Towards Uniform UV-spectra (UV2NIST)

Annemieke Kolkman, Erik Emke, Albert Brandt

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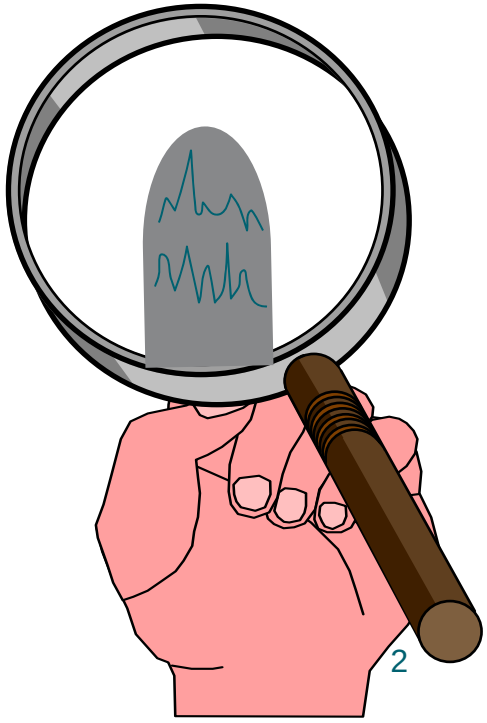
KWR Watercycle Research Institute

Outline

- HPLC-UV fingerprint
  - Harmonized method
  - *KRretI*
- UV spectrum library (UV2NIST)
  - Requirements
  - Development

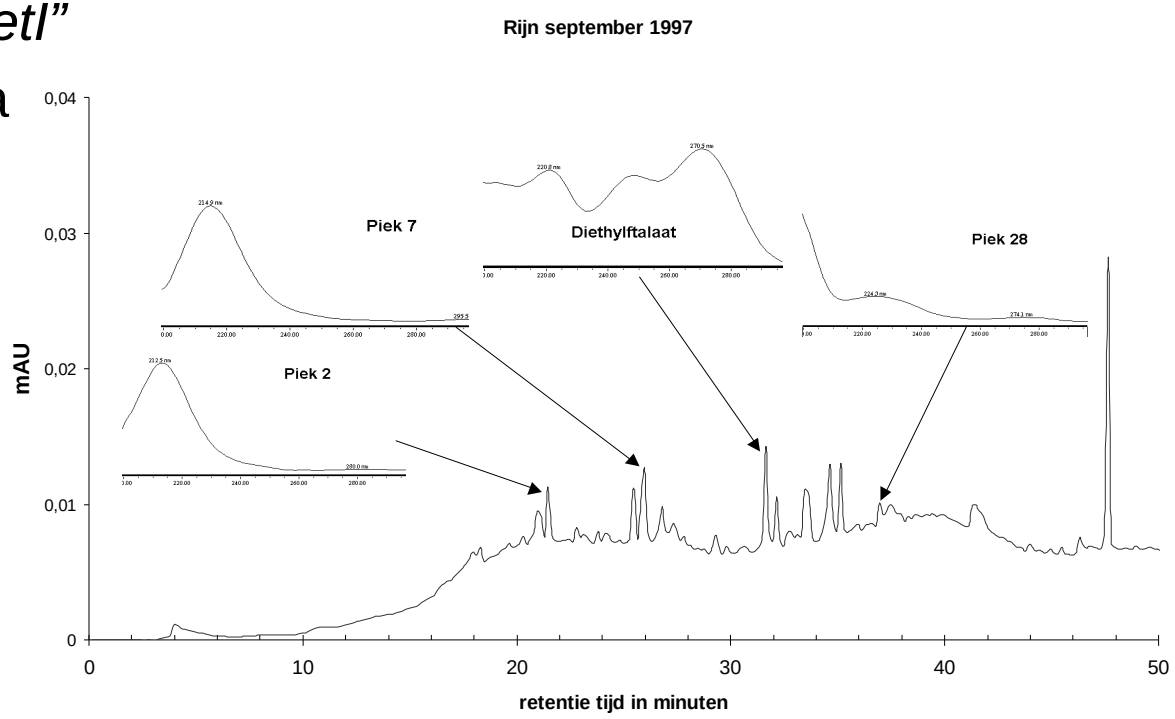


Significance of HPLC/UV fingerprinting in monitoring water quality

- Monitoring organic compounds in time and place (daily at monitoring station in Bimmen and Eijsden, Keizersveer)
- Comparison of raw water, water types and products
- Assessment of water treatment
- Early-warning system
  - Unknown and known compounds
- Direction of identification of unknown substances by other techniques (TOF/Orbitrap)

HPLC-UV fingerprint (1997)

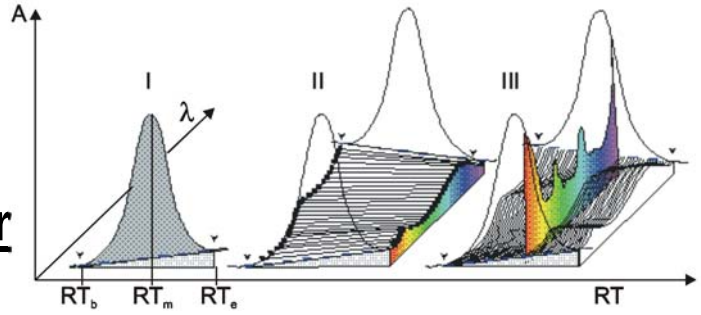
- Harmonized method
- Retention index “*KRetI*”
- Store the UV-spectra



Harmonizing HPLC-UV Fingerprint method (basic requirements)

-Use a **C18 column** 5µm of 250 * 4,6 mm or one with equivalent separation power

- The gradient between the internal standards is linear
- The pH is kept constant with **0,05% formic acid** in both eluents
- The DAD is set at a:
  - acquisition rate of **at least 1 Hz**
  - with a set resolution of **< 1,5 nm**
  - Spectral range **200-350 nm or higher**
- The **"trigger"** wavelength is at **215 nm**
- **Normalize** the concentration to response of chloorxuron at 215 nm



KRretI: Retention index

$$KRretI = Rty_a = Rt_{Fn} + \frac{\left(Rt_{Nb} - Rt_{Fn}\right)}{\left(Rt'_{Nb} - Rt'_{Fn}\right)}\left(Rtx_a - Rt'_{Fn}\right)$$

Rt_{Fn} and Rt_{Nb} = Determined standard retention time of fenuron (21.12 min) and neburon (43.6 min)

Rt'_{Nb} and Rt'_{Fn} = Measured retention time of the two internal standards

Rt_{ya} = Corrected retention time for the compound with retention time Rt_{Xa}

Rt_{Xa} = Actual measured retention time compound X

Results round robin tests 2011

	KRetI (min.)	Intralab (min.) precision (n=4)	Interlab (min.) precision (n=6)
Caffeïne	14,98	0.29	0.02
Fenuron	21,12	I.S	I.S
Carbamazepine	28,05	0,10	0.01
Methabenzthiazuron	30,76	0,04	0.01
TPPO	33,13	0,70	0.01
Chloorxuron	38,24	0,11	0.01
Linuron	38,98	0,51	0.01
Metolachlor	42,83	0,03	0.01
Neburon	43,60	I.S	I.S

Store the UV-spectra in a database

- Libraries are not easily transferred from one DAD to the other (history is lost)
- Exchangeability between DAD from different manufacturers is difficult
- Building a new library is time-consuming
- sharing data of known compounds with other participants
- New spectra from “unknown” compounds can be shared in a uniform way

Requirements UV-spectra database

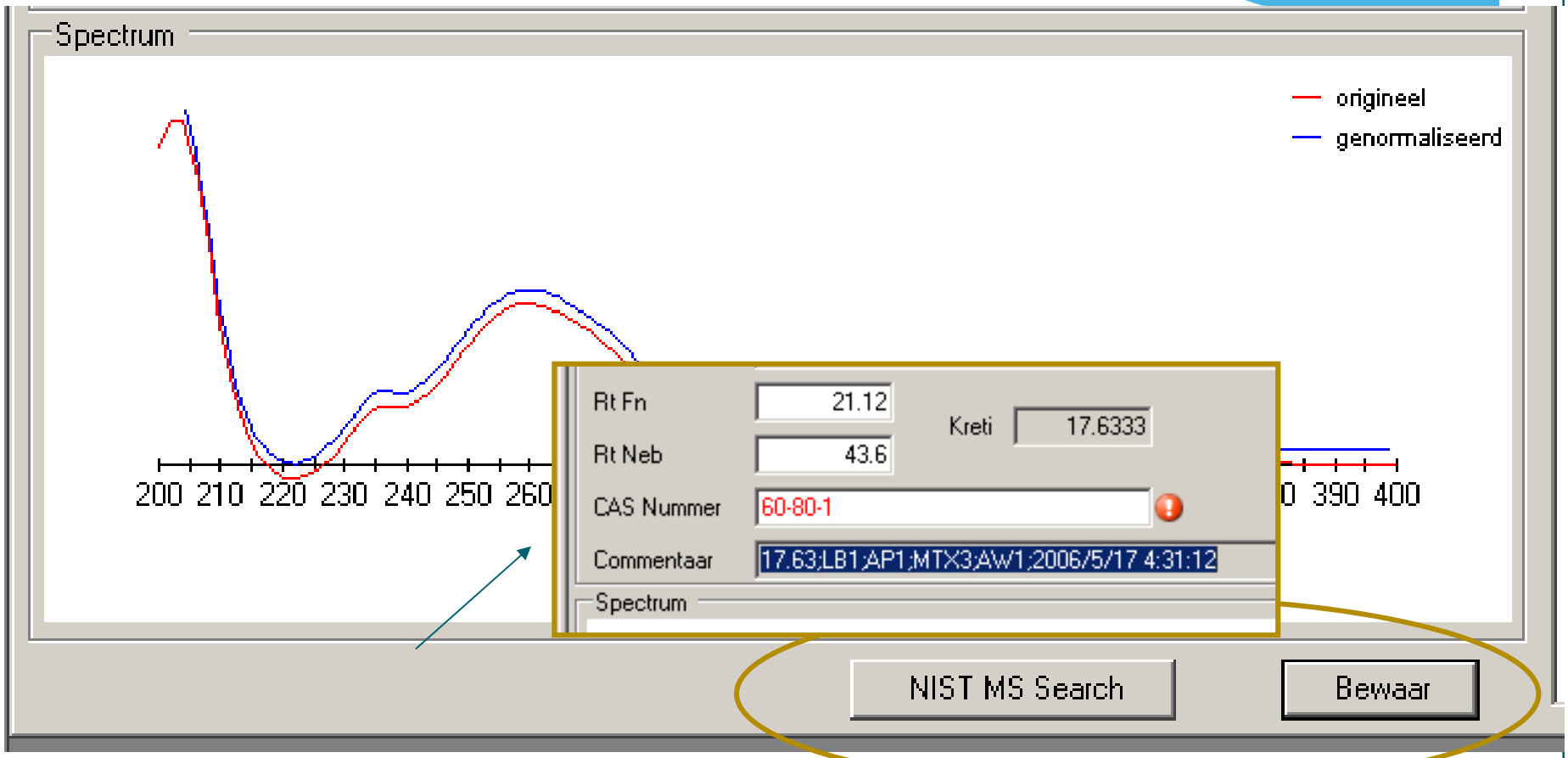
- UV-spectra should have a uniform format
  - Correction for wavelength deviations per DAD
  - Wavelength scaling, in steps of 1 nm
  - Normalization of absorption
  - Addition extra information parameters in a comment line
    - *KRetI*; Laboratory; Instrument; Matrix;…….

- Uniform text format to make it accessible

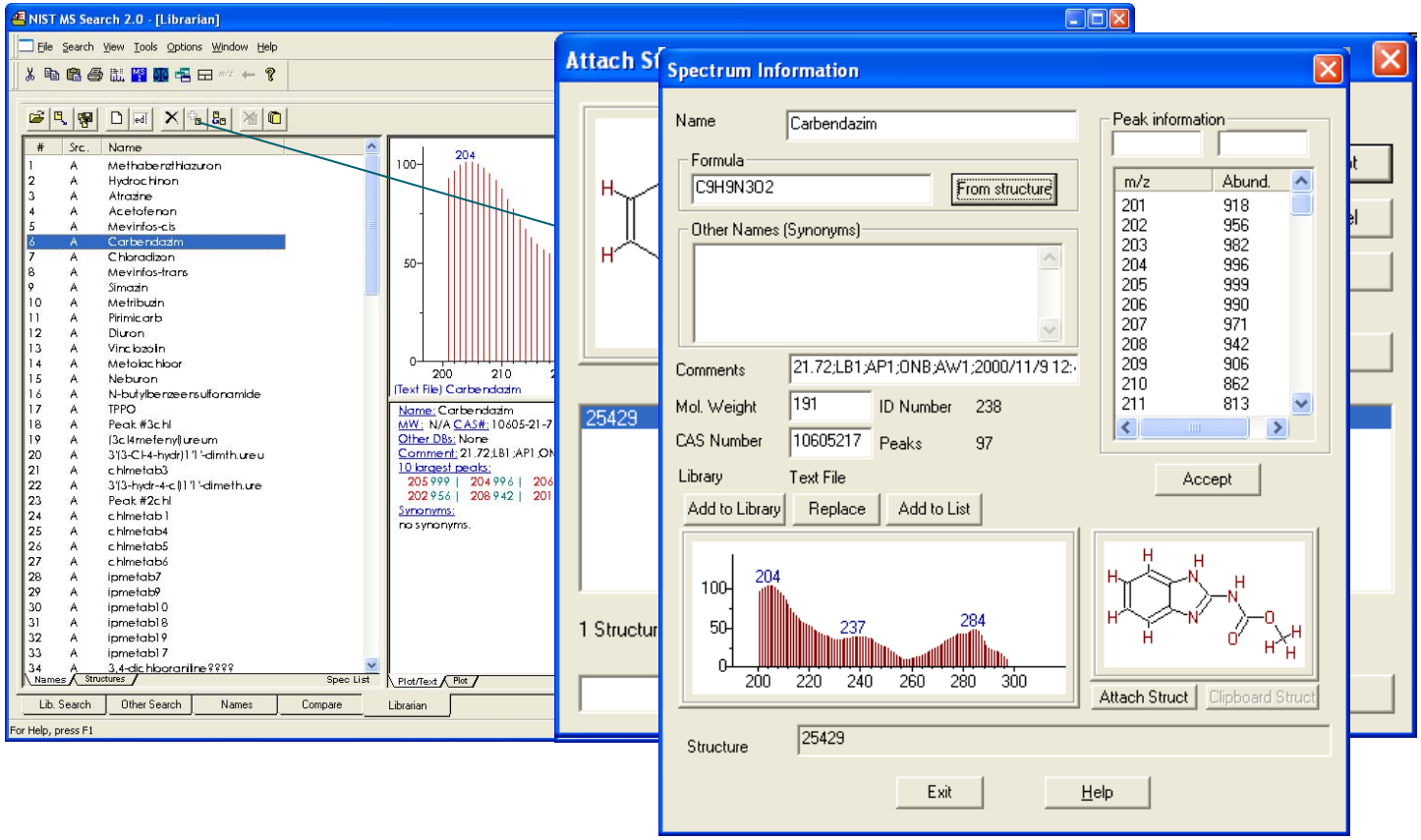
UV2NIST convertor (Step by step)

1. Read the output of DAD detector
   - Currently support for Agilent and Waters equipment
2. Correct the wavelength for instrument depended wavelength accuracy
3. Scale the wavelength to 1 nm
4. Normalize the absorbance
5. Calculate the *KRetI* retention index
6. Add a comment with the origins of the spectra
7. Save it in MSP format (compatible with NIST-MS search)

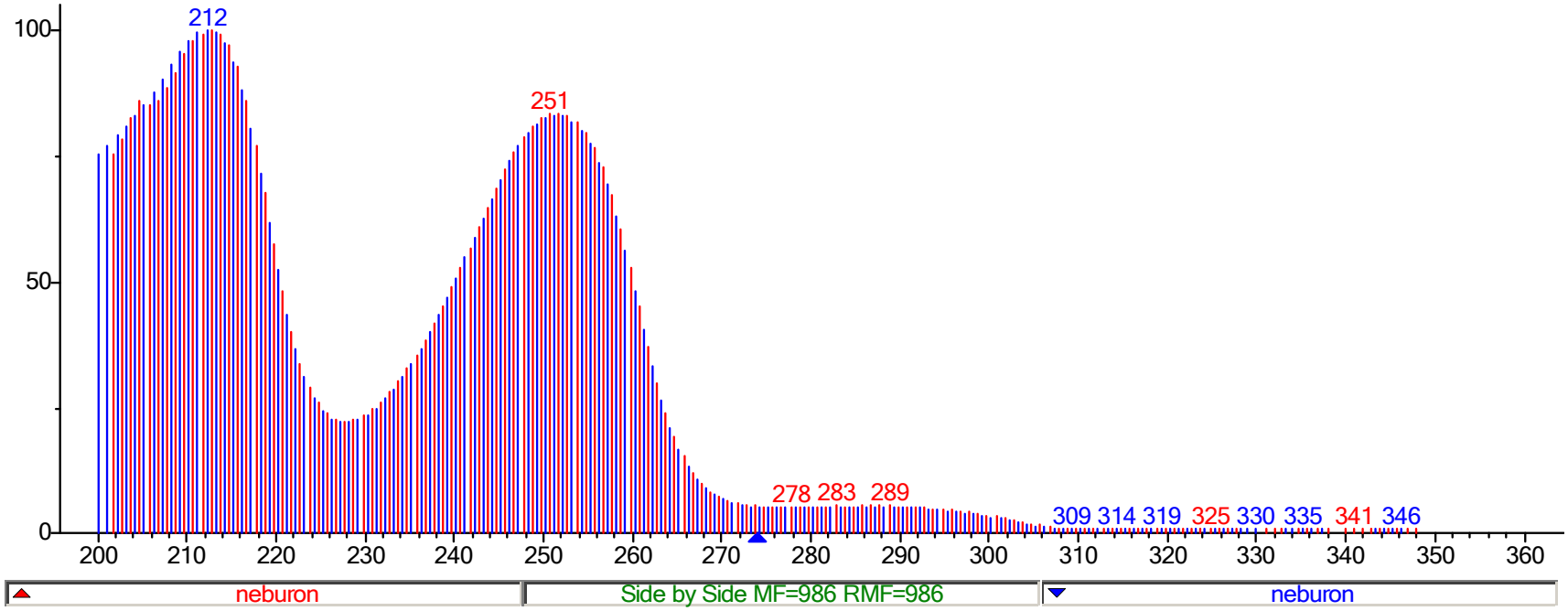
Import data and prepare for conversion



Import in NIST-MS search



Search for “unknown”



UV2MassBank?

Index Type :
Contributor: **UV2NIST Spectra from KWR**

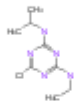
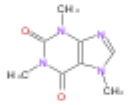
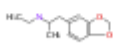
Edit / Resubmit Query

Results : 3 Hit. (1 - 3 Displayed)

Open All Tree | Multiple Display | Spectrum Search

First Prev **1** Next Last (Total **1** Page)

▼ Results End

Name	Formula / Structure	ExactMass	ID
Atrazine 1 spectrum UV: N/A	**C8H14ClN5** 	**215.09380**	UV000079
Caffeine 1 spectrum UV: N/A	**C8H10N4O2** 	**194.08040**	UV000054
MDEA 1 spectrum UV: N/A	**C12H17NO2** 	**207.12590**	UV000001

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▲ Results Top

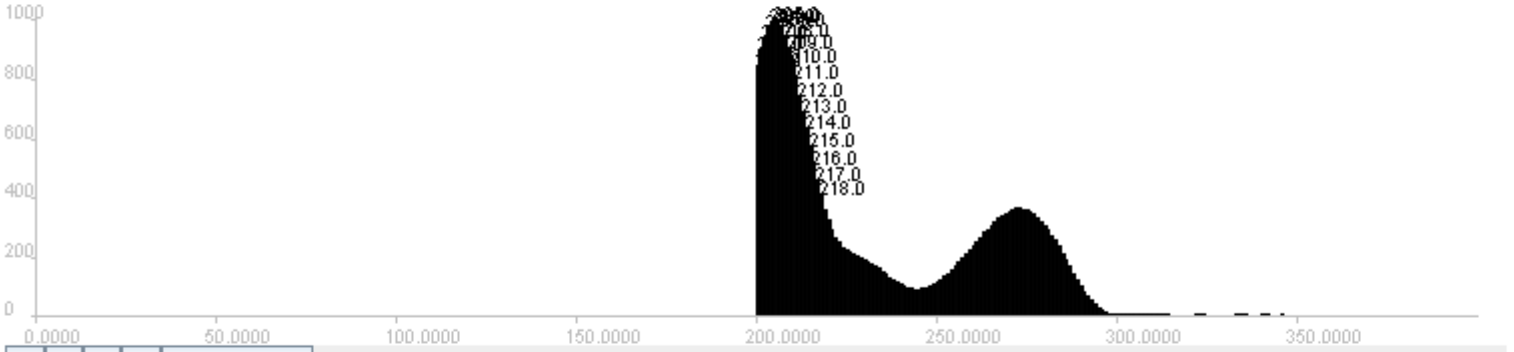
UV2MassBank

MassBank Record: UV000054

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

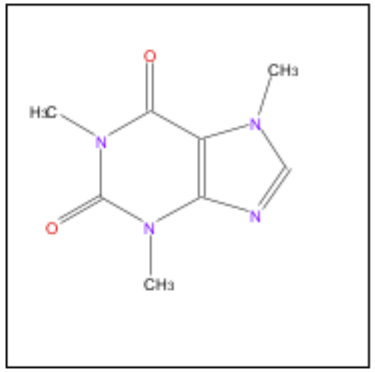
Caffeine; UV; N/A;

Mass Spectrum



<< < > >> show all m/z

Chemical Structure

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ACCESSION: UV000054
RECORD_TITLE: Caffeine; UV; N/A;
DATE: 2009.08.31 (Created 2012.11.16)
AUTHORS: E. Emke: KWR, NL (modified by E. Schymanski: Eawag, CH)
LICENSE: CC BY-SA
COPYRIGHT: KWR
COMMENT: 14.82;LB1;AP1;ONB;AW1;2009/8/31 3:32:08
COMMENT: UV2NIST2MassBank
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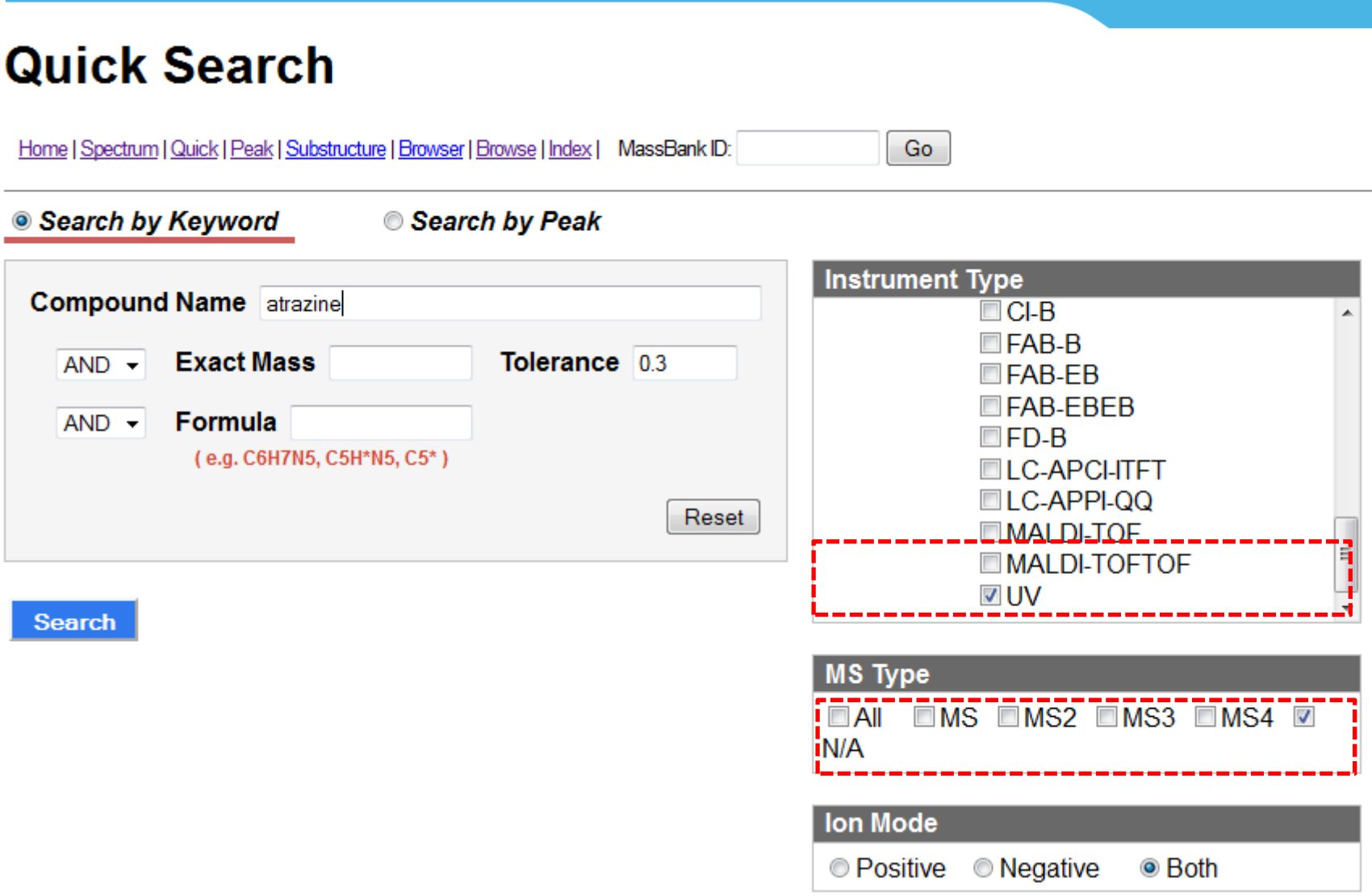
Searching UV2MassBank



Final Conclusions

- A uniform database of UV spectra has been developed
  - Instrument independent (Wasters, Agilent, Thermo,…)
  - Data sharing is possible
  - Database contains 103 compounds (DOA's, pesticides, pharmaceutical, industrial compounds)
- The database is stored in text format
- Data can be searched with free software (e.g. NIST-MS-search demo version)
- Ready to export to other database tools (e.g. Massbank)

Acknowlegments