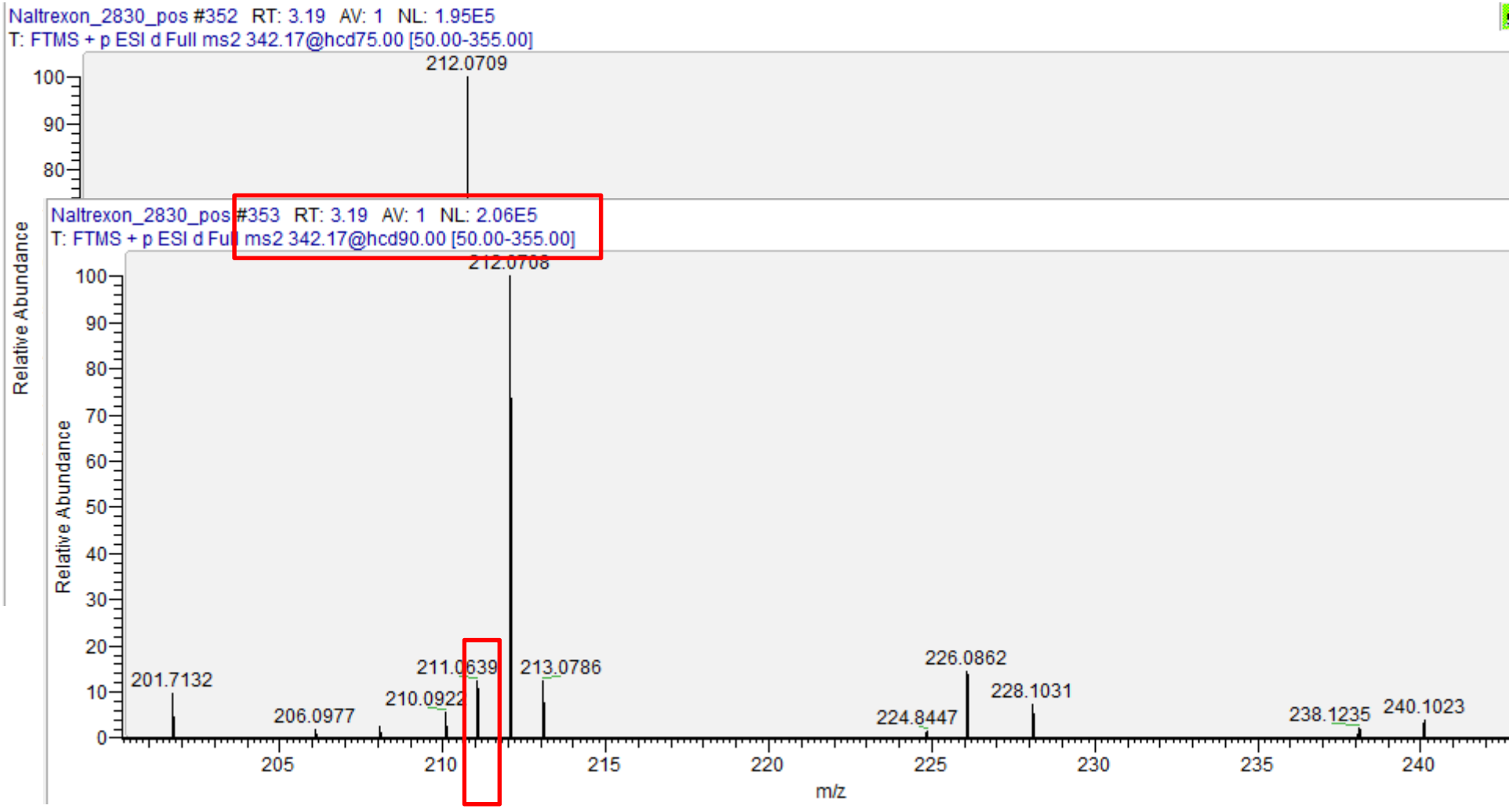
(with formula)





Mass Spectrometry Workflow

Fail peak (with formula)



Mass Spectrometry Workflow

Fail Peaks

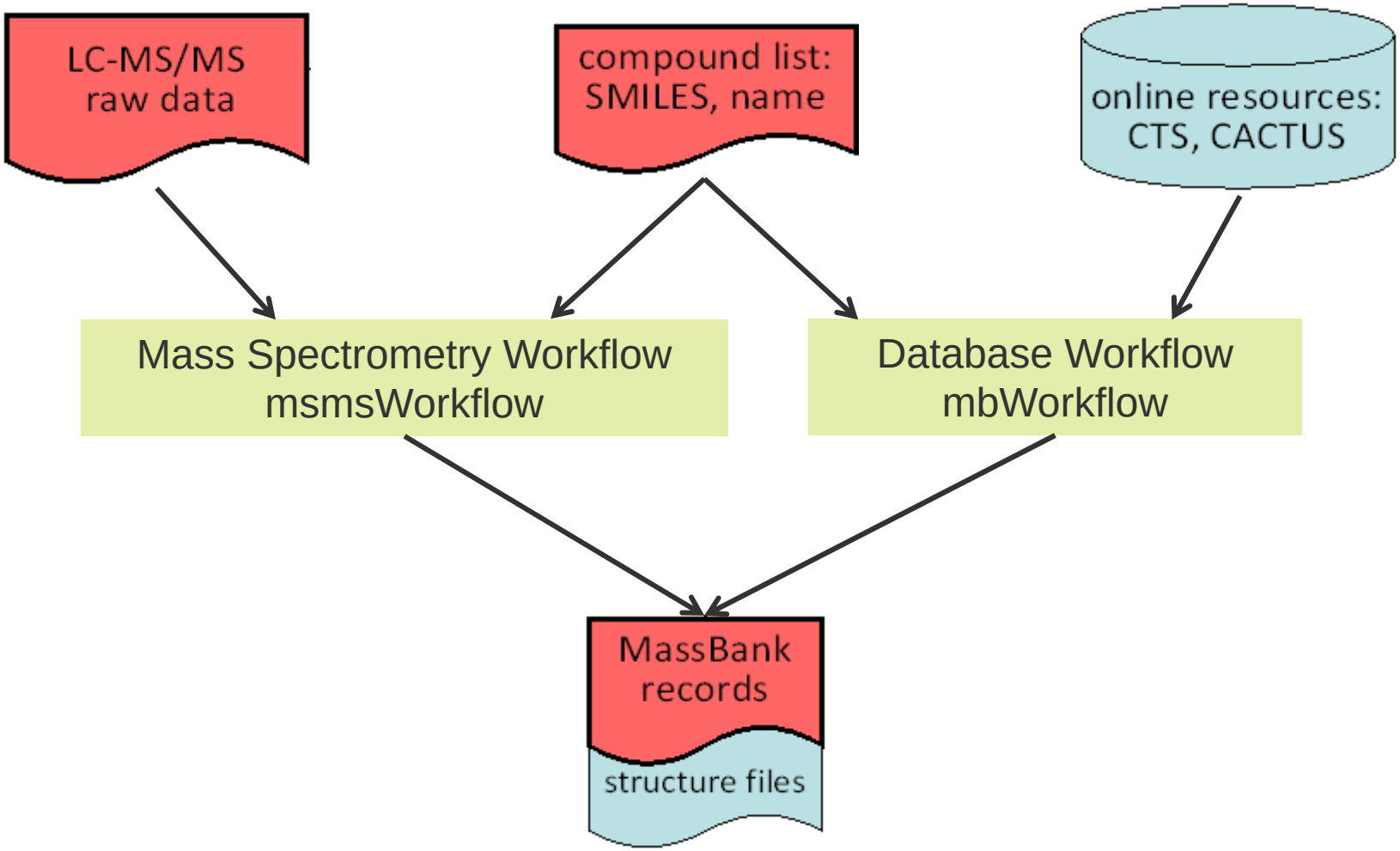
Include this “fail peak” by entering a 1 in the OK column

- Save under a new name…
  - e.g. “pH_narcotics_Failpeaks_wOKs.csv”

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	OK	name	cpdID	scan	mzFound	formula	mzCalc	dppm	dbe	mz	int	formulaCc	parentSca	aMax	mzCe
2	0	407	2817	558	182.1191	NA	NA	NA	NA	182.1193	117786.4	0	556	9902212	304.1
3	0	2231	2821	963	233.1351	NA	NA	NA	NA	233.1355	255822.6	0	961	16383558	278.1
4	0	3491	2824	723	215.1468	NA	NA	NA	NA	215.1471	242559.4	0	721	15875343	272.1
5	0	3988	2825	725	249.1548	NA	NA	NA	NA	249.1553	516037.1	0	723	23457888	278.1
6	0	4110	2825	730	193.0993	NA	NA	NA	NA	193.0995	138038.3	0	723	5673618	278.1
7	0	5543	2828	938	265.1612	NA	NA	NA	NA	265.1618	146493.5	0	933	4158767	310.1
8	1	6503	2830	352	211.0738	C12H9N3C	211.074	-0.9633	13	211.0741	24055.95	1	346	195149.4	34
9															

We are now ready to go onto the Record Creation part of the workflow…

RMassBank Workflow – Simple Form



MassBank Record Workflow

Start a new MB workspace and reset the “Infolists” (compound information)

- Reset and load “Infolists” (compound information) – we will use a part-filled one

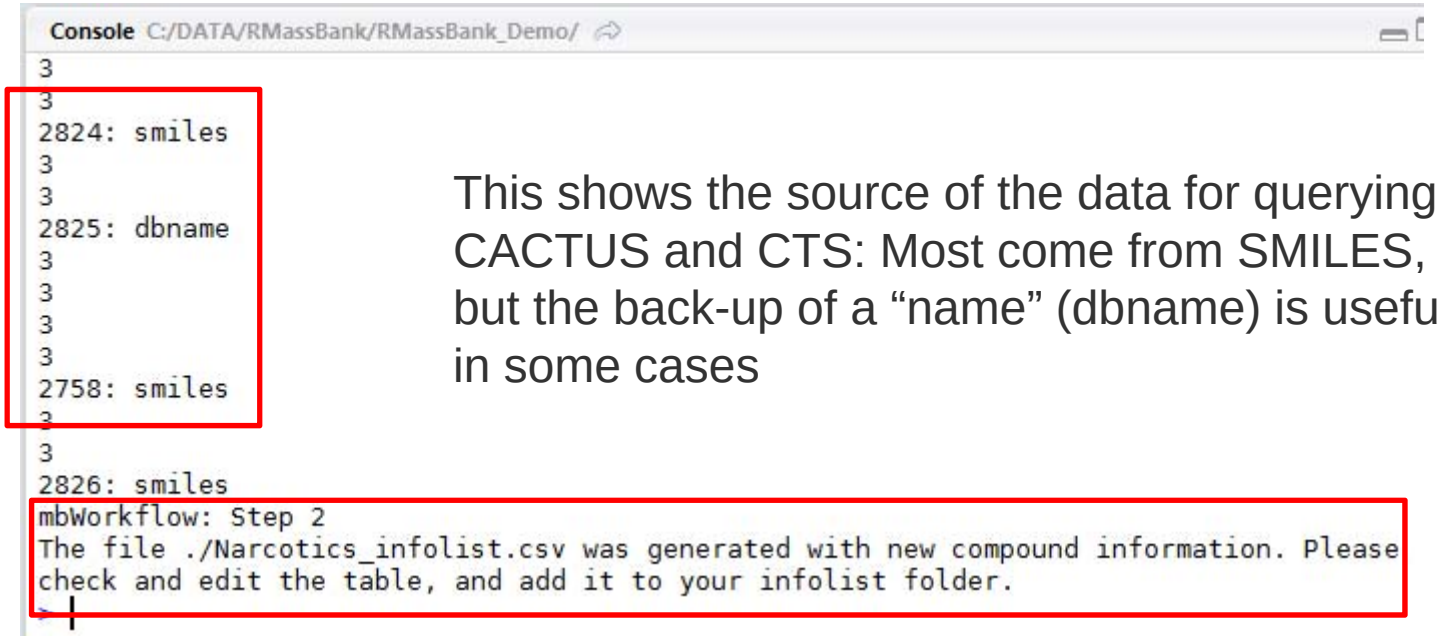
```
79
80 # Next, start the MassBank Record Workflow:
81 mb <- newMbWorkspace(w)
82 mb <- resetInfolists(mb)
83 # To speed up this example, we have provided a partially complete list
84 mb <- loadInfolists(mb, system.file("infolists_incomplete",
85                                     package="RMassBankData"))
```

- Add fail peaks, if applicable, and start the workflow:

```
90 # now add the fail peaks from above, if you have included any
91 mb <- addPeaks(mb, "pH_narcotics_Failpeaks_wOKs.csv")
92
93 # Start the record generation workflow
94 mb <- mbWorkflow(mb, infolist_path="./Narcotics_infolist.csv")
95
```

MassBank Record Workflow

Progress:



This shows the source of the data for querying CACTUS and CTS: Most come from SMILES, but the back-up of a “name” (dbname) is useful in some cases

There is new information to check manually
Please open the table in Excel/OpenOffice…

MassBank Record Workflow

Infolist Editing (1) - Names

	A	B	C	D	E	F	G	H	I	J	K	L	M
1		id	dbcas	dbname	dataused	COMMEN	COMMEN	CH$NAME	CH$NAME	CH$NAME	CH$COMP	CH$FORM	CH$EXACT
2	1	2819	15532-75-9	1-(3-Triflu	smiles	standard c	2819	1-[3-(triflu	1-(3-(Trifluoromethy		N/A; Envir	C11H13F3I	230.1031
3	2	2821	50-48-6	Amitriptyl	smiles	standard c	2821				N/A; Envir	C20H23N	277.183
4	3	2822	300-62-9	Amphetar	smiles	standard c	2822	Amphetar	Amfetami	(1-methyl	N/A; Envir	C9H13N	135.1048
5	4	2823	519-09-5	Benzoylec	dbname	standard c	2823	Benzoylec	None	3-benzoy	N/A; Envir	C16H19NC	289.1314
6	5	2817	50-36-2	Cocaine	dbname	standard c	2817	Cocaine	Unknown	(1S,3S,4R,	N/A; Envir	C17H21NC	303.1471
7	6	2824	125-71-3	Dextrome	smiles	standard c	2824				N/A; Envir	C18H25NC	271.1936
8	7	2825	30223-73-5	EDDP (2-E	dbname	standard c	2825				N/A; Envir	C20H23N	277.183
9	8	2758	299-42-3	Ephedrine	smiles	standard c	2758	Ephedrine	(1R,2S)-2-(methylam		N/A; Envir	C10H15NC	165.1154
10	9	2826	6740-88-1	Ketamine	smiles	standard c	2826				N/A; Envir	C13H16ClN	237.092
11													

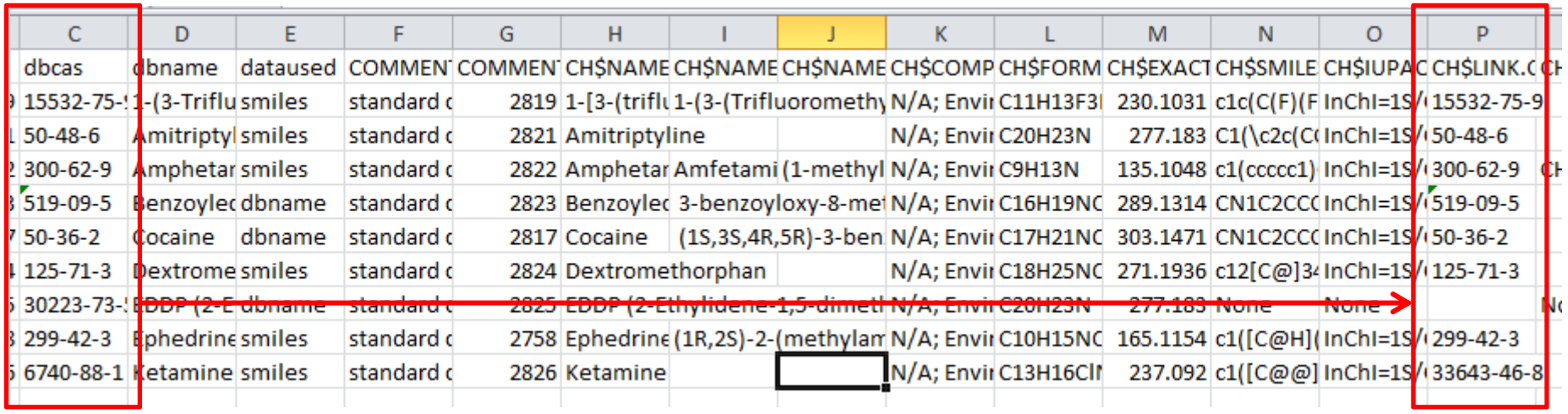
Fix up the names here

- “None”, “Unknown” unnecessary
- At least one name should be present (copy from dbname)

	H	I	J	K
1	CH$NAME	CH$NAME	CH$NAME	CH$COMP
2	1-[3-(triflu	1-(3-(Trifluoromethy		N/A; Envir
3	Amitriptyline			N/A; Envir
4	Amphetar	Amfetami	(1-methyl	N/A; Envir
5	Benzoylec	3-benzoyloxy-8-met		N/A; Envir
6	Cocaine	(1S,3S,4R,5R)-3-ben		N/A; Envir
7	Dextromethorphan			N/A; Envir
8	EDDP (2-Ethylidene-1,5-dimeth			N/A; Envir
9	Ephedrine	(1R,2S)-2-(methylam		N/A; Envir
10	Ketamine			N/A; Envir

MassBank Record Workflow

Infolist Editing (2) – double-check CAS Numbers (if they were in original csv)



	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
	dbcas	dbname	dataused	COMMEN	COMMEN	CH$NAME	CH$NAME	CH$NAME	CH$COMP	CH$FORM	CH$EXACT	CH$SMILE	CH$IUPA	CH$LINK.C	CH
9	15532-75-9	1-(3-Triflu	smiles	standard c	2819	1-[3-(triflu	1-(3-(Trifluoromethy		N/A; Envir	C11H13F3I	230.1031	c1c(C(F)(F	InChI=1S/(	15532-75-9	
1	50-48-6	Amitriptyl	smiles	standard c	2821	Amitriptyline			N/A; Envir	C20H23N	277.183	C1(\c2c(C(	InChI=1S/(	50-48-6	
2	300-62-9	Amphetar	smiles	standard c	2822	Amphetar	Amfetami	(1-methyl	N/A; Envir	C9H13N	135.1048	c1(ccccc1)	InChI=1S/(	300-62-9	CH
3	519-09-5	Benzoylec	dbname	standard c	2823	Benzoylec	3-benzoyloxy-8-met		N/A; Envir	C16H19NC	289.1314	CN1C2CCC	InChI=1S/(	519-09-5	
7	50-36-2	Cocaine	dbname	standard c	2817	Cocaine	(1S,3S,4R,5R)-3-ben		N/A; Envir	C17H21NC	303.1471	CN1C2CCC	InChI=1S/(	50-36-2	
4	125-71-3	Dextrome	smiles	standard c	2824	Dextromethorphan			N/A; Envir	C18H25NC	271.1936	c12[C@]34	InChI=1S/(	125-71-3	
5	30223-73-5	EDDP (2-E	dbname	standard c	2825	EDDP (2-Ethylidene-1,5-dimeth			N/A; Envir	C20H23N	277.183	None	None		No
8	299-42-3	Ephedrine	smiles	standard c	2758	Ephedrine	(1R,2S)-2-(methylam		N/A; Envir	C10H15NC	165.1154	c1([C@H]((	InChI=1S/(	299-42-3	
6	6740-88-1	Ketamine	smiles	standard c	2826	Ketamine			N/A; Envir	C13H16ClN	237.092	c1([C@@]	InChI=1S/(	33643-46-8	

MassBank Record Workflow

Infolist Editing (3) – reality-checking entries & delete “rubbish”

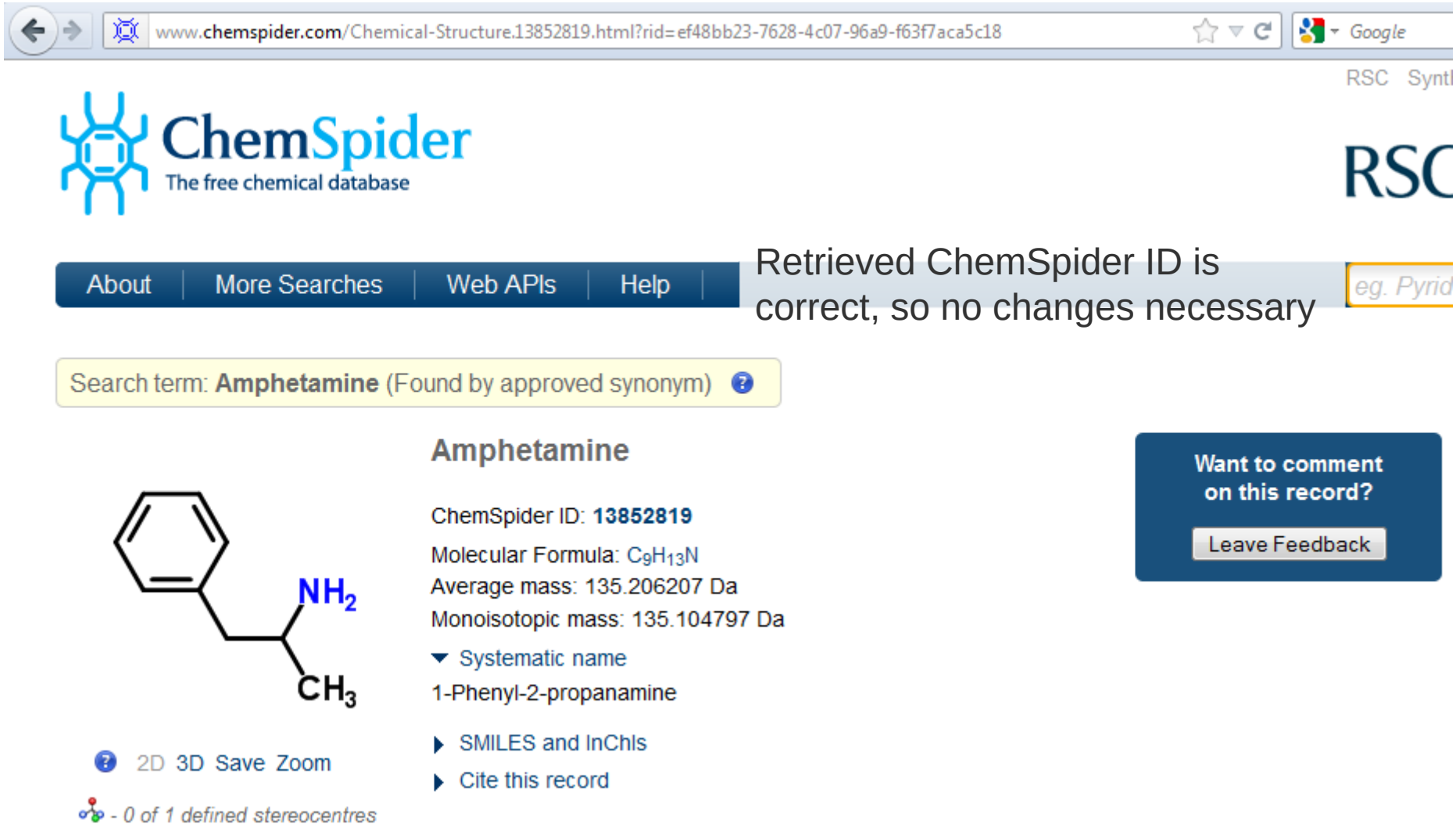
M	N	O	P	Q	R	S	T			
CH$EXACT	CH$SMILE	CH$IUPAC	CH$LINK.C	CH$LINK.C	CH$LINK.I	CH$LINK.K	CH$LI			DER
230.1031	c1c(C(F)(F	InChI=1S/(	15532-75-9							
277.183	C1(\c2c(C(	InChI=1S/(	50-48-6	2666		D07448		CID:2160	KRMDCWI	2075
135.1048	c1(ccccc1)	InChI=1S/(	300-62-9	CHEBI				CID:3007	KWTSXDU	13852819
289.1314	CN1C2CCC	InChI=1S/(	519-09-5					CID:2340	GVGYEFKI	2250
303.1471	CN1C2CCC	InChI=1S/(	50-36-2	27958		C01416		CID:44622	ZPUCINDJ	10194104
271.1936	c12[C@]3(	InChI=1S/(	125-71-3			D03742		CID:69161	MKXZASYA	13109865
277.183	None	None		None	None	None	None	CID:None	None	
165.1154	c1([C@H]((	InChI=1S/(	299-42-3	15407		C01575		CID:9294	KWGRBVC	8935
237.092	c1([C@@](	InChI=1S/(	33643-46-8						YQEZLKZA	158414

Delete this

Check this - ChemSpider & PubChem ID usually similar

MassBank Workflow

Infolist Editing (4) – checking “Amphetamine” in ChemSpider



Retrieved ChemSpider ID is correct, so no changes necessary

MassBank Record Workflow

Infolist Editing (5) – fill in missing entries for EDDP - search by CAS

M	N	O	P	Q	R	S	T	U	V	W	X
CH$EXACT	CH$SMILE	CH$IUPAC	CH$LINK.C	CH$LINK.C	CH$LINK.H	CH$LINK.K	CH$LINK.L	CH$LINK.P	CH$LINK.I	CH$LINK.CHEMSPIDER	
230.1031	c1c(C(F)(F	InChI=1S/(	15532-75-9					CID:4296	KKIMDKM	4145	
277.183	C1(\c2c(C(	InChI=1S/(	50-48-6	2666		D07448		CID:2160	KRMDCWI	2075	
135.1048	c					D07445		CID:3007	KWTSXDU	13852819	
289.1314	C	N1C2CCC	InChI=1S/	519-09-5				CID:2340	GVGYEFKI	2250	
303.1471	C	N1C2CCC	InChI=1S/	50-36-2		27958		C01416	CID:44622	ZPUCINDJ	10194104
271.1936	c12[C@]3(	InChI=1S/(	125-71-3			D03742		CID:69161	MKXZASV	13109865	
277.183	None	None		None	None	None	None	CID:None	None		
165.1154	c1([C@H]((	InChI=1S/(	299-42-3	15407		C01575		CID:9294	KWGRBVC	8935	
237.092	c1([C@@]	InChI=1S/(	33643-46-8						YQEZLKZA	158414	

Search ChemSpider and
PubChem with CAS 30223-73-5 (in csv)

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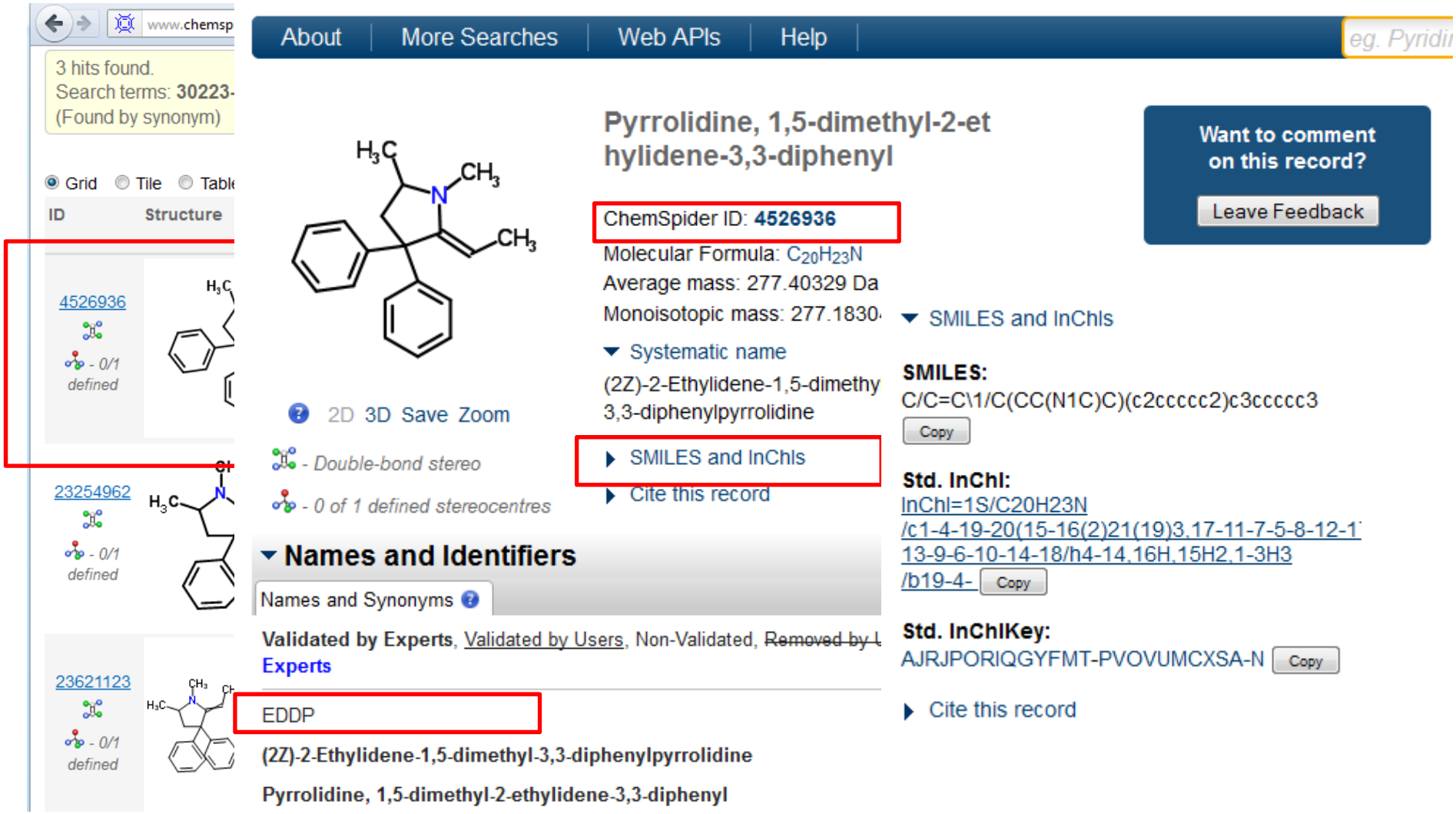
MassBank Record Workflow

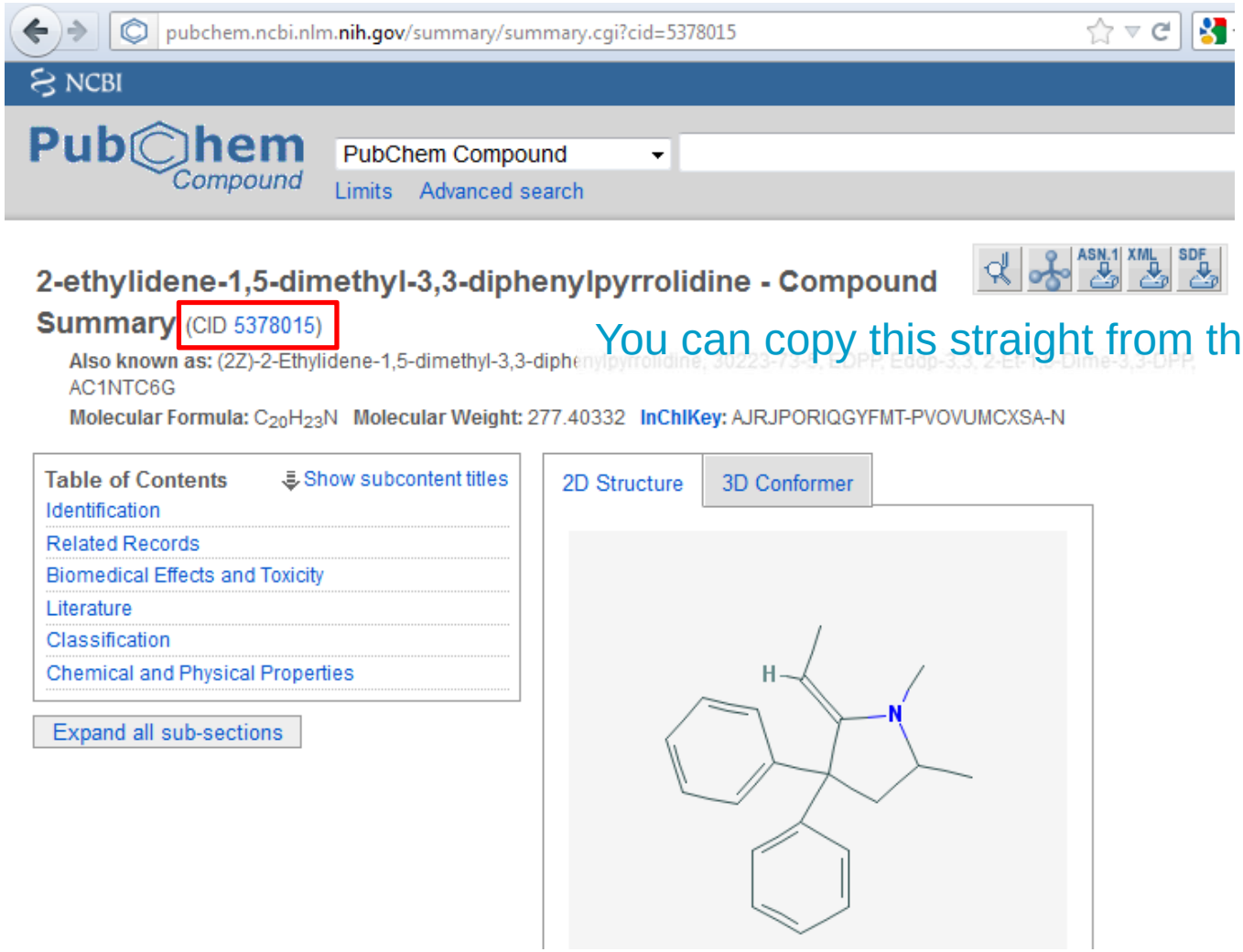
Infolist Editing (5) – EDDP - search ChemSpider by CAS: 30223-73-5



MassBank Record Workflow

=> http://pubchem.ncbi.nlm.nih.gov/search/search.cgi#

Infolist Editing (5) – EDDP - search PubChem by CAS: 30223-73-5



MassBank Record Workflow

Infolist Editing (5) – fill in missing entries for EDDP – now filled in?

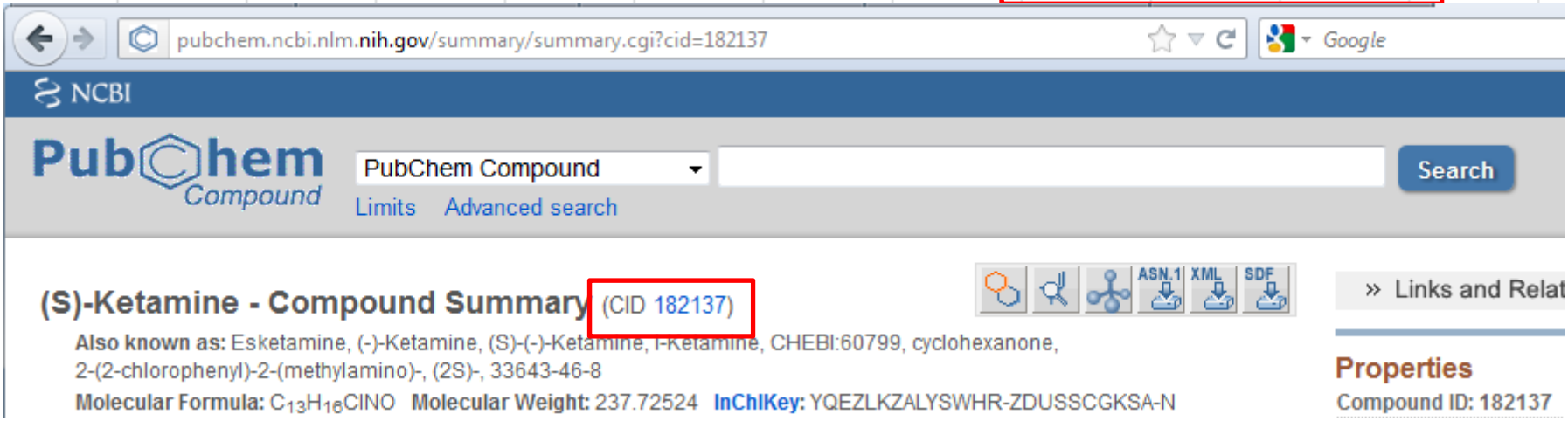
135.1048	c1(ccccc1)	InChI=1S/(	300-62-9	CHEBI		D07445		CID:3007	KWTSXDU	13852819	
289.1314	CN1C2CC(	InChI=1S/(	519-09-5					CID:2340	GVGYEFKI	2250	
303.1471	CN1C2CC(	InChI=1S/(	50-36-2	27958		C01416		CID:44622	ZPUCINDJ	10194104	
271.1936	c12[C@]3(	InChI=1S/(	125-71-3			D03742		CID:69161	MKXZASY	13109865	
277.183	None	None		None	None	None	None	CID:None	None		
165.1154	c1([C@H](	InChI=1S/(	299-42-3	15407		C01575		CID:9294	KWGRBVC	8935	
237.092	c1([C@@]	InChI=1S/(	33643-46-8						YQEZLKZA	158414	

M	N	O	P	Q	R	S	T	U	V	W	X
CH$EXACT	CH$SMILE	CH$IUPAC	CH$LINK.C	CH$LINK.C	CH$LINK.I	CH$LINK.K	CH$LINK.L	CH$LINK.I	CH$LINK.I	CH$LINK.CHEMSPIDER	
230.1031	c1c(C(F)(F	InChI=1S/(	15532-75-9					CID:4296	KKIMDKM	4145	
277.183	C1(\c2c(C(	InChI=1S/(	50-48-6	2666		D07448		CID:2160	KRMDCWI	2075	
135.1048	c1(ccccc1)	InChI=1S/(	300-62-9			D07445		CID:3007	KWTSXDU	13852819	
289.1314	CN1C2CC(	InChI=1S/(	519-09-5					CID:2340	GVGYEFKI	2250	
303.1471	CN1C2CC(	InChI=1S/(	50-36-2	27958		C01416		CID:44622	ZPUCINDJ	10194104	
271.1936	c12[C@]3(	InChI=1S/(	125-71-3			D03742		CID:69161	MKXZASY	13109865	
277.183	C/C=C\1/C	InChI=1S/(	30223-73-5					CID:53780	AJRJPORI(	4526936	
165.1154	c1([C@H](	InChI=1S/(	299-42-3	15407		C01575		CID:9294	KWGRBVC	8935	
237.092	c1([C@@]	InChI=1S/(	33643-46-8						YQEZLKZA	158414	

MassBank Record Workflow

Infolist Editing (6) – Check missing PubChem entry?

M	N	O	P	Q	R	S	T	U	V	W	X
CH$EXACT	CH$SMILE	CH$IUPAC	CH$LINK.C	CH$LINK.C	CH$LINK.H	CH$LINK.K	CH$LINK.L	CH$LINK.P	CH$LINK.I	CH$LINK.CHEMSPIDER	
230.1031	c1c(C(F)(F	InChI=1S/(	15532-75-9					CID:4296	KKIMDKM	4145	
277.183	C1(\c2c(C(	InChI=1S/(	50-48-6	2666		D07448		CID:2160	KRMDCWI	2075	
135.1048	c1(ccccc1)	InChI=1S/(	300-62-9			D07445		CID:3007	KWTSXDU	13852819	
289.1314	CN1C2CCC	InChI=1S/(	519-09-5					CID:2340	GVGYEFKI	2250	
303.1471	CN1C2CCC	InChI=1S/(	50-36-2	27958		C01416		CID:44622	ZPUCINDJ	10194104	
271.1936	c12[C@]34	InChI=1S/(	125-71-3			D03742		CID:69161	MKXZASYA	13109865	
277.183	C/C=C\1/C	InChI=1S/(	30223-73-5					CID:53780	AJRJPORIC	4526936	
165.1154	c1([C@H]((	InChI=1S/(	299-42-3	15407		C01575		CID:9294	KWGRBVC	8935	
237.092	c1([C@@]	InChI=1S/(	33643-46-8						YQEZLKZA	158414	



MassBank Record Workflow

Infolist editing finished! Save under a new name and restart workflow

- Moving Infolists into a separate Infolist folder is recommended!

```
100
101 mb <- resetInfolists(mb)
102 mb <- loadInfolists(mb, "C:/DATA/RMassBank/InfoLists_demo")
103 # NOTE: if you didn't want to correct the whole list, use this one:
104 #mb <- loadInfolists(mb, system.file("infolists", package="RMassBankData"))
105 # and start the workflow again - "no new data added" is a good sign!
106 mb <- mb
107
```

```
> mb <- resetInfolists(mb)
> mb <- loadInfolists(mb, "C:/DATA/RMassBank/InfoLists_demo")
> # NOTE: if you didn't want to correct the whole list, use this one:
> #mb <- loadInfolists(mb, system.file("infolists", package="RMassBankData"))
> # and start the workflow again - "no new data added" is a good sign!
> mb <- mbWorkflow(mb)
mbWorkflow: Step 1
mbWorkflow: Step 2
No new data added.
mbWorkflow: Step 3
mbWorkflow: Step 4
Compiling: 1_3_Chlorophenyl_piperazin_2818_pos.mzML
Compiling: 1_3_Trifluoromethylphenyl_piperazin_2819_pos.m
Compiling: 1_Benzylpiperazin_2820_pos.mzML
Compiling: Amitriptylin_2821_pos.mzML
Compiling: Amphetamin_2822_pos.mzML
Compiling: Benzoylecgonin_2823_pos.mzML
```

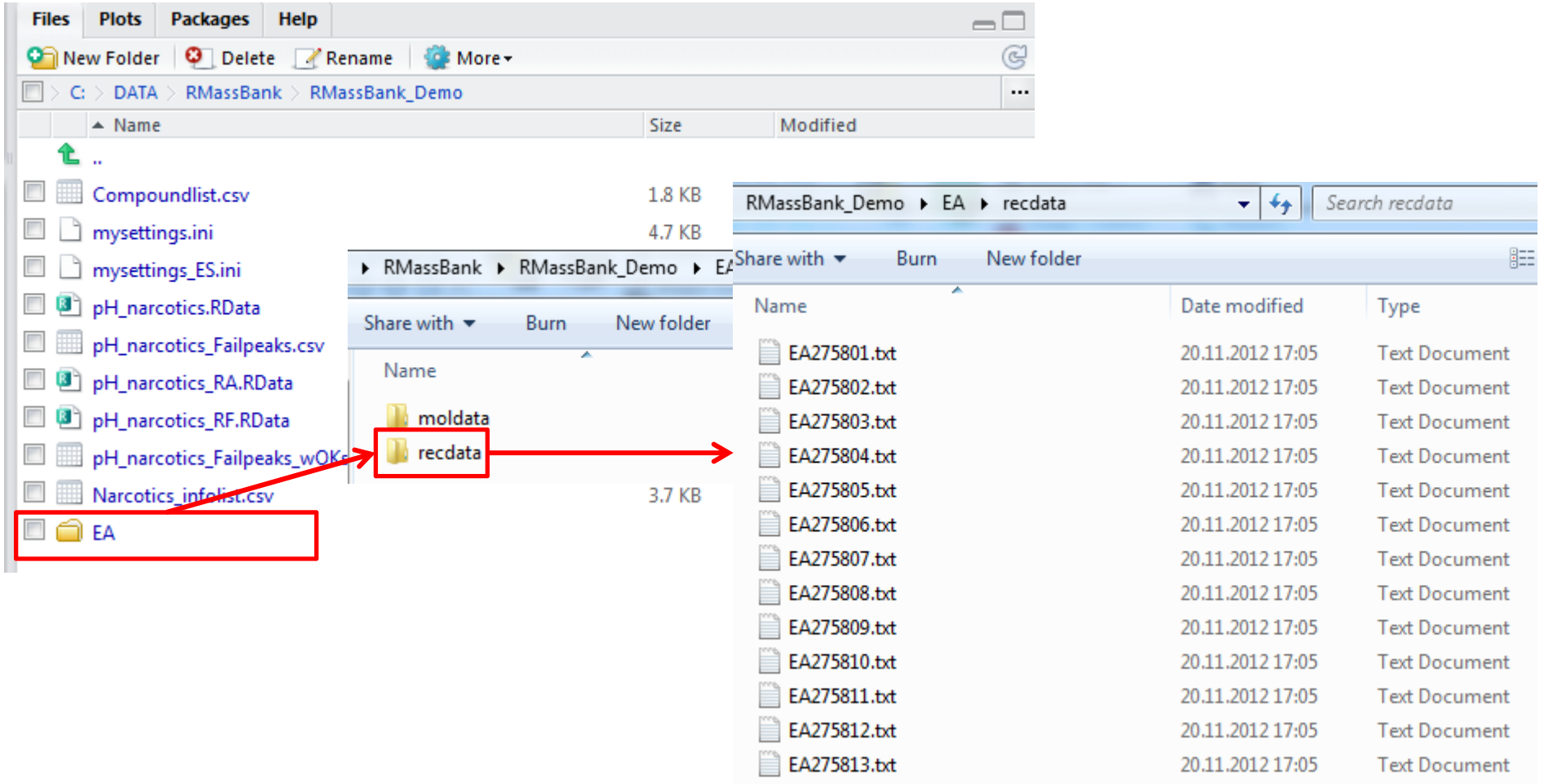
```
3
3
3
mbWorkflow: Step 5
mbWorkflow: Step 6
mbWorkflow: Step 7
mbWorkflow: Step 8
>
```

And that's it – DONE!

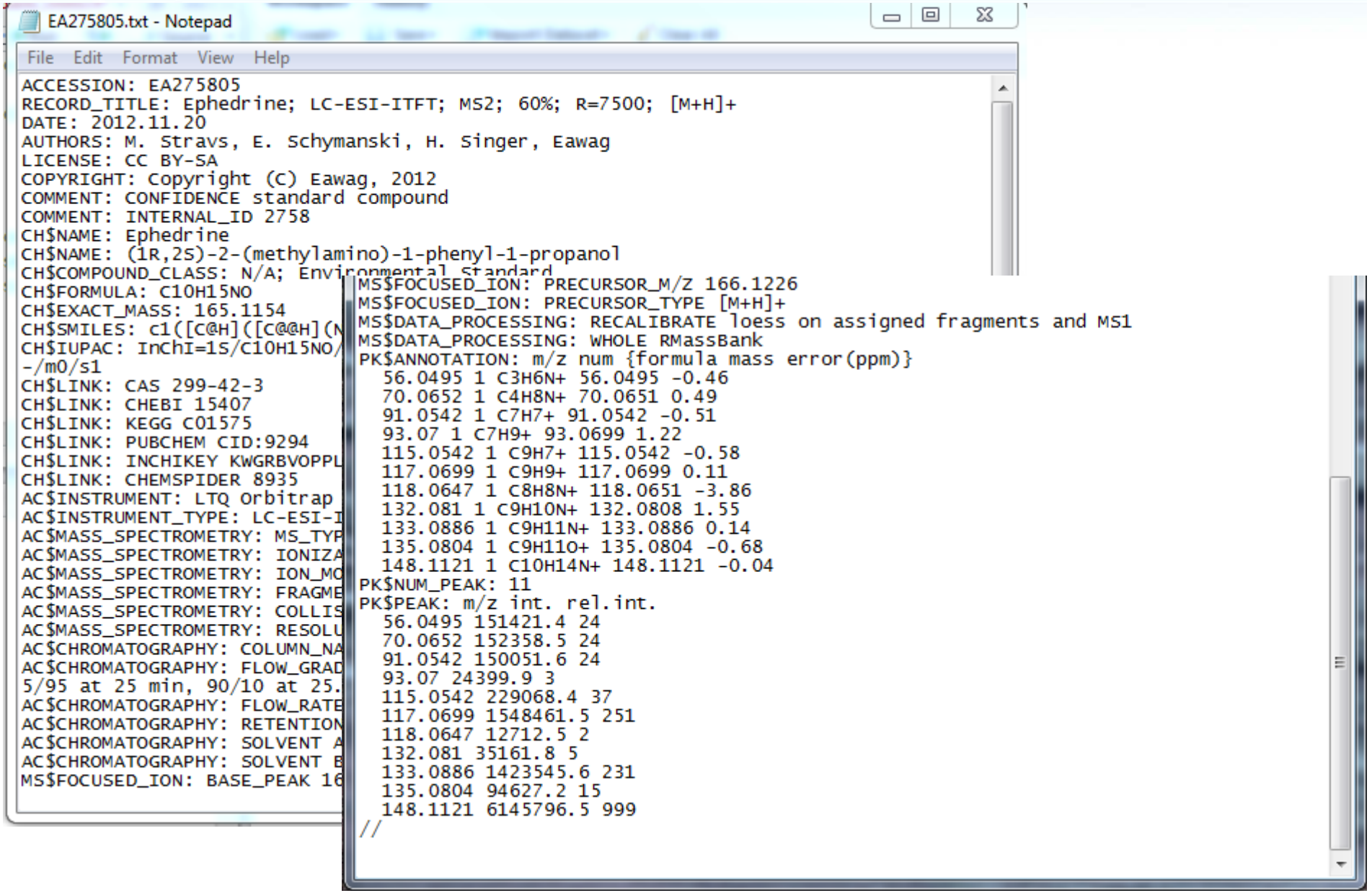
MassBank Record Workflow

Take a look at your new records!

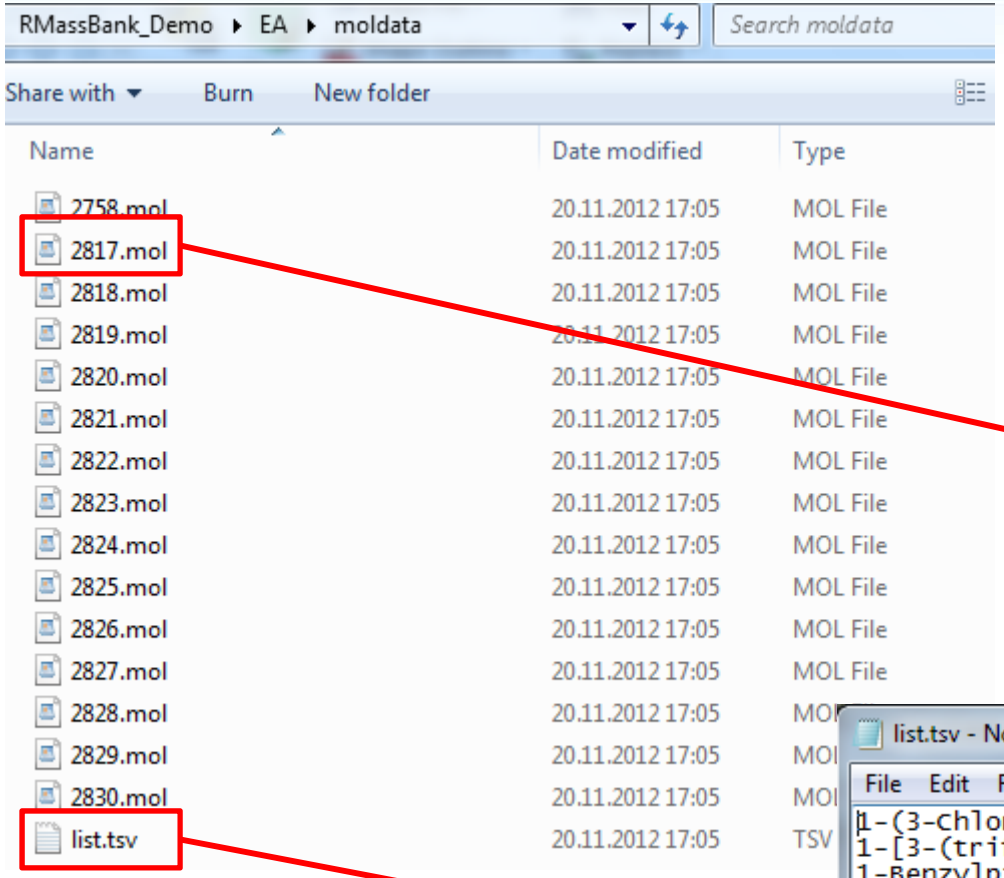
- You can open these in any text editor

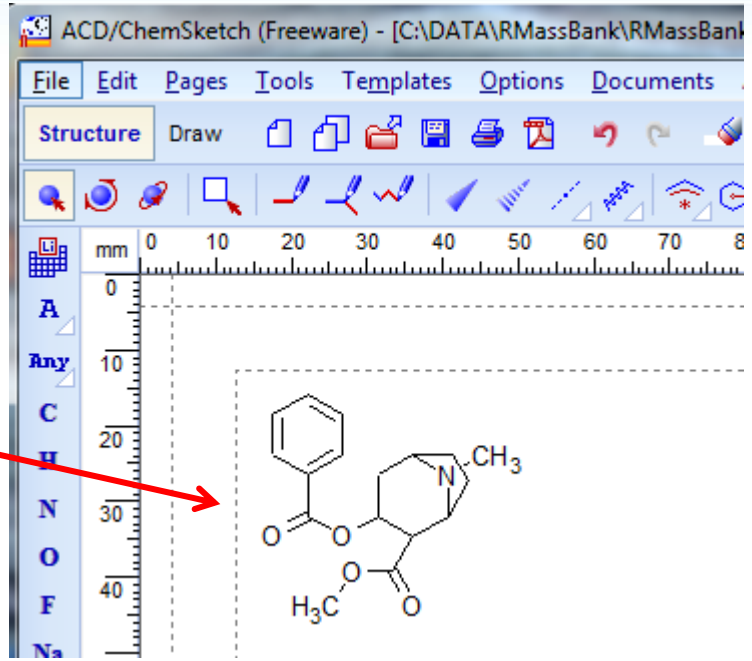


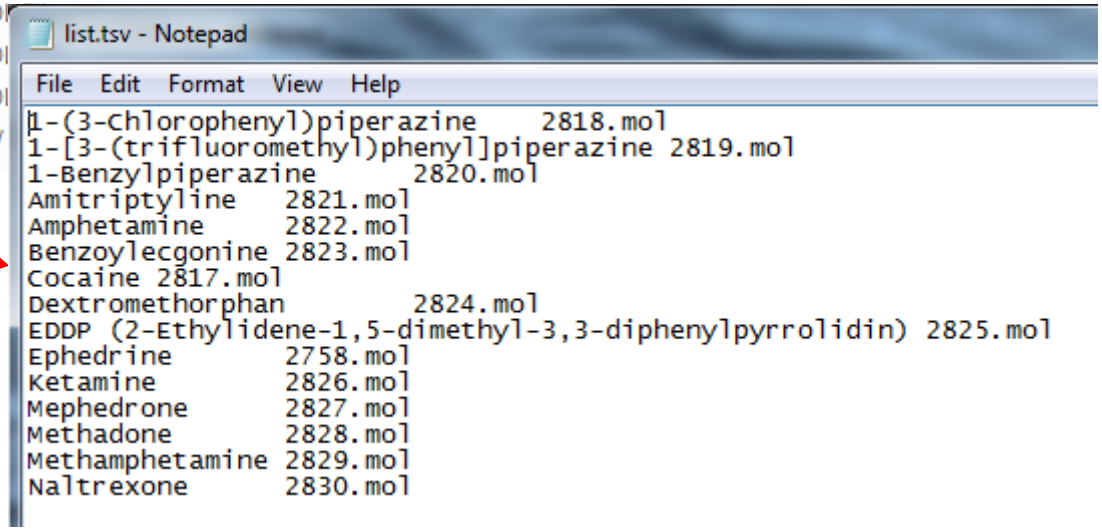
MassBank Records “recdata”



MassBank Record Structures “moldata”





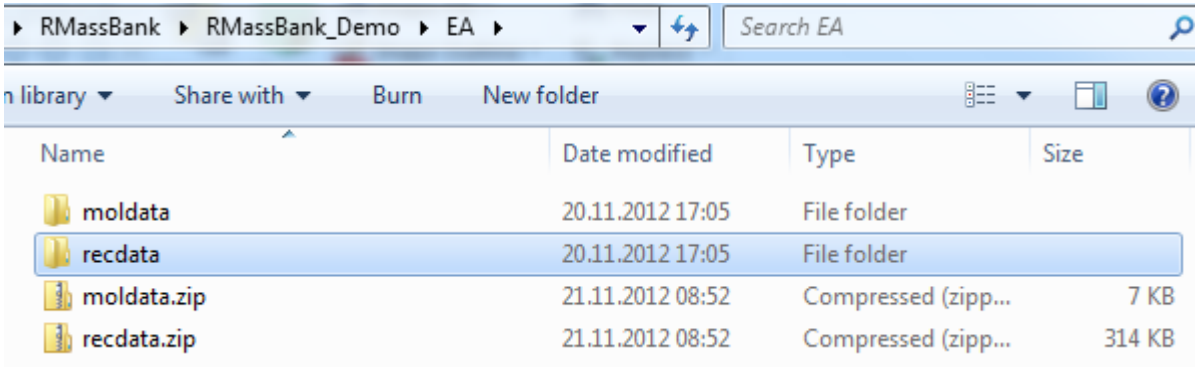


This is the list that connects structure and record

Final Tasks for MassBank records:

Create “recdata.zip” and “moldata.zip” (e.g. with 7Zip)

- This wasn’t automated as this requires additional packages for Windows

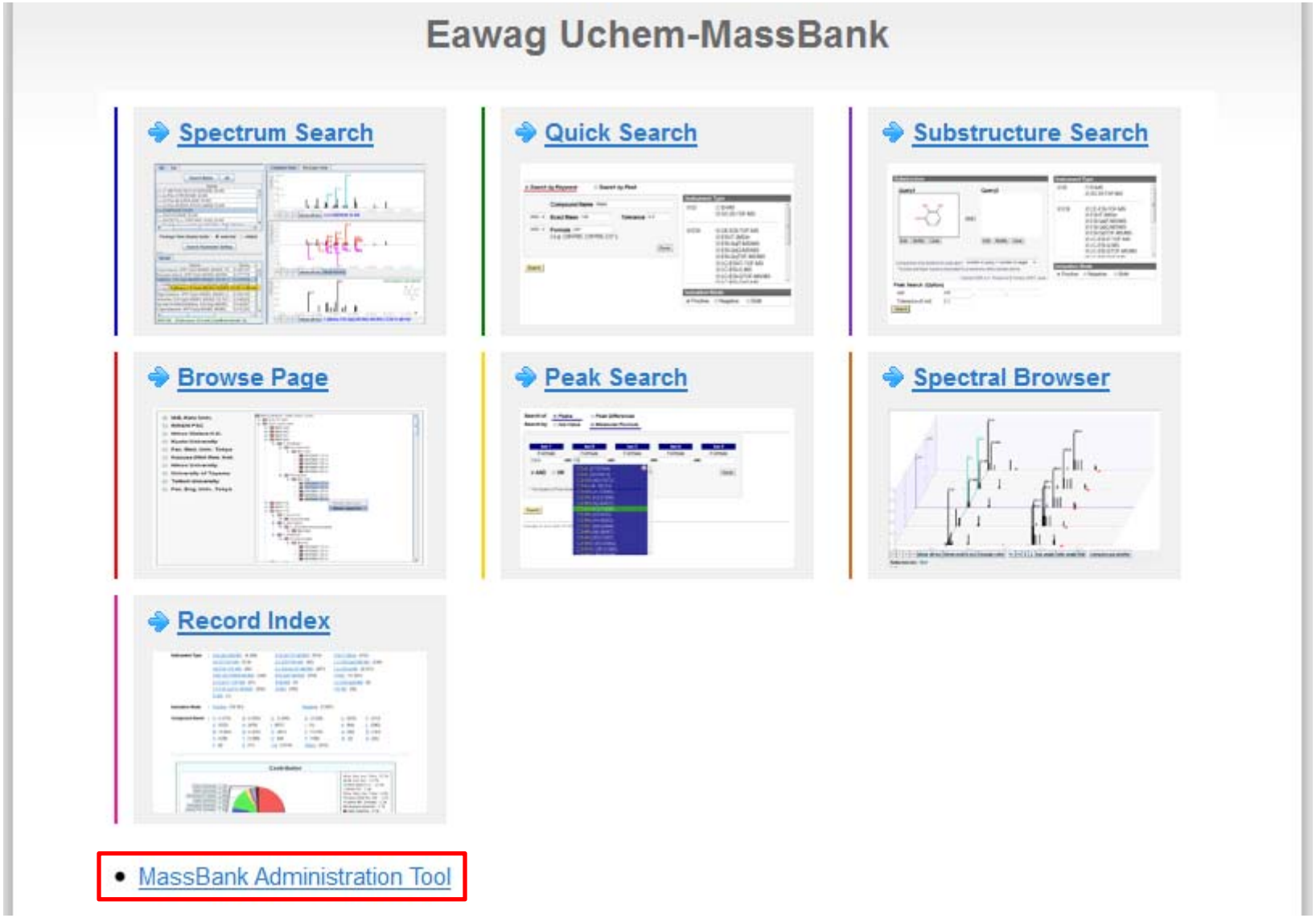


These zip files are now ready for upload to MassBank

- If these are new records for NORMAN MassBank
  - Email these to massbank@normandata.eu
- If these are private records, you can upload to your own MassBank
  - I’ll give a few screenshots in the next few slides

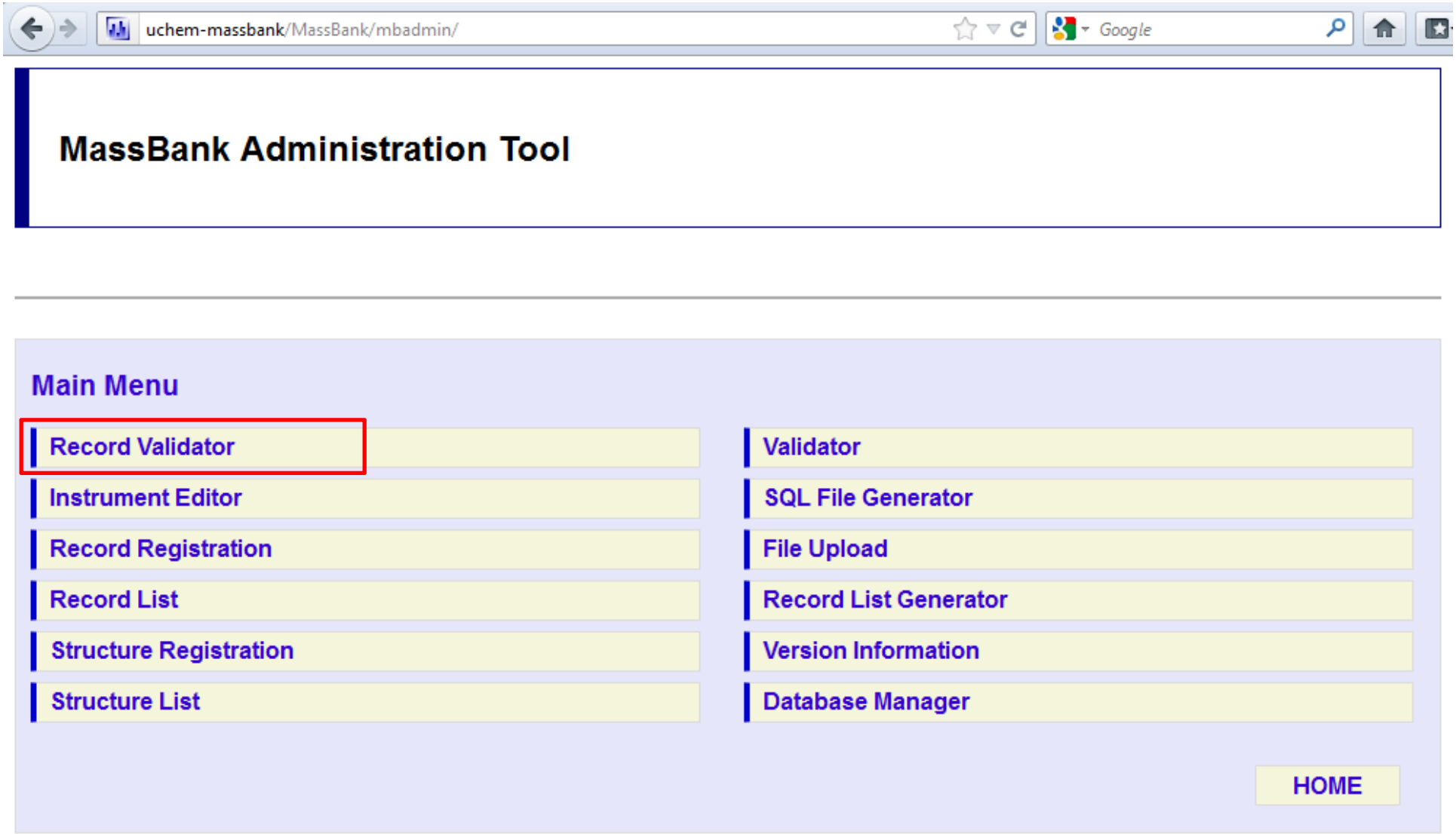
Uploading MassBank Records

Example with “uchem-massbank” screenshots



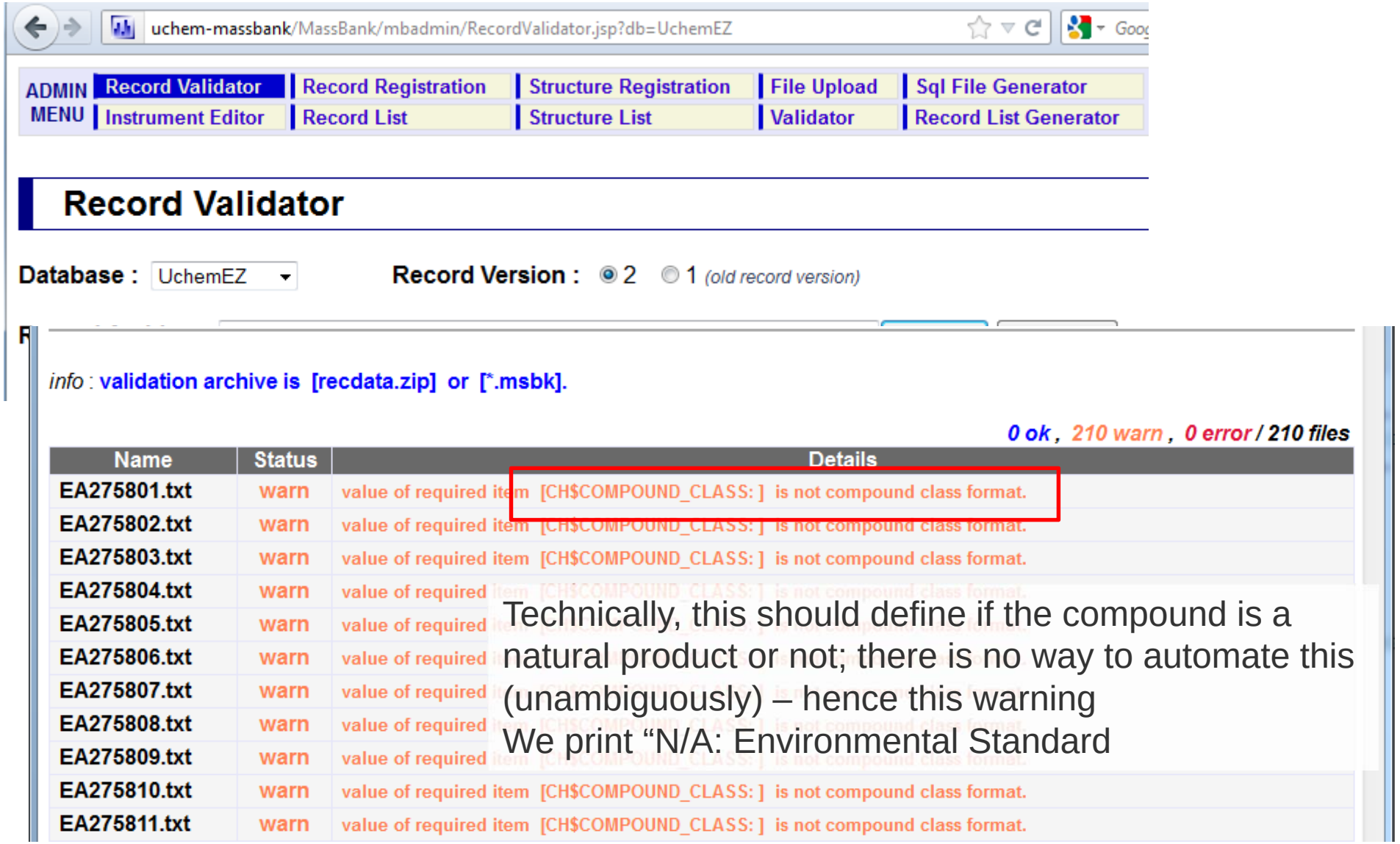
Uploading MassBank Records

Example with “uchem-massbank” screenshots



Uploading MassBank Records

Record validation



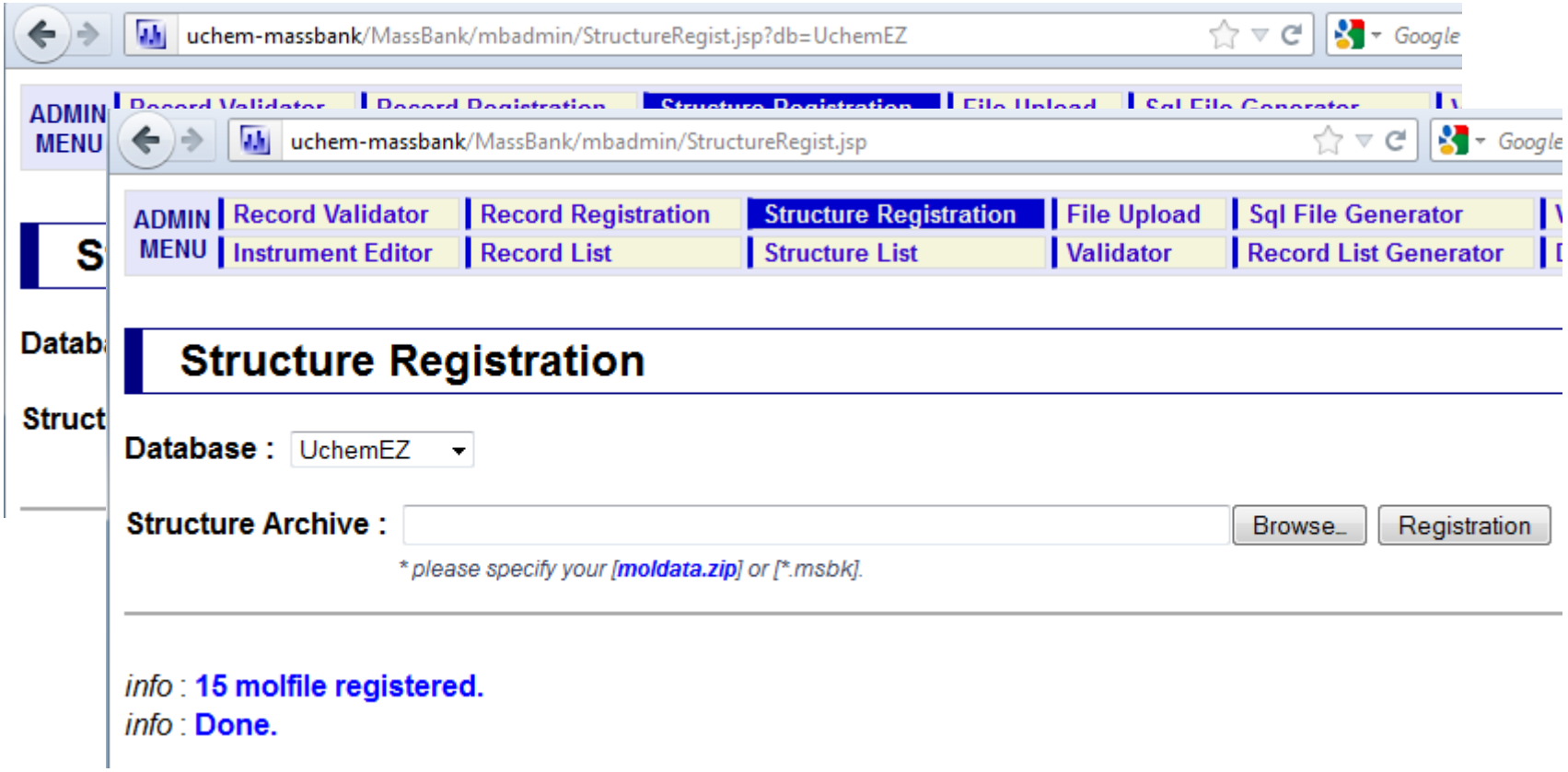
Technically, this should define if the compound is a natural product or not; there is no way to automate this (unambiguously) – hence this warning
We print “N/A: Environmental Standard

Uploading MassBank Records

Record Registration

Uploading MassBank Records

Structure Registration



Records Registered

Browse MassBank to see what they look like!

Record Index

mass calculator

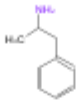
Home | Spectrum | Quick | Peak | Substructure | Browser | Browse | Index | MassBank ID: Go

Contributor :

CASMI (42) | Chubu Univ. (2,628) | EA Uchem Orbi (5,132)

EA Uchem Orbi Test (641) | EQ Uchem Q Ex (1,262) | Eawag Uchem Adducts (153)

Fukuyama Univ. (340) | IMM, CAMS & PUMC, China (192) | IPB Halle (528)

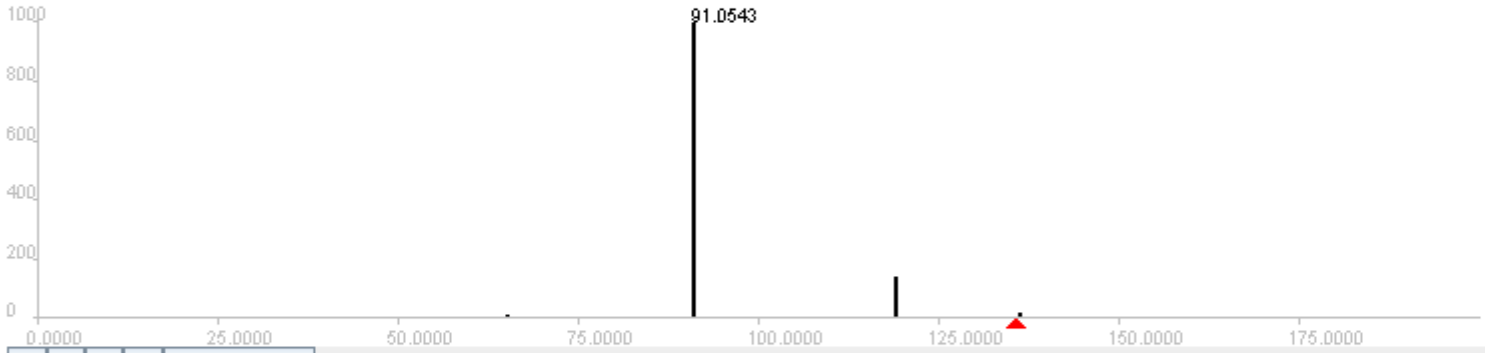
Amphetamine	14 spectra	**C9H13N** 	**135.10480**	
LC-ESI-ITFT; MS2; 15%; R=15000; [M+H]+				EA282208
LC-ESI-ITFT; MS2; 15%; R=7500; [M+H]+				EA282202
LC-ESI-ITFT; MS2; 30%; R=15000; [M+H]+				EA282209
LC-ESI-ITFT; MS2; 30%; R=7500; [M+H]+				EA282203
LC-ESI-ITFT; MS2; 35%; R=15000; [M+H]+				EA282214
LC-ESI-ITFT; MS2; 35%; R=7500; [M+H]+				EA282201
LC-ESI-ITFT; MS2; 45%; R=15000; [M+H]+				EA282210
LC-ESI-ITFT; MS2; 45%; R=7500; [M+H]+				EA282204
LC-ESI-ITFT; MS2; 60%; R=15000; [M+H]+				EA282211
LC-ESI-ITFT; MS2; 60%; R=7500; [M+H]+				EA282205
LC-ESI-ITFT; MS2; 75%; R=15000; [M+H]+				EA282212
LC-ESI-ITFT; MS2; 75%; R=7500; [M+H]+				EA282206
LC-ESI-ITFT; MS2; 90%; R=15000; [M+H]+				EA282213
LC-ESI-ITFT; MS2; 90%; R=7500; [M+H]+				EA282207

uchem-massbank/MassBank/jsp/Dispatcher.jsp?type=disp&id=EA282210&site=1

MassBank Record: EA282210

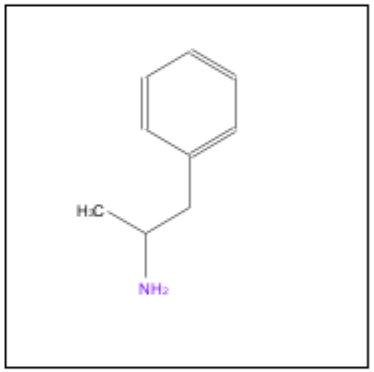
Amphetamine; LC-ESI-ITFT; MS2; 45%; R=15000; [M+H]+

Mass Spectrum



<< < > >> show all m/z

Chemical Structure



ACCESSION: EA282210
RECORD_TITLE: Amphetamine; LC-ESI-ITFT; MS2; 45%; R=15000; [M+H]+
DATE: 2012.11.20
AUTHORS: M. Stravs, E. Schymanski, H. Singer, Eawag
LICENSE: CC BY-SA
COPYRIGHT: Copyright (C) Eawag, 2012
COMMENT: CONFIDENCE standard compound
COMMENT: INTERNAL_ID 2822

CH$NAME: Amphetamine
CH$NAME: Amfetamine

Take-Home Messages: RMassBank Demo

That was a 61-slide introduction to RMassBank

- Install (and update) RMassBank and necessary programs
- Run RMassBank for trial data
  - Get a basic understanding for the workflow
- Understand the manual checking required
  - “Fail peaks” – checking these in raw data
  - Automatic annotation with CTS – searching/editing this data
- Generate records with RMassBank
- A quick impression how to upload these records

It will be impossible to remember everything!

- vignette(“RMassBank”) – this contains an extensive explanation
- Stravs et al. 2012, *J. Mass Spectrom.*, DOI: 10.1002/jms.3131

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- Marie-Curie Post Doctoral Fellowship (E. Schymanski),
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- Discussions: Matthias Ruff, Martin Loos (Eawag);

  Tobias Schulze, Martin Krauss, Werner Brack (UFZ)
- MassBank & Naming Rights: Prof. Takaaki Nishioka
- NORMAN Association

- And thank you all for listening!

RMassBank

eawag
aquatic research

Any Questions?

massbank@normandata.eu (Tobias – record upload)

massbank@eawag.ch (RMassBank: E. Schymanski, M. Stravs)

http://metabolomics-forum.com/viewforum.php?f=29

emma.schymanski@eawag.ch

DOI: 10.1002/jms.3131

All Details Contained Within!