Targeted & Untargeted analysis of volatiles & T&O

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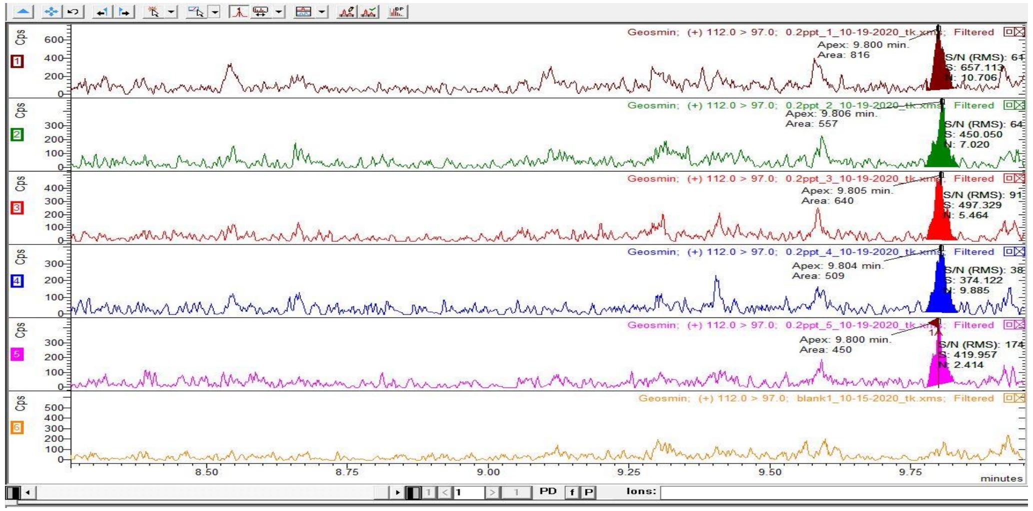


Training School "AOPs for water T&O"
5-9 Sep 2022, Athens, Greece

Targeted vs untargeted analysis

- **Targeted** analysis is focused on determination of certain compounds, e.g. geosmin and MIB.
- Targeted analysis is usually carried out in “selected ion monitoring – SIM” mode, since we know the identity of compounds. SIM achieves better sensitivity (increased signal-to-noise, S/N).
- **Untargeted** analysis (NTA) aims to find what is in the sample without focusing on specific compounds.
- Untargeted analysis is used for diagnostic and forensic-type investigations.
- Untargeted analysis uses the “full-scan” mode, because we want to obtain mass spectra to compare them with spectra from the NIST library.
- In untargeted analysis we must be aware of the limitations of our extraction technique and chromatographic method (not all compounds are extracted, retained-eluted from the column and detected). We must use “wide-range” methods or multiple methods.

Confirmation of identity (targeted analysis)

Targeted analysis in SIM mode:

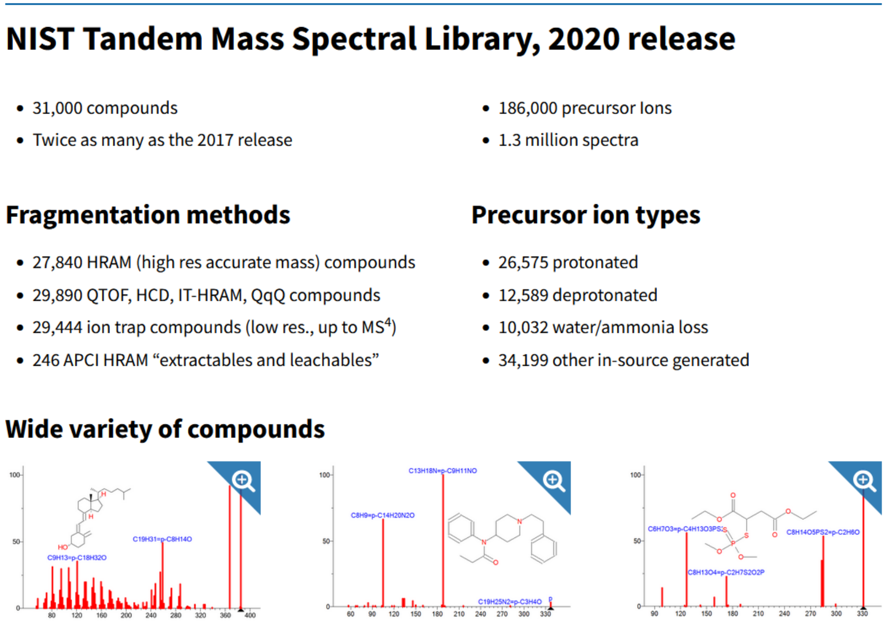
- The Retention Time (RT) of the peak should be within ± 0.1min of the RT of a standard solution of the targeted compound.
- The ion ratios (q/Q) of at least two qualifier ions (q) to the base peak (Q) should be within 30% of the respective rations in a standard solution of the targeted compound.
- Such criteria can be found e.g. in EC-SANTE 11945/2015 “Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed”

Confirmation of identity (untargeted analysis)

- Search in databases (e.g. NIST) and see if mass spectrum matches with a compound included in NIST. This is a “suspect” compound.
- Carry out mass spectral deconvolution (e.g. with AMDIS) to achieve a better evaluation of mass spectral matching of suspect compounds according to defined criteria.
- Estimate the “Retention Index-RI” of the suspect compound and see if it matches the RI found in databases (e.g. NIST web book).
- If all of the above are achieved, still the compound is “tentatively confirmed”
- A compound is considered **“confirmed”** only if you have a reference standard in the lab, you inject it in GC-MS and you get a match of both the Retention Times (RT) and the mass spectra according to defined criteria.

Is a standard commercially available?
Can someone synthesize it ?

Databases with GC and MS data of T&O



NIST Tandem Mass Spectral Library, 2020 release

- 31,000 compounds
- Twice as many as the 2017 release
- 186,000 precursor Ions
- 1.3 million spectra

Fragmentation methods

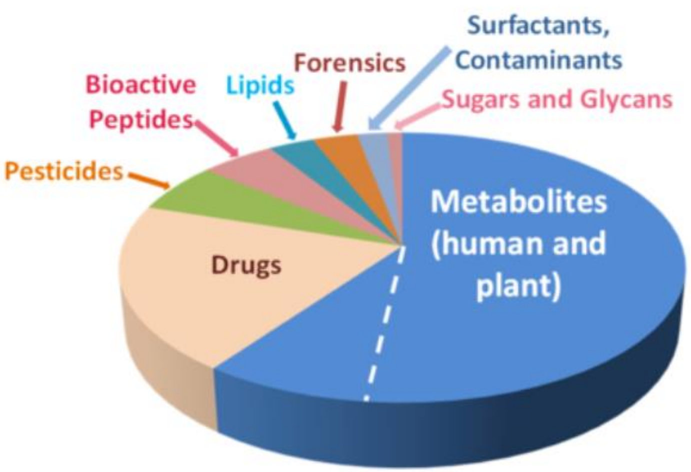
- 27,840 HRAM (high res accurate mass) compounds
- 29,890 QTOF, HCD, IT-HRAM, QqQ compounds
- 29,444 ion trap compounds (low res., up to MS^4)
- 246 APCI HRAM “extractables and leachables”

Precursor ion types

- 26,575 protonated
- 12,589 deprotonated
- 10,032 water/ammonia loss
- 34,199 other in-source generated

Wide variety of compounds

Types of compounds and spectra



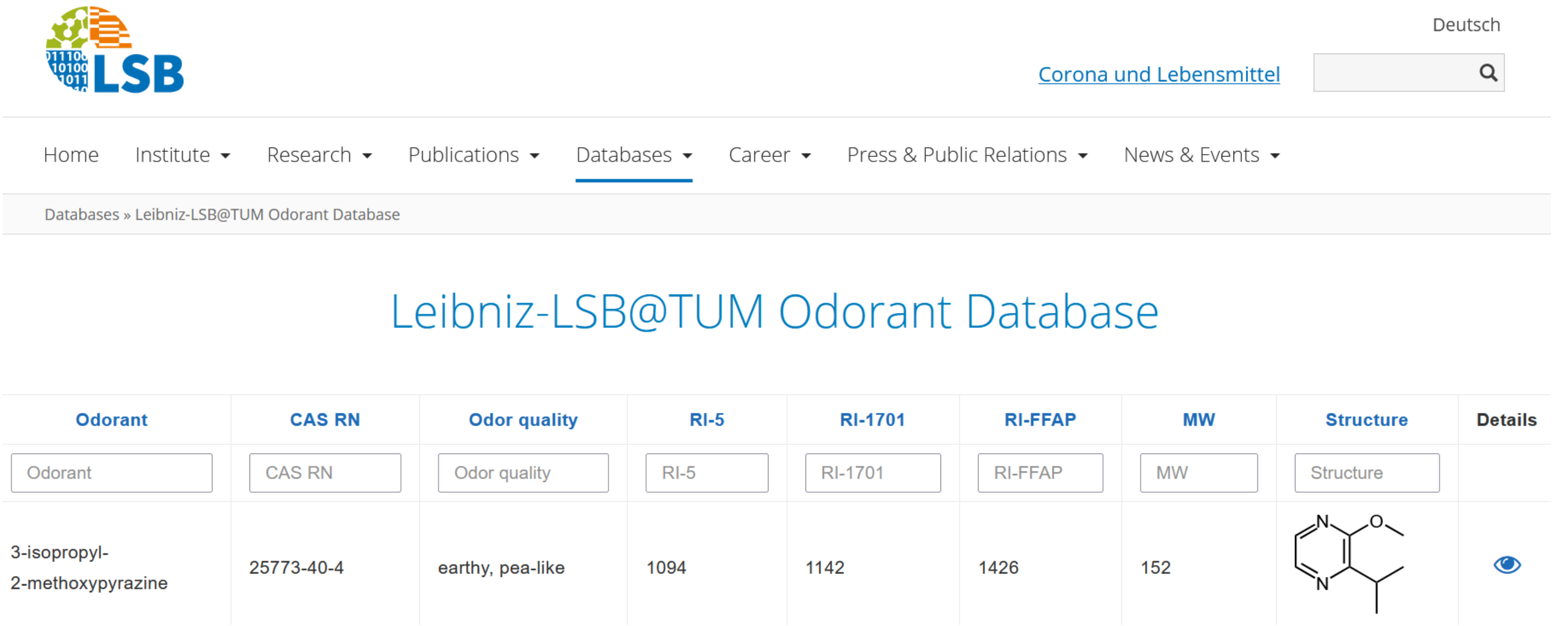
75% (+), 25% (-)

32% MS^2 of in-source

8% of MS^n

6,000 human metabolites

https://chemdata.nist.gov



Deutsch

Corona und Lebensmittel

Databases » Leibniz-LSB@TUM Odorant Database

Leibniz-LSB@TUM Odorant Database

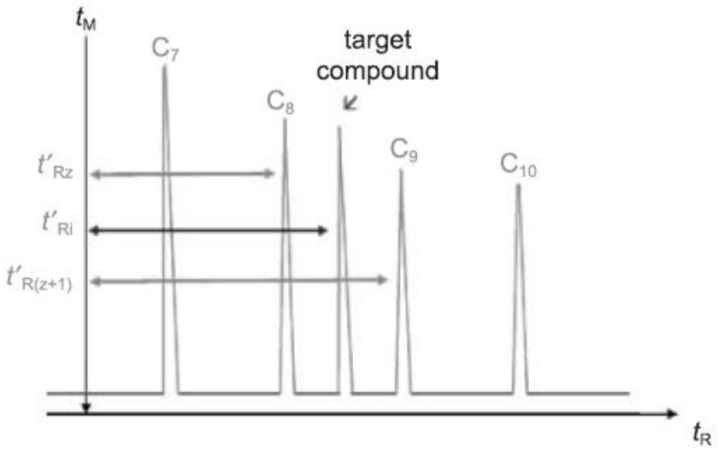
Odorant	CAS RN	Odor quality	RI-5	RI-1701	RI-FFAP	MW	Structure	Details
Odorant	CAS RN	Odor quality	RI-5	RI-1701	RI-FFAP	MW	Structure	
3-isopropyl-2-methoxypyrazine	25773-40-4	earthy, pea-like	1094	1142	1426	152		👁

Kreissl J, Mall V, Steinhaus P, Steinhaus M. Leibniz-LSB@TUM Odorant Database, Version 1.2. Leibniz Institute for Food Systems Biology at the Technical University of Munich: Freising, Germany, 2022 https://www.leibniz-lsb.de/en/databases/leibniz-lsbtum-odorant-database

Retention Time Indices

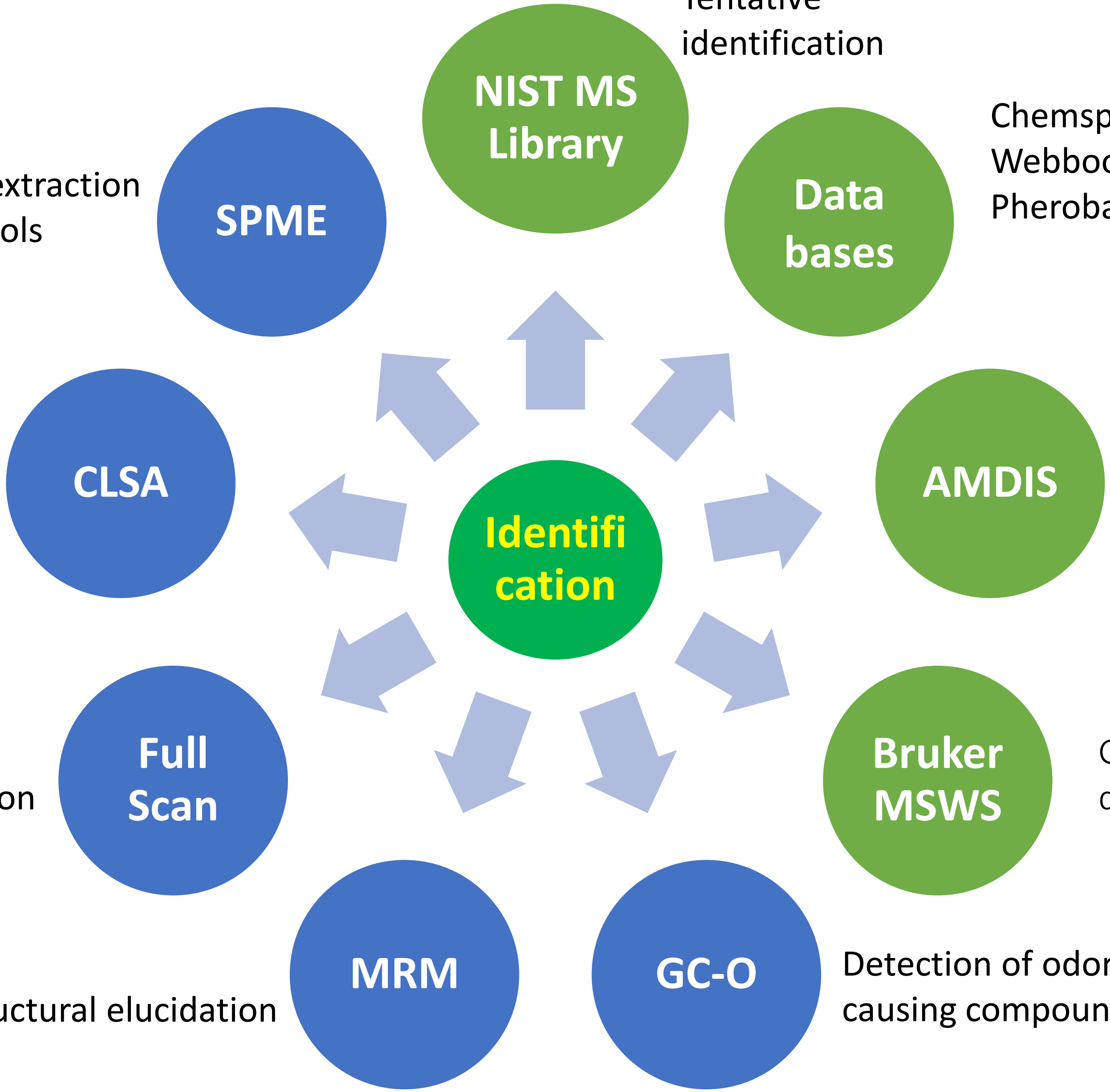
- Retention Times (RT) of compounds depend on chromatographic conditions (e.g. column characteristics, temperature program, flow rate).
- Retention Time Indices (RTI) are used to standardize retention time data when using different chromatographic methods, for identification purposes.
- Developed by Kovats (1958) for isothermal conditions and later by van den Dool and Kratz (1963) for temperature-programming conditions (Linear Retention Index, LRI).
- The calculation of carried out LRI is based on the comparison of the retention time of the compound of interest to retention times of a series of n-alkanes (e.g. C7-C30) that are analyzed according to the exact conditions with the compounds of interest.

$$I^T = 100[z + (t^T_{Ri} - t^T_{Rz}/t^T_{R(z+1)} - t^T_{Rz})]$$



- The LRI is characteristic of the compound for columns of the same phase.
- LRIs of a vary large number of compounds are reported in databases (e.g. NIST web book).
- AMDIS now includes LRI matching.
- LRIs “match” when their difference is less than ± 5 or ± 10 depending on column phase.

Tools for non-targeted analysis of T&O



Identifi
cation

NIST MS Library
Tentative
identification

Data bases
Chemspider,NIST
Webbook, PubChem,
Pherobase etc

AMDIS
Spectral
deconvolution,
Retention
Indices,
confirmation.

Bruker MSWS
GC and MS
data analysis

GC-O
Detection of odor-
causing compounds

MRM
Structural elucidation

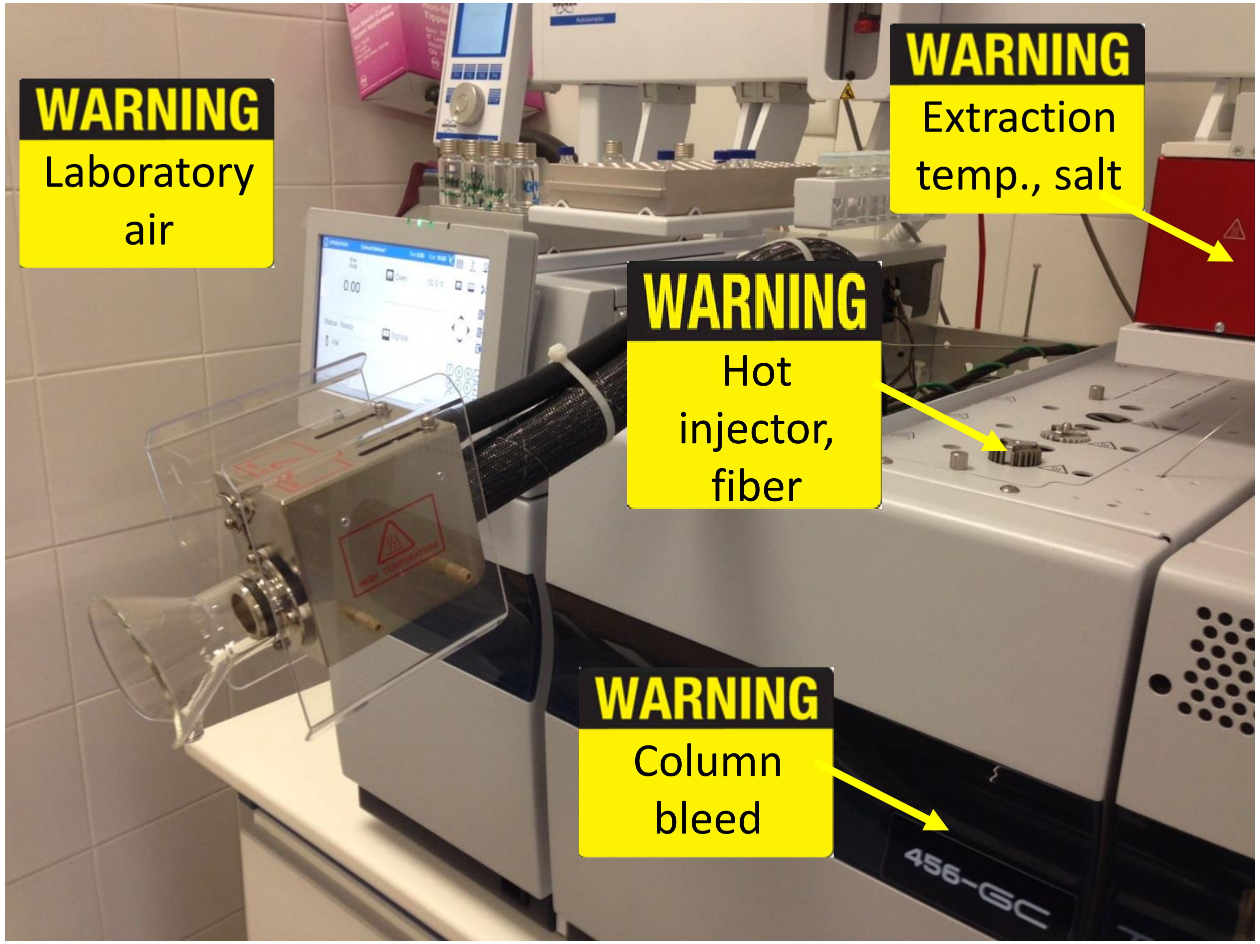
Full Scan
Tentative
identification

CLSA
Extremely low
concentrations

SPME
Basic extraction
protocols

Artifacts

Something observed in a scientific investigation or experiment that is not naturally present but occurs as a result of the preparative or investigative procedure” Oxford dictionary



WARNING
Laboratory air

WARNING
Extraction temp., salt

WARNING
Hot injector, fiber

WARNING
Column bleed

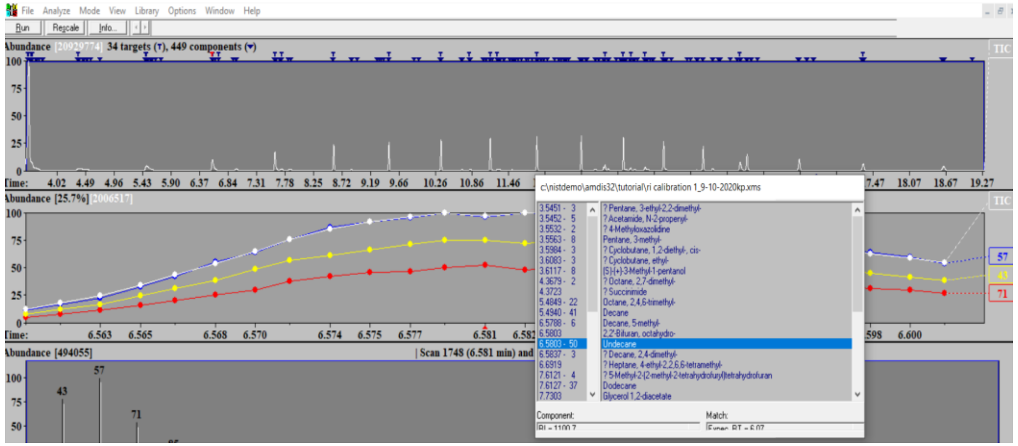
WARNING
Culture media, plastic

Analyze all culture media, ingredients and blanks. Keep temperatures as low as possible.

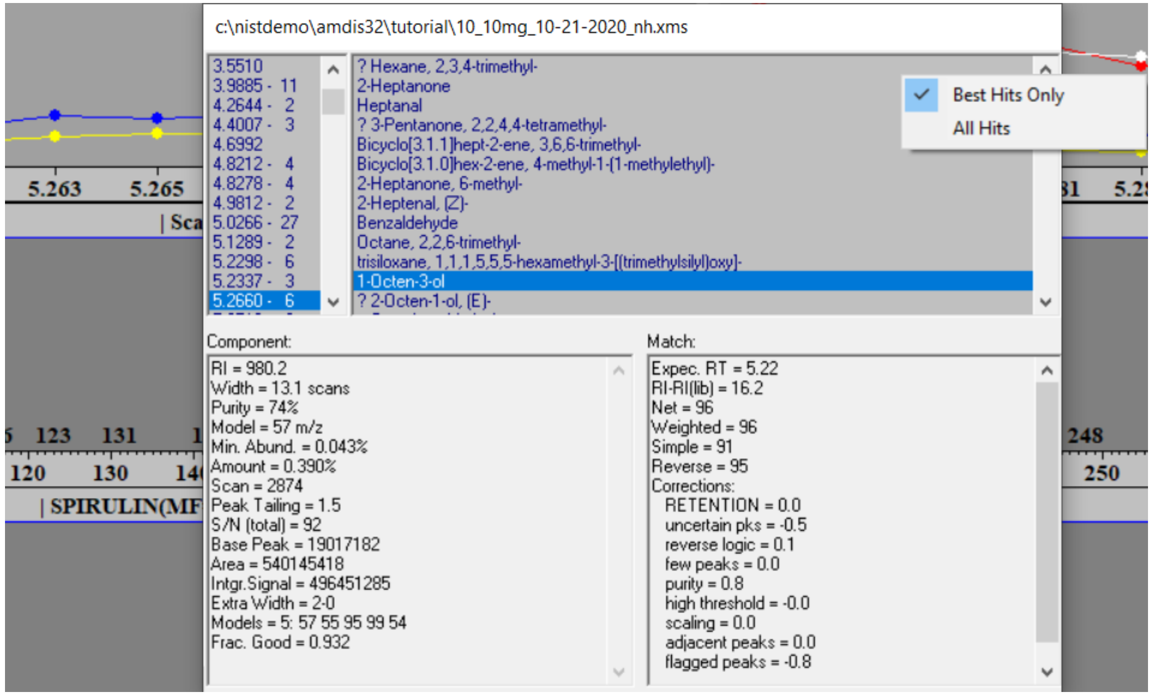
GC-EI-MS and data processing (NTA)

Paraskevopoulou et al. 2022, in prep.

- Non-polar (5 MS) and semi-polar columns (Rxi 624 Sil MS).
- Retention Index calibration with n-alkanes (C7-C30).
- Mass spectral deconvolution with AMDIS (NIST).
- Annotation by combined RI-MS matching scores and 2nd column.
- Unambiguous identification with CRM, upon availability.
- Sub-ppb levels (10ml sample, HS-SPME)



RI calculation (Van den Dool & Kratz, 1963, J Chrom A)


c:\nistdemo\amdis32\tutorial\10_10mg_10-21-2020_nh.xms

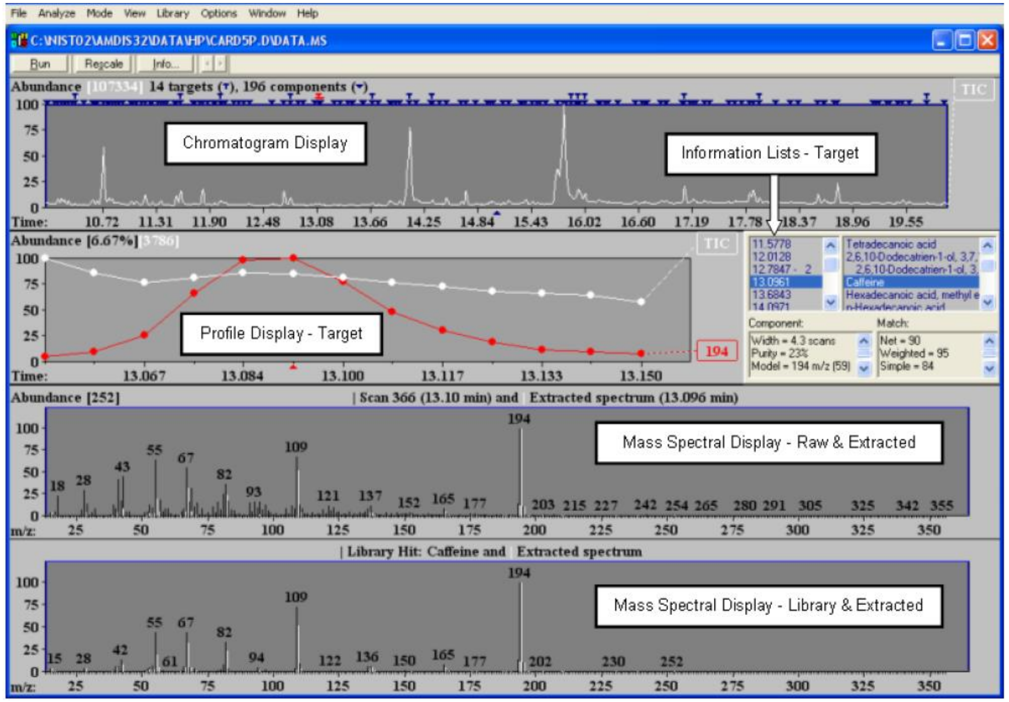
3.5510
3.9885 - 11
4.2644 - 2
4.4007 - 3
4.6992
4.8212 - 4
4.8278 - 4
4.9812 - 2
5.0266 - 27
5.1289 - 2
5.2298 - 6
5.2337 - 3
5.2660 - 6

? Hexane, 2,3,4-trimethyl-
2-Heptanone
Heptanal
? 3-Pentanone, 2,2,4,4-tetramethyl-
Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-
2-Heptanone, 6-methyl-
2-Heptenal, (Z)-
Benzaldehyde
Octane, 2,2,6-trimethyl-
trisiloxane, 1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy]-
1-Octen-3-ol
? 2-Octen-1-ol, (E)-

Component:
RI = 980.2
Width = 13.1 scans
Purity = 74%
Model = 57 m/z
Min. Abund. = 0.043%
Amount = 0.390%
Scan = 2874
Peak Tailing = 1.5
S/N (total) = 92
Base Peak = 19017182
Area = 540145418
Intgr.Signal = 496451285
Extra Width = 2-0
Models = 5: 57 55 95 99 54
Frac. Good = 0.932

Match:
Expec. RT = 5.22
RI-RI(lib) = 16.2
Net = 96
Weighted = 96
Simple = 91
Reverse = 95
Corrections:
RETENTION = 0.0
uncertain pks = -0.5
reverse logic = 0.1
few peaks = 0.0
purity = 0.8
high threshold = -0.0
scaling = 0.0
adjacent peaks = 0.0
flagged peaks = -0.8

Combined RI-MS matching score (Mallard & Reed, 2017, NIST)



11.5778
12.0128
12.7847 - 2
13.0961
13.6843

Tetradecanoic acid
2,6,10-Dodecatrien-1-ol, 3,7
2,6,10-Dodecatrien-1-ol, 3
Caffeine
Hexadecanoic acid, methyl e
n-Hexadecanoic acid

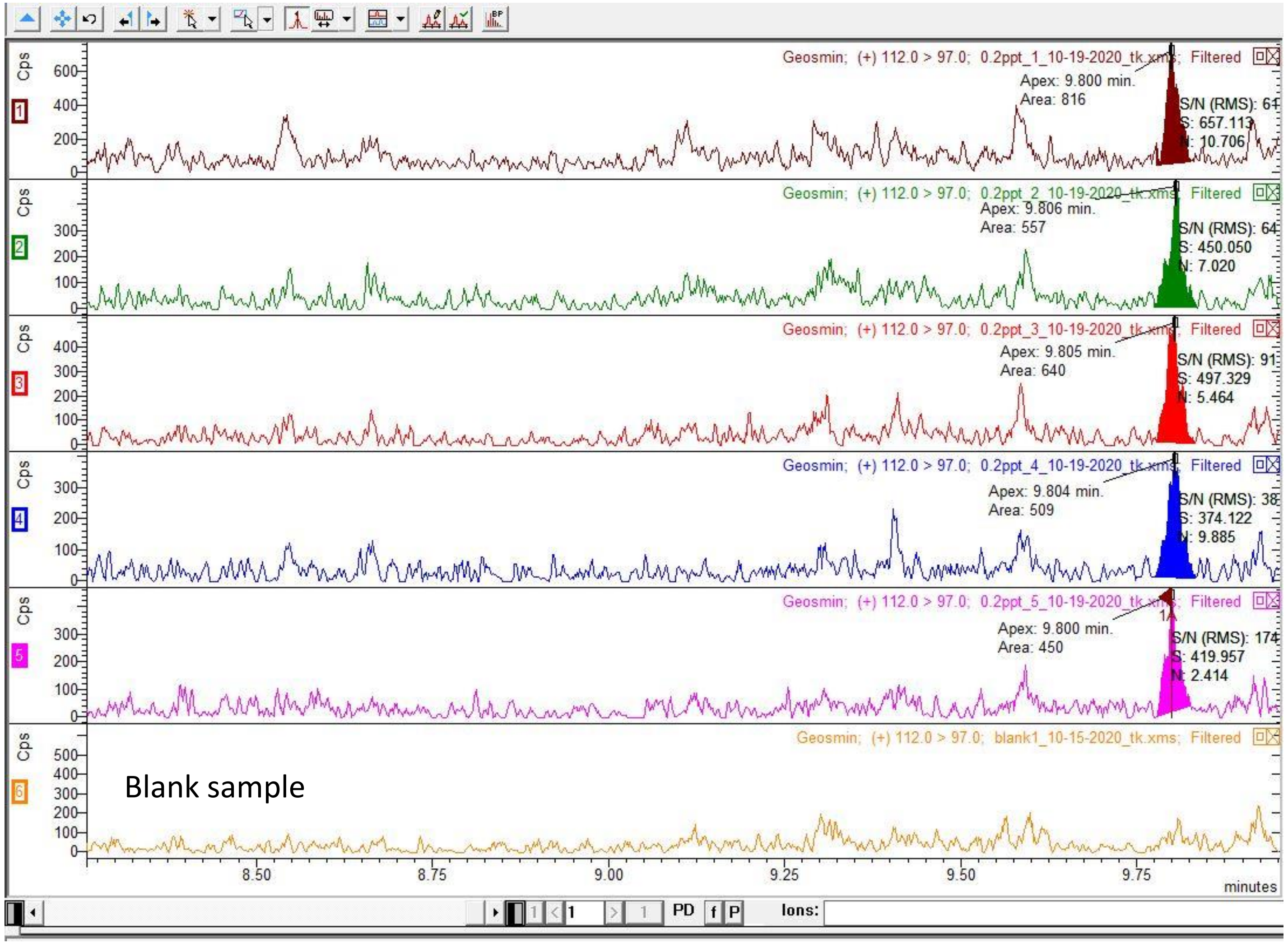
Component:
Width = 4.3 scans
Purity = 23%
Model = 194 m/z (59)

Match:
Net = 90
Weighted = 95
Simple = 84

Mass Spectral deconvolution (Stein 1999, J Am Soc Mass Spec)

GC-EI-MS targeted analysis

- Sensitivity (ng/L or lower levels).
- GC-MS and GC-MS/MS.
- Optimized HS-SPME conditions.
- Quantitation with “bracketed calibrations” by spiking samples in the same sample matrix.
- Routine monitoring.
- Automated, fast analysis.



Blank sample

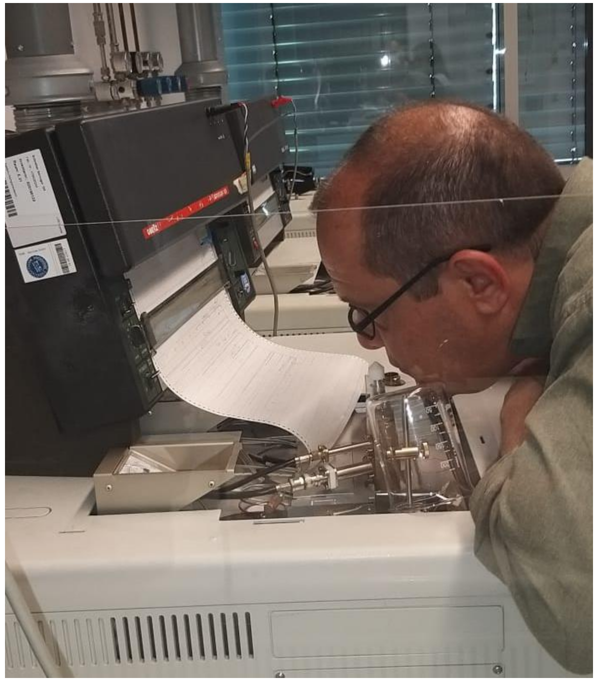
Example of geosmin detection at 0.2 ng/L by optimized HS-SPME-GC-EI-MS/MS (PAL LSI, Bruker TQ). Accredited method (ISO 17025).

Validation, Quality Control, Proficiency Testing

- Validation carried out using a defined workflow (protocol), databases, tools and parameters.
- Confidence levels of annotation/identification are defined and explicitly mentioned in results.
- Quality control using CRMs and in-house prepared T&O mixtures.
- Proficiency testing schemes for targeted analysis at environmentally relevant concentrations.
- Proficiency testing schemes for “emergency T&O episodes” involving T&O.
- Our intension is to include untargeted screening of water T&O in the scope of accreditation (ISO 17025).

Ongoing and future work

- Include **GC-Olfactometry** in the workflow (odor-active volatiles).
- Accreditation of water T&O workflow (NTA).
- Development of a WaterTOP open-access database of water T&O (sensory quality, chromatography, mass spectral information).
- Translation of TOW in European languages (open-access, WaterTOP).



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LABORATORY OF ORGANIC MICROPOLLUTANTS

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