Evaluation of GC/MS Data in Forensic Analyses by Chemometric Methods

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Systematic Toxicological Analysis

A main objective of forensic toxicological analysis is the identification of general unknown poisons in biological material. A reproducible and automatic extraction procedure for the analysis of drugs in post-mortem samples of blood and tissue has been developed [1]. Extracts are analyzed by GC/MS producing more than 2000 spectra per sample.

The resulting total ion current (TIC) chromatograms contain many substances from the biological material (liver sample). The distinction between negative and positive cases and identification of unknown poisons is very time consuming using routine software.

A successful identification depends on the availability of the corresponding mass spectra in the applied libraries. In this work the MS library for toxicologically interesting substances by Pfleger, Maurer and Weber (PMW-TOX) [2] is used.
Difficulties arise in cases, where comprehensive mass spectral data of the unknown compound are not available. A software was developed to filter the total number of acquired mass spectra in general unknown cases and to simplify the TIC chromatogram by an automated, computer-assisted subtraction procedure.

This procedure allows the forensic toxicologist to focus on mass spectra of uncommon compounds, and therefore improves the chance for the identification of a "general unknown" in complex biological materials.

The Software FORGE (**For**ensic **G**C/MS-Data **E**xploration) was realized in MATLAB to test new algorithms. Development of a Software in Visual C++ is in progress.

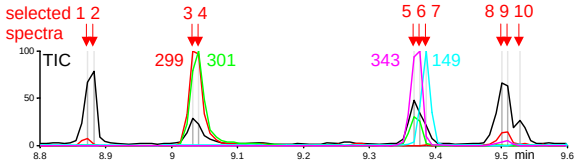
Stimpfl T., Jurenitsch J., Vycudilik W.: *J. Anal. Toxicol.* **25,** 125 (2001)
Pfleger K., Maurer H.H., Weber A.W.: Mass spectral and GC data of drugs, poisons, pesticides, pollutants and their metabolites; 2nd ed., VCH Publishers, Weinheim, Germany (1992)

Software FORGE
Forensic **G**C/MS-Data **E**xploration

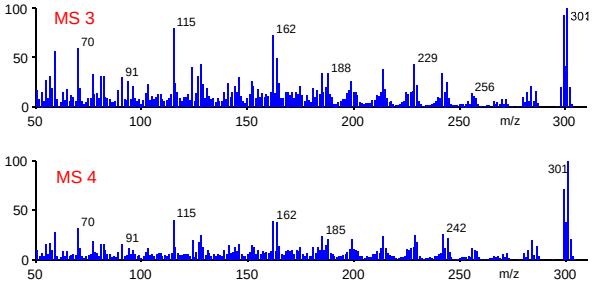
- **Baseline Subtraction**
  Performed separately for each mass chromatogram.
- **Selection of Characteristic Spectra**
  Automatic selection of approx. 50 mass spectra from GC/MS data.
- **Retention Index Calibration**
  Linear or quadratic regression model using alkanes and relevant test compounds
  $x = a.t^2 + b.t + c$ x = retention index t = retention time
- **Tools for Building Negative Libraries**
  Characteristic spectra of negative reference samples (no intoxication) are collected to eliminat unsuspicious spectra.
- **Subtraction of Spectra present in Negative Library**
  Spectra of matrix components are filtered to clean the TIC and to facilitate interpretation.
- **Forward Search**
  Identification of compounds with spectra selected.
- **Target Search**
  Search for maximal occurrence of selected target spectra in chromatogram with optionally considering retention indices.
- **Calculation of Spectral Features**
  Various features can be generated from mass spectral data to obtain suitable variables for analysis of spectra-structure relationships.
- **Exploratory Data Analysis of Spectra or Features**
  Spectral features or MS peak intensities are used for exploratory data analysis by PCA.

Selection of Characteristic Spectra

GC/MS analyses of a blood sample spiked with standard mixture of toxins. Selected range of TIC after baseline subtraction:



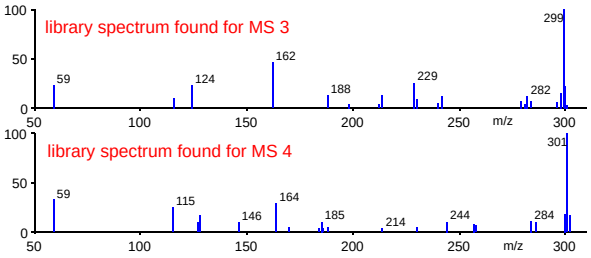
Selection Method: Selection of characteristic spectra by searching for local maxima in all mass chromatograms.
Two positive slopes followed by two negative slopes with an intensity above a threshold are considered a maximum in a mass chromatogram. A characteristic spectrum must contain maxima in at least three mass chromatograms.



Selected mass spectra 3 and 4 are very similar because of an overlap of two substances. The developed method allows the detection of substances hidden in the TIC trace.

Identification of Spectra

A “foreward search” is used to identify the selected characteristic spectra. The search algorithm tries to fit the library spectrum into the spectrum of the unknown. Only small intensities of the library spectrum are allowed not to be present in the unknown. Higher intensities in the spectrum of the unknown are tolerated (caused by matrix or overlaps).

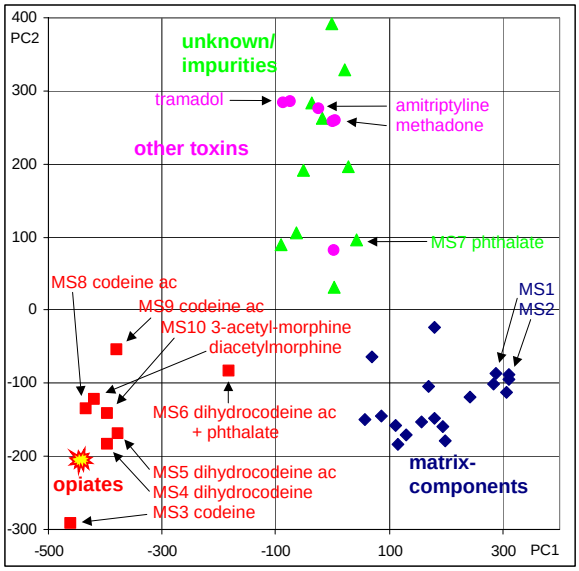


Results of Identification for all selected characteristic mass spectra:
All added toxins where identified correctly (spiked sample!)

MS	retention time (min)	Identification by library PMW-TOX	Substance
1	8.87	Tricosane	matrix
2	8.88	Tricosane	matrix
3	9.03	Codeine	toxin
4	9.04	Dihydrocodeine	toxin
5	9.37	Dihydrocodeine AC	toxin
6	9.38	Dihydrocodeine AC	toxin + impurity
7	9.39	Diisooctylphthalate	impurity
8	9.50	Codeine AC	toxin
9	9.51	Codeine AC	toxin
10	9.53	3-acetyl-morphine (Heroin)	toxin

AC ….. acetylated (sample preparation)

PCA of Characteristic Spectra



PCA: 42 objects (characteristic spectra) 862 features
Variances of PC1, PC2 are 28% and 20%

This representation shows the structural similarity of found compounds derived from mass spectral features. The added toxins can be easily recognized.

Such PCA plots can be used as templates for interpretation of forensic GC/MS analyses.

Heroin found in real forensic blood sample.