RMassBank

eawag
aquatic research

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

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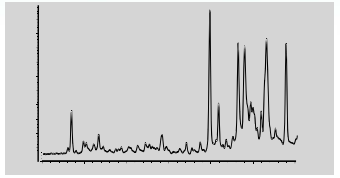
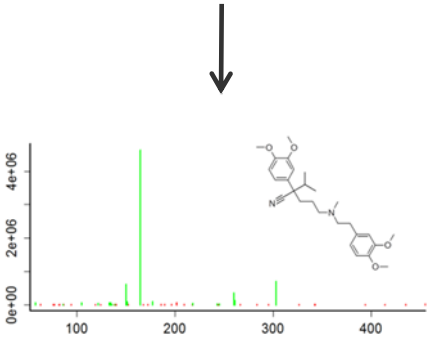
MARIE CURIE ACTIONS

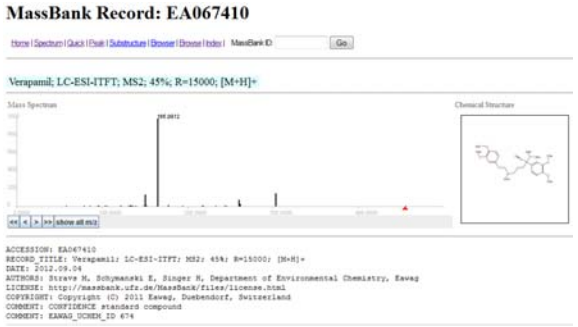
Presentation Overview

Contents

- Introduction and Problem Formulation
  - Spectral clean-up (garbage in => garbage out!)
  - Spectral annotation (compound-spectrum link)
- The RMassBank Workflow
  - Data processing
  - Record creation
- Results of RMassBank Processing
  - Proof-of-concept on 70 pesticide spectra
  - Spectral Interpretation







Spectral Clean-up

Garbage in = garbage out!

The General Problem

- No time: many want to add spectra in principle, no-one has time to do it
- Manual data entry is tedious, repetitive, prone to errors
- Full manual quality control of spectra is tedious and resource-consuming

The Result* – Database becomes a “spectral dump”**

- “We’ll just use a noise cut-off, that’ll be fine” – e.g. WA001201
  - Cut-off at 5 ‰ (MassBank reporting can go down to 1 ‰)
- “We’ll just use the peak list from our software” – e.g. CE000143
  - Many low intensity noise peaks, including peaks above $[M+H]^+$
- “Annotation is tedious” – e.g. JEL00007
  - Minimum amount of information provided only

* These examples are demonstrations only and no offense intended!

** Thanks to Oliver Fiehn for this apt description!

Spectral Clean-up

MassBank Record: WA001201

```
MS$DATA_PROCESSING: FIND_PEAK ignore rel.int. < 5

PK$NUM_PEAK: 19
PK$PEAK: m/z int. rel.int.
  102 63 63
  105 8 8
  130 133 133
  131 8 8
  139 20 20
  162 12 12
  163 12 12
  166 834 834
  167 59 59
  178 12 12
  180 63 63
  182 24 24
  184 999 999
  185 63 63
```

* These examples are demonstrations only and no offense intended!

Spectral Clean-up

MassBank Record: CE000143

```
MS$FOCUSED_ION: PRECURSOR_M/Z 268.10404
MS$FOCUSED_ION: PRECURSOR_TYPE [M+H]+

PK$NUM_PEAK: 20
PK$PEAK: m/z int. rel.int.
  76.423012 1164.474609 2
  79.144173 972.286438 2
  88.860359 1310.202271 2
  106.183556 1195.565674 2
  135.887161 3993.299072 7
  135.992874 3979.442627 7
  136.034454 2340.972656 4
  136.041092 1992.728271 4
  136.061371 568236.125 999
  136.08461 4257.030762 7
  136.130478 2980.912598 5
  136.228577 1192.598145 2
  136.234634 1166.915161 2
  197.613419 1193.385498 2
  203.694138 1189.134888 2
  219.079987 21971.183594 39
  237.09053 180033.921875 317
  292.21759 1154.94812 2
  293.092346 1334.223755 2
  293.587189 1281.828003 2
//
```

These examples are demonstrations only and no offense intended!

Spectral Clean-up – Example of Orbitrap Spectra

Garbage in = garbage out!

Measurement artefacts include

- Systematic increase in ppm error with low *m/z* values
  - 5 ppm accuracy => 15 ppm at *m/z* < 100
- Satellite or shoulder peaks – result of FT instrument processing
- Consistent electronic or measurement noise peaks
- Noise peaks at a ~fixed level hiding real peaks of lower intensity
  - A strict noise cut-off will result in a loss of information

High mass accuracy standard spectra have advantages:

- Meringer et al. (2011) showed a missing subformula indicates instrument noise or interfering peaks
  - Use subformula assignment to perform spectral clean-up!

Meringer et al. 2011, *MATCH Commun. Math. Comput. Chem*. 65, 259-290.

Spectral Annotation

Matching Spectrum and Compound Information

User needs to contribute a bare minimum of information

- Only the user knows what compound has been measured
  - At least one form of unambiguous compound identifier is required
  - e.g. internal ID, name, SMILES and retention time
- Measurement parameters / methods / settings are relatively consistent
  - These can be added in batch form, not individually

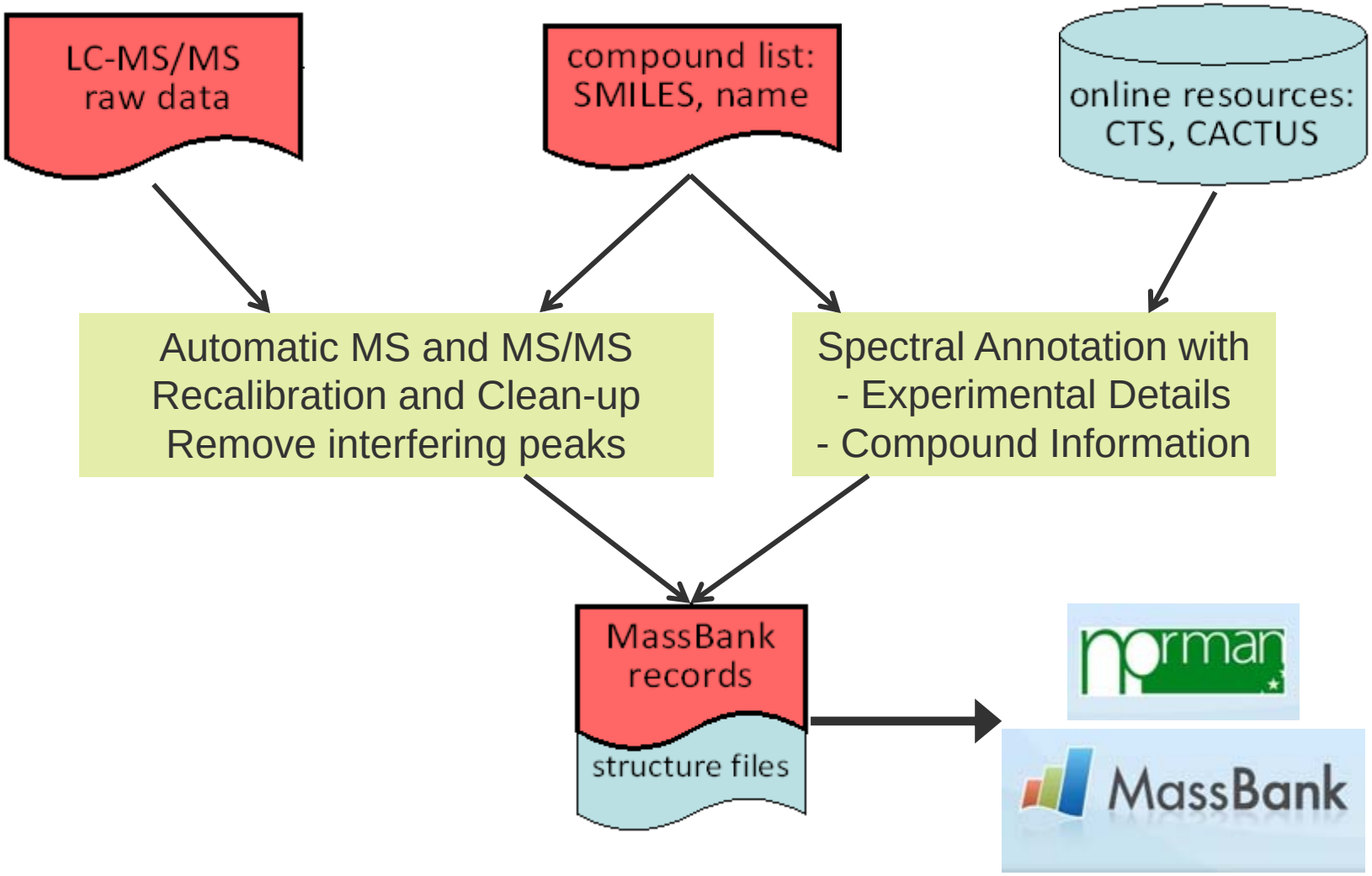
Internet Services: Let search engines do the work for you!

- CACTUS Chemical Identifier Resolver[1]
  - SMILES (c1ccccc1) to InChI Key (UHOVQNZJYSORNB-UHFFFAOYSA-N)
- Chemical Translation Service (CTS)[2] to do the rest
  - Names, CAS #, InChI and Identifiers (IDs, if available): PubChem CID, ChemSpider, ChEBI, HMDB, KEGG, LipidMaps

[1] http://cactus.nci.nih.gov/chemical/structure

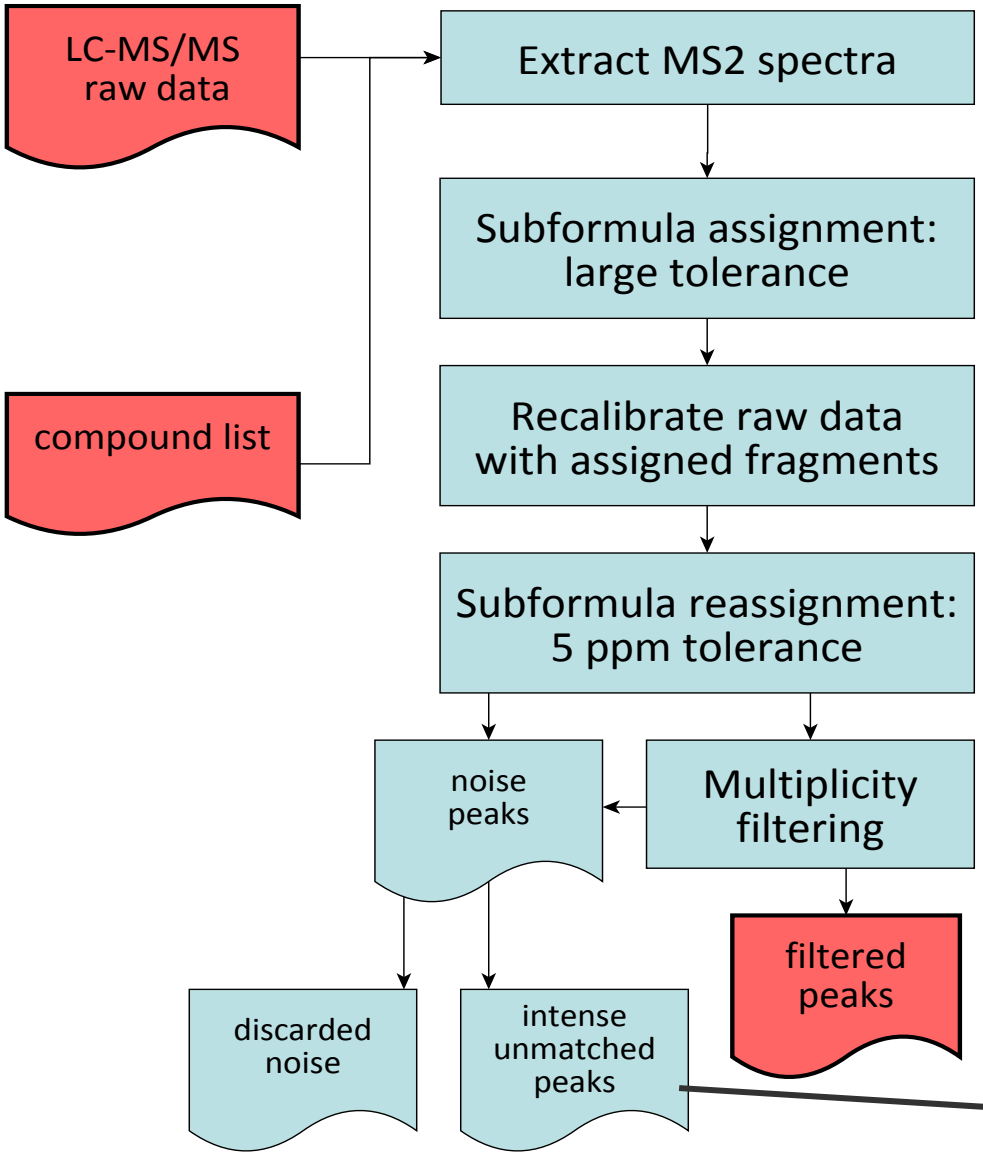
[2] http://uranus.fiehnlab.ucdavis.edu:8080/cts/homePage

RMassBank Workflow – Simple Form



Stravs, Schymanski, Singer and Hollender, 2012,
Journal of Mass Spectrometry, accepted. DOI: 10.1002/jms.3131

RMassBank – Clean-up and Recalibration



Automated extraction by accurate m/z and RT.
FT-satellite removal (± 0.5 Da; I < 5 % of peak)

Elements of molecular formula ± adduct
ppm ≤ 15 (m/z < 120) or ≤ 10 (m/z > 120)

Use only peaks with unique formula calculated

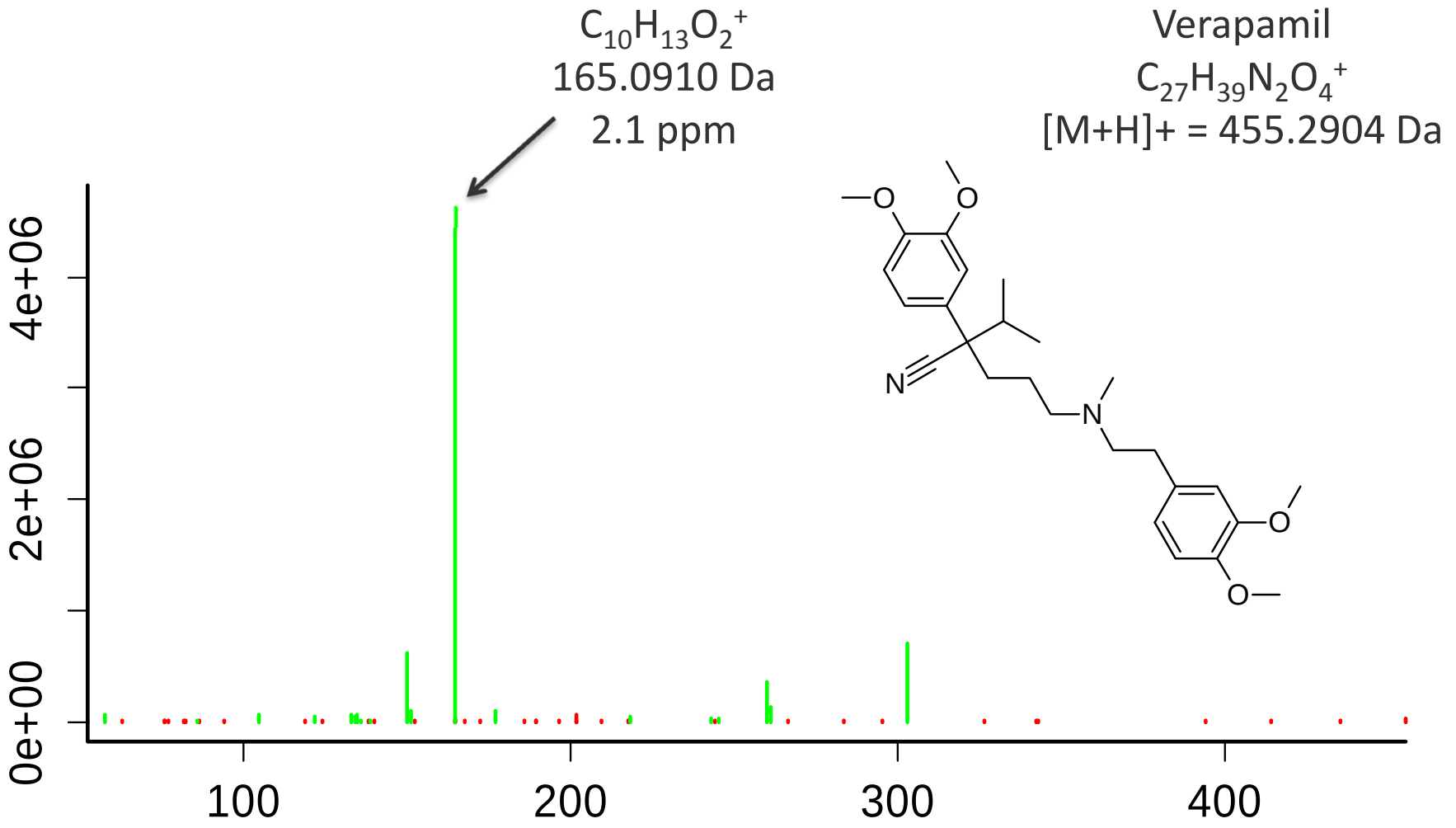
Formulas recalculated post calibration.
For "Fail Peaks", recalculate with +2N & O

Peaks are retained if they occur ≥2x per compound (in e.g. 14 recorded spectra)

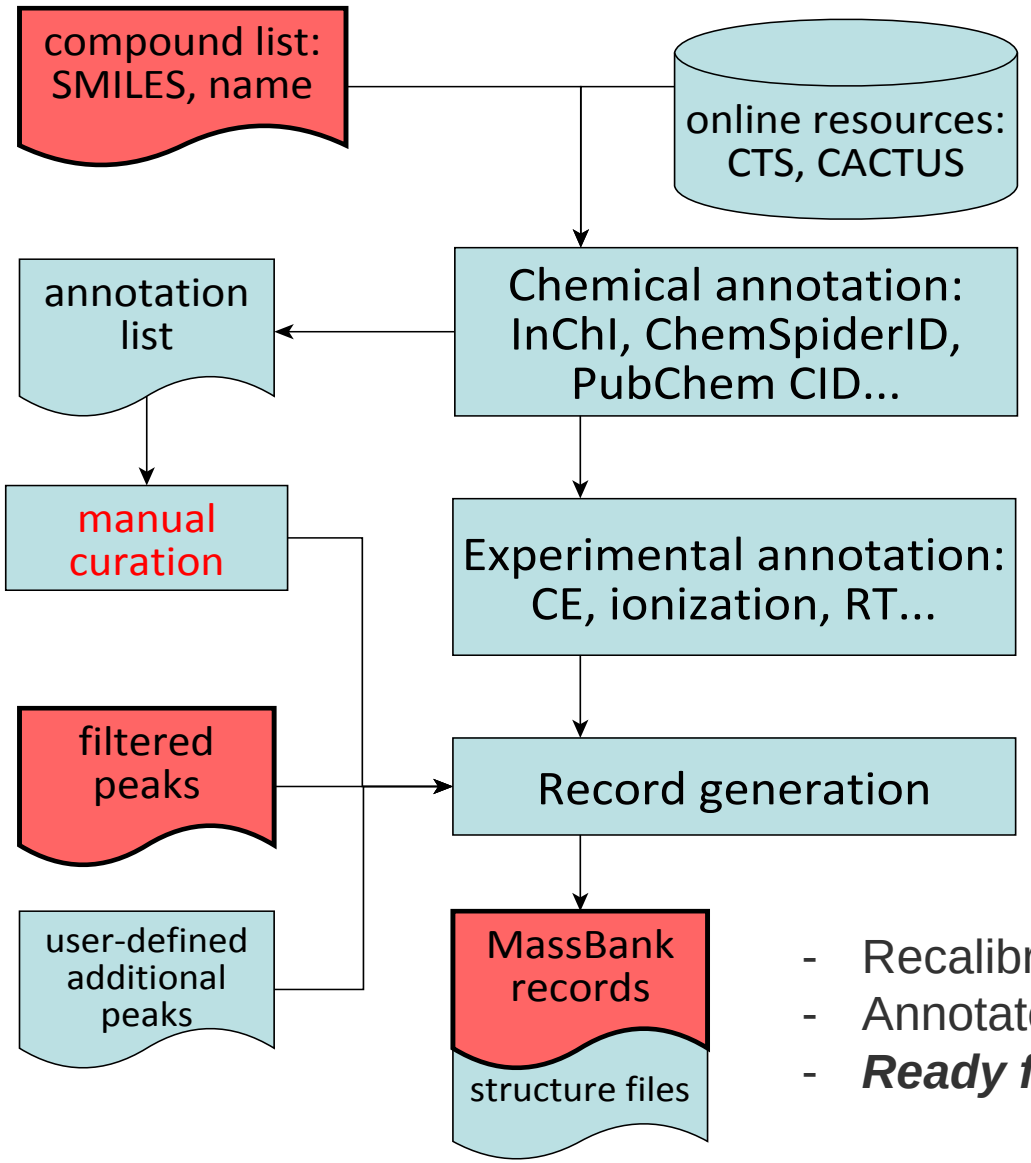
Filtered peaks go through to Records

Intense unmatched peaks for manual check

RMassBank – Example Clean-up



RMassBank – Spectrum Annotation



- Recalibrated, cleaned-up
- Annotated
- ***Ready for upload!***

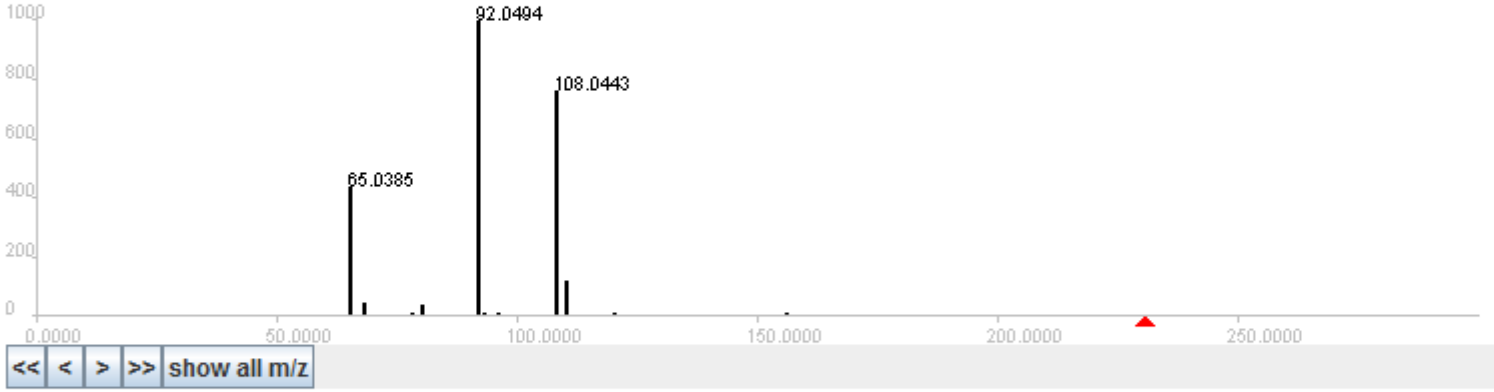
Example MassBank Record

MassBank Record: EA015612

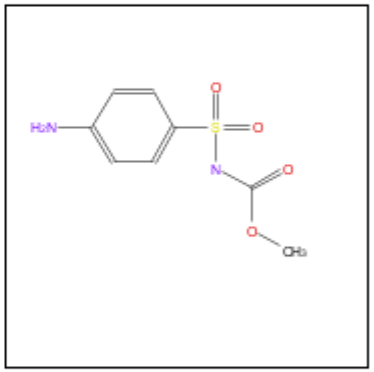
Home | Spectrum | Quick | Peak | Substructure | Browser | Browse | Index | MassBank ID: Go

Asulam; LC-ESI-ITFT; MS2; 75%; R=15000; [M+H]+

Mass Spectrum



Chemical Structure

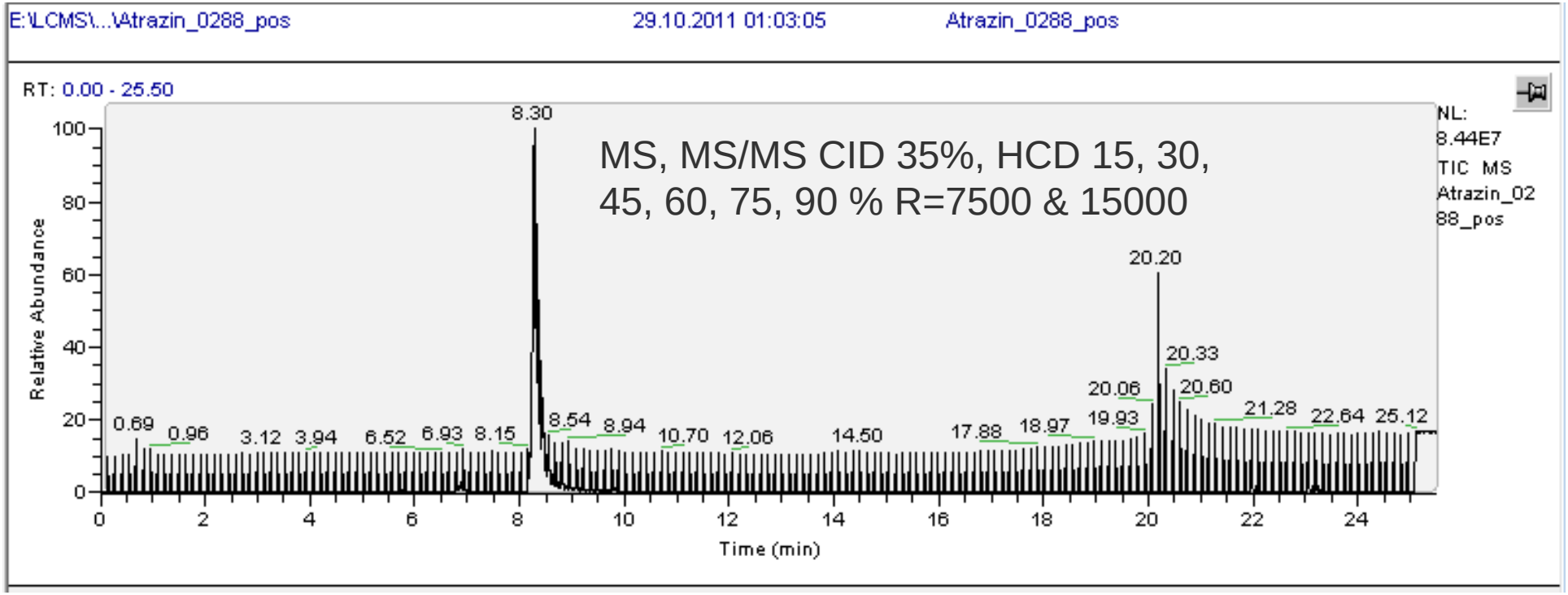


```
ACCESSION: EA015612
RECORD_TITLE: Asulam; LC-ESI-ITFT; MS2; 75%; R=15000; [M+H]+
DATE: 2012.03.16
AUTHORS: Stravs M, Schymanski E, Singer H, Department of Environmental Chemistry, Eawag
LICENSE: http://massbank.ufz.de/MassBank/files/license.html
COPYRIGHT: Copyright (C) 2011 Eawag, Duebendorf, Switzerland
COMMENT: CONFIDENCE standard compound
COMMENT: EAWAG_UCHEM_ID 156
```

RMassBank with 70 Eawag Pesticide Spectra

Experimental

- Individual Injection; routine chromatography; Orbitrap XL with ESI + / -
- Daily vendor-recommended calibration



Screened for $[M+H]^+$ precursor within RT ± 0.3 min
MS/MS retrieved from MS with highest intensity

RMassBank with 70 Eawag Pesticide Spectra

Processing: The Numbers

- 68 of 70 pesticides with sufficient $[M+H]^+$ for processing
- 55,594 peaks present following satellite removal
  - 14,699 wit
  - 13,305 of t
- 454 peaks with
  - 256 of thes
- Only 44 peaks remained for “manual inspection”
- No difference observed between spectra with different resolutions

~76 % of peaks are noise!

Formation of N_2 and H_2O adducts is relevant in MS/MS!

Additional modes (results not shown here)

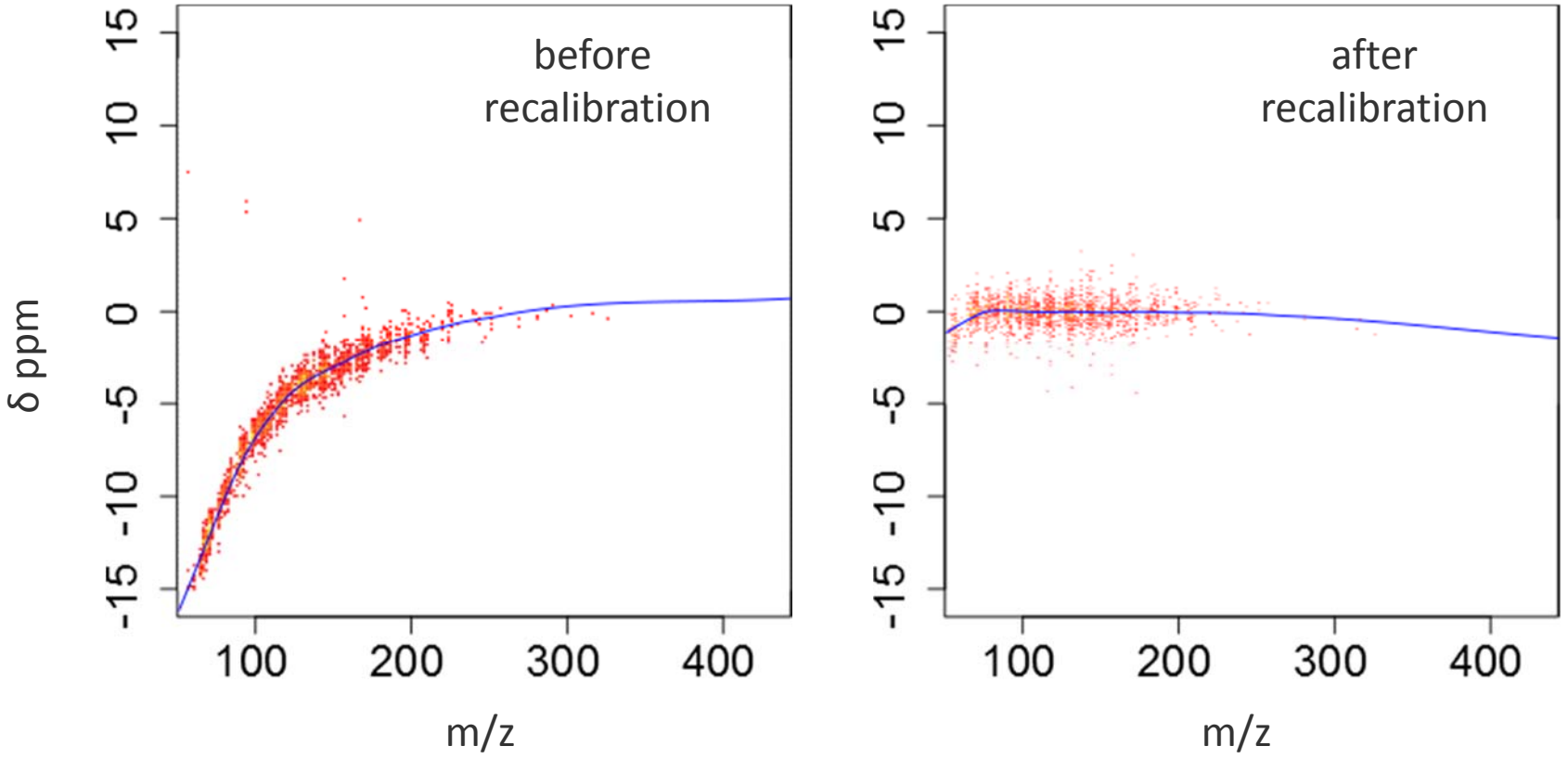
- M^+, $[M+Na]^+$, $[M-H]^-$, M^-, $[M+FA]^-$,

Effect of Recalibration

- Shown in the next few slides…

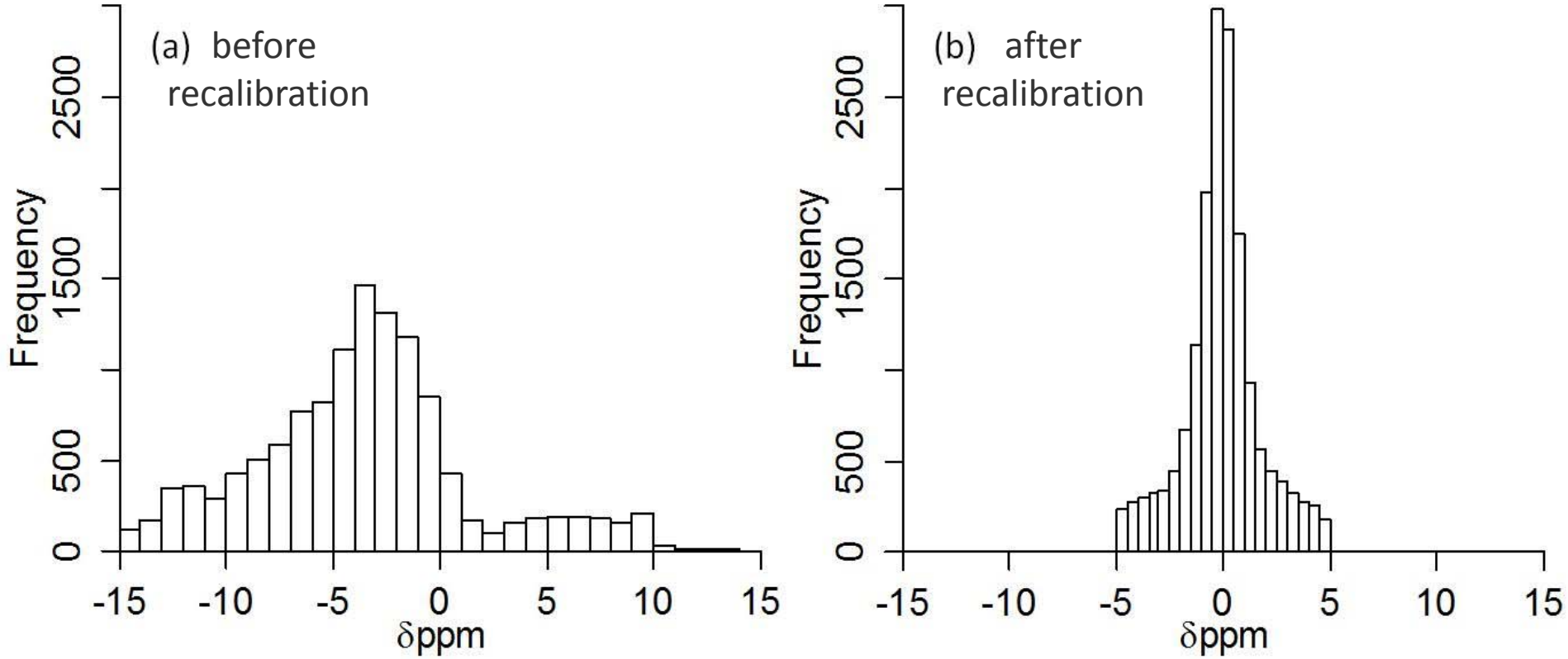
RMassBank with 70 Eawag Pesticide Spectra

Recalibration Curve: Relative mass deviation over *m/z*



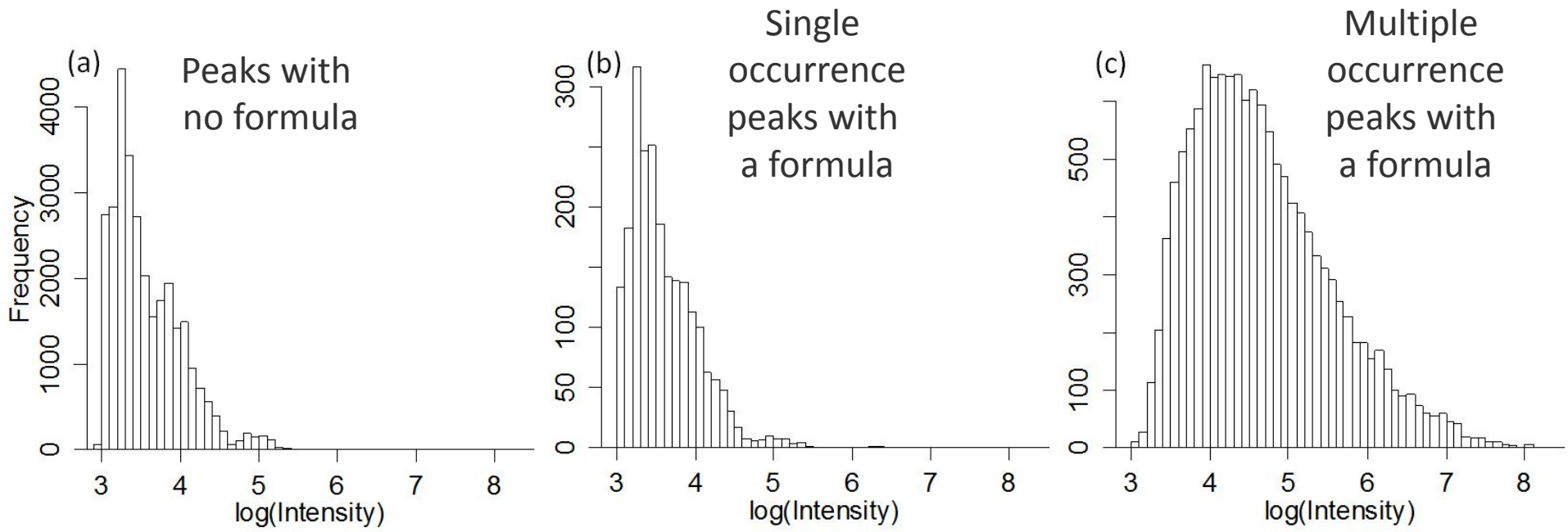
RMassBank with 70 Eawag Pesticide Spectra

Recalibration: Relative Mass Deviation Distribution



RMassBank with 70 Eawag Pesticide Spectra

Frequency of Occurrence of Peaks by Intensity: Multiplicity Filtering



RMassBank with 70 Eawag Pesticide Spectra

944 MS/MS spectra from $[M+H]^+$ of 70 pesticides

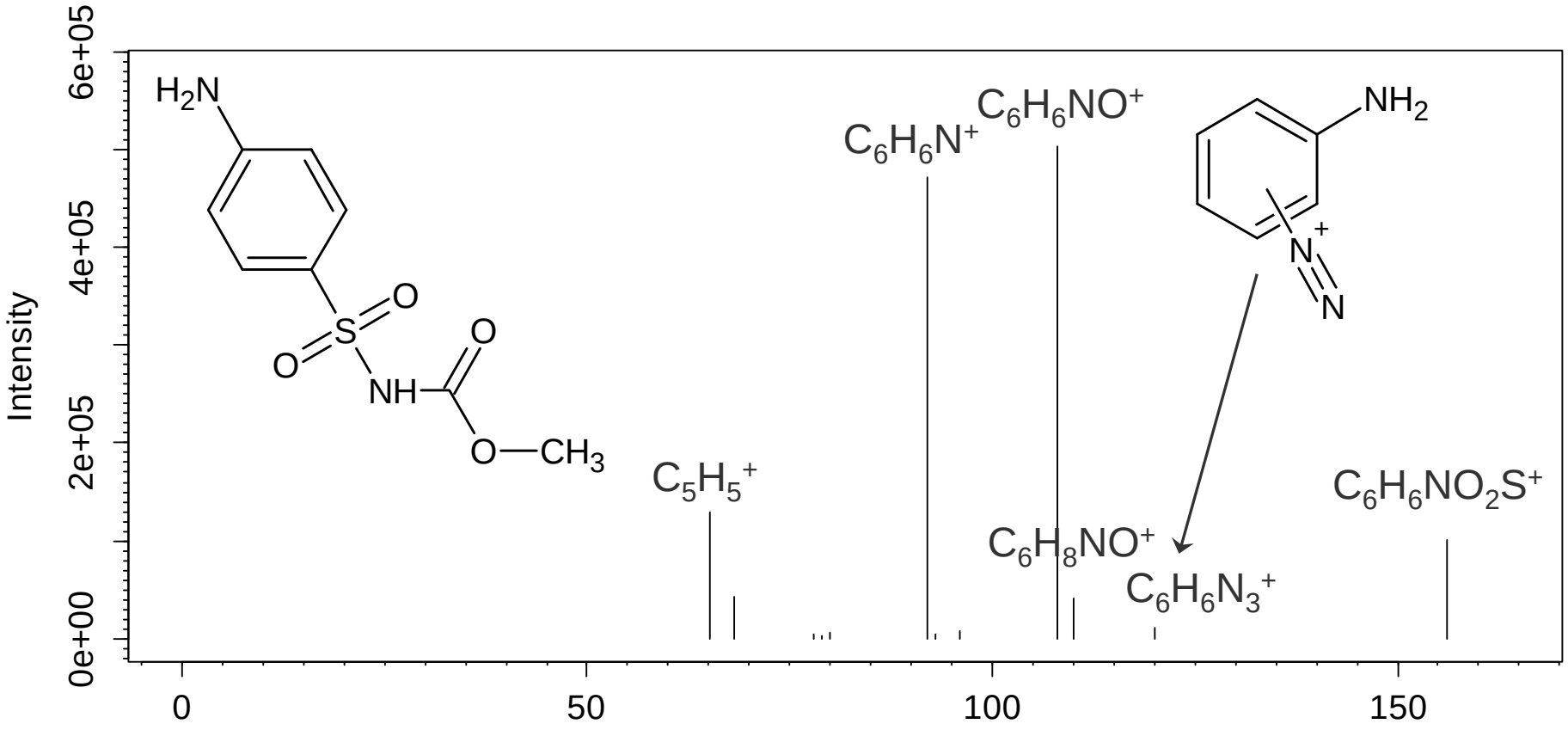
- www.massbank.jp
- http://massbank.normandata.eu/MassBank

Total Number of “RMassBank Spectra”

- RMassBank records in Uchem-MassBank:
  - 5,312 records (374 compounds) LTQ Orbitrap XL
  - 153 records (12 compounds) Orbitrap Adducts
  - 1,262 records (151 compounds) Q Exactive Orbitrap
- RMassBank records in NORMAN MassBank:
  - 3,193 records (158 compounds) from UFZ Orbitrap XL
  - 3,102 records (226 compounds) from Eawag Orbitrap XL

RMassBank with 70 Eawag Pesticide Spectra

For those online: pull up MassBank record EA015611



Conclusions: RMassBank

The RMassBank Workflow

- Reduces much manual work associated with bulk creation of many records
- Creates high quality MS/MS spectra
- Annotation with formula value-adds the spectra
- Works very well for the spectra it was developed on (Orbitrap)
- BUT: Every mass spectrometer is different:
  - Processing and measurement steps will probably need adjusting

Benefit for Contributors

- We have learnt a lot about our spectra and compounds (e.g. MS/MS adducts!)
- MassBank is being using within our department
- If you want to know more about what recalibration can do for your data:
  - 29-30th November: Emerging Pollutants Workshop, Non-target session
  - Stravs et al. 2012, *J. Mass Spectrom.*, DOI: 10.1002/jms.3131

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- Discussions: Matthias Ruff, Martin Loos (Eawag); Tobias Schulze, Martin Krauss, Werner Brack (UFZ)
- Programs: Markus Meringer (MOLGEN-MS/MS), Florian Rasche (Sirius), Miguel Rojas-Cherto & Egon Willighagen (MEF)
- MassBank & Naming Rights: Prof. Takaaki Nishioka
- NORMAN Association

RMassBank

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Any Questions?

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All Details Contained Within!