THE SPECTRUM FACTORY

A new workflow for the creation of libraries of unknown mass spectra from known precursor compounds

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Overview

- Chosen precursor molecules are subjected to a variety of physicochemical stimuli in order to induce structural changes (pseudo-metabolism)
- Reaction mixtures analyzed by GC/MS
- Libraries of precursor-tagged unknown spectra ("unknowns", known precursor structure) are created using AMDIS and can be compared with other libraries worldwide.

Introduction

The large number of unidentified metabolites encountered during the analysis of biological materials is a significant problem in metabolite profiling. Further to previous initiatives providing curated libraries of recurring unknown spectra (RUS) and addressing their quality using GC stationary phases having slightly different polarities [1,2], the current initiative aims to create libraries generated from known precursor molecules following a variety of physicochemical stimuli. Thus, the structures of all entries in the libraries must be related to the precursor molecules. Matching of such spectra against other unknown spectra will assist their structure elucidation.

The current project is limited to GC/MS but the principle applies to related techniques such as LC/MS.

Materials and Methods

Materials and sample preparation:

Chosen precursors such as glucosamine and a combination with simple mixtures of sugars and amino acids (Maillard-type) are subjected to physicochemical stimuli (acid, base, heat, light etc) and analysed by GC/MS following chemical derivatization as methoxyamine-TMS.

GC:

Agilent 6890 systems 5% arylene-methylsiloxane columns.
Temperature program: 50°(1 min)-10°/min-320 °C (10 min)).
Split (1:10, 1:20 and splitless injections.
n-Alkane mixtures (C8-C40) analysed under identical conditions for automated RI calculation by AMDIS [3].

MS:

Agilent 5973 mass spectrometers (70 eV), m/z 33 -800.

Data processing: data files were subjected to NIST database searching and AMDIS analysis for the creation of user libraries.

Data processing and library creation:

Agilent Chemstation and Masshunter.

Data files analysed using AMDIS [3] and matched against the current NIST database [4].

Spectrum Factory Workflow

Precursor **X** (for example, glucosamine, ribose, mixtures)

"reactions" enzymes, heat, acid, base, oxidizing agents, microwave, light, ultrasound, pH, catalysts, irradiation, combinational reactants (e.g. Maillard), electrochemical reactions/simulated metabolism….

optional fractionation steps (SPE, liquid-liquid…) incl. sequential

GC/MS, LC/MS

LIB_X Spectra related to compound X

compare library spectra

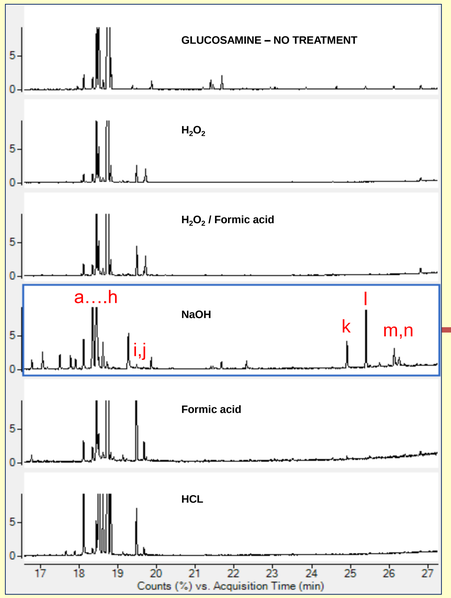
NIST, etc, & LIB_R (Library of recurring unknowns [6] and web-based database such as [5])

If no match, add X-related unknown spectrum to LIB_R

If match in LIB_R, info gained about nature of unknown, i.e. related to X

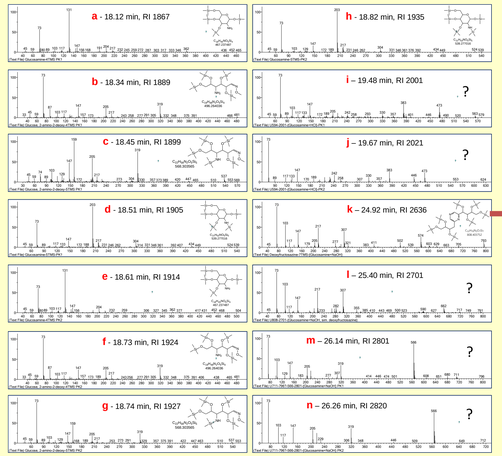
Example - Glucosamine

Partial chromatograms (TIC, split 10 injection) obtained from glucosamine subjected to a variety of stimuli: aqueous solutions left for a week at ambient temperature.

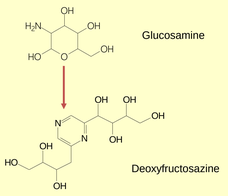


Glucosamine Library

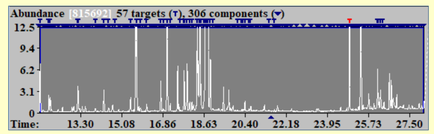
Major component spectra were compared with the NIST and other databases and placed into a glucosamine-related library:



Deoxyfructosazine tentatively identified by matching in NIST17 (not present in NIST14). Originally named F-26255;{D1-2726-D5-2645-H5-2717} (Food RUS library, verified using 3 stationary phases [2].
Found in aged ginger [6].
Trace amount also found in glucosamine standard.

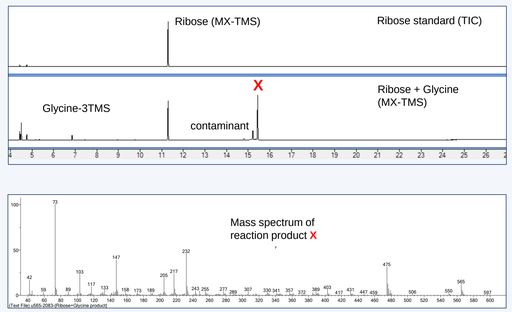


Splitless injection reveals many more candidates (NaOH treated samples after 2 weeks/ ambient): Indicated matches are against the library of Food RUS [6].



The mass spectrum of unknown n, above, matched unknown F-28880;{D1-2951-D5-2817-H5-2928} found in potato [6].

Example – Ribose+Glycine

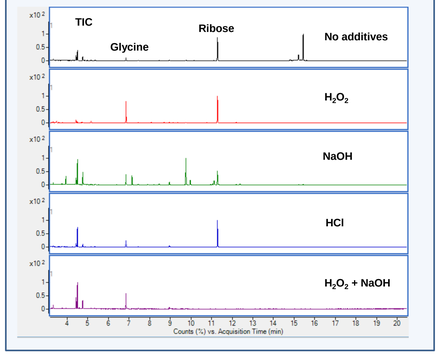


Analysis of reaction mixture

Ribose + Glycine (2 weeks, aqueous, ambient temp., natural light

Mass spectrum of reaction product **X** added to precursor-tagged library

Chromatograms – Ribose+Glycine



Conclusions

- ❑ The method is suitable for automation (combinatorial methods).
- ❑ Should support the automated RUS library creation procedures under development [1,2]
- ❑ Data suitable for publication in a web-based query service to assist the elucidation of unknowns [5].

References

[1] Mallard, WG, et al., **Creation of libraries of recurring mass spectra from large data sets assisted by a dual-column workflow,** Anal. Chem. 86, 10231 (2014)

[2] Halket, JM, et al., **Creation of libraries of recurring mass spectra: triple column workflow for improved characterisation and mapping of unknowns across multiple samples**, Proc. 64th ASMS, San Antonio, TX, 2016

[3] Stein, SE, **An integrated method for spectrum abstraction and compound identification from gas chromatography/mass spectrometry data**, JASMS 10, 770 (1999).

[4] NIST/EPA/NIH Mass Spectral Library with Search Program: http://www.nist.gov/srd/nist1a.cfm

[5] Askenazi, M, Stein, SE, **The Fragmentarium: A Universal Query Service Enabling Partial Matching of Unidentified Spectra across the Full Gamut NIST MS Spectral Libraries,** Proc. 64th ASMS, San Antonio, TX, 2016

[6] RUS libraries in progress for food components, essential oils and paediatric urine are available: **https://tinyurl.com/ycfh8fhb**