



Target screening and quantification software for GCxGC-TOF-MS data

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Introduction

GCxGC-TOF-MS is a very powerful tool for screening and determination of low levels of contaminants in complex matrices. The GCxGC separation results in clean EI-mass spectra which are very suited for automated identification against existing target libraries. In the raw data files obtained, information on potential presence of thousands of contaminants is available. However, limitations in current data handling software (performance and speed) seriously limits the use of the technique for routine comprehensive target compound analysis.

Aim

- Evaluation of in-house developed software module based on *MetAlign* [1] for suitability of automated identification of residues and contaminants in complex food and feed matrices
- Quantification using predefined masses. [1] (<http://www.metalign.nl/UK/>)

Analytical approach

Sample

- Extraction: soak with water; ethyl acetate
- Clean up: GPC, dispersive SPE (PSA)
- Final extract 1 g/ml
- Spike with 356 pesticides/PCB/PAH/PBDE

GCxGC-TOF-MS

- Injection: 10 µl (PTV solvent vent mode)
- Separation: 1st: 30 m x 0.25 mm, 0.25 µm RTX-CLPesticides; 2nd: 2 m x 0.1 mm, 0.1 µm BPX-50
- EI, fullscan MS data; m/z 50-1000, 200 scans/s

Data processing routine

Prior to processing

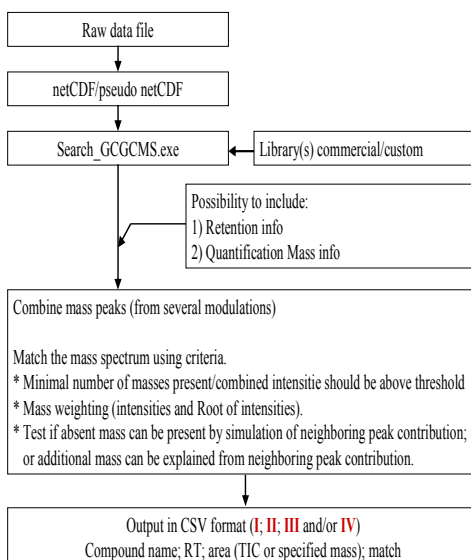
- Conversion GCxGC-TOF-MS into (pseudo-)netCDF format.
- Flexible library construction from NIST format into more straightforward library format.
- Retention times with allowed deviation / references / internal standard(s).
- Possibility to update retention time file using unlimited number of references.
- Quan mass listed in CSV file format (in library).

Processing (pseudo-)netCDF

- Split into GC-TOF-MS netCDF files.
- Run in batch through *MetAlign*, 200-fold data reduction.
- Concatenate redms files into one file using original order.
- Search redms files using Search_GCGCMS.exe identification program.

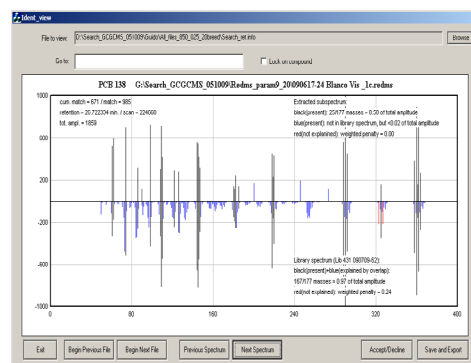
Output

- Qualitative table (all_qual; **I**) containing compounds only meeting search criteria.
- Qualitative table (all_qual_ret; **II**) containing compounds additionally meeting the retention time (1D&2D).
- Quantitative table (all_quan_ret; **III**) containing reconstructed TIC "area/signal" for compounds meeting the retention time criteria.
- Quantitative table (all_quan_mass_ret; **IV**) containing the "area/signal" for the pre-defined quantification mass.



Spectrum visualization

- The mass spectra can be reviewed in the program "Ident_view.exe", a viewer to compare the extracted mass spectrum with the library spectrum.



Performance of the screening and quantitation

- Performance is checked using 3 fortified samples at a level of 10 ng/g (356 compounds).
- The processing was against a library and a retention file containing 463 spectra and times.
- Processing is done using optimized settings.

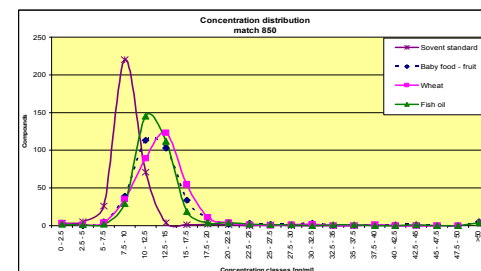
Results

Table 1 shows the number of compounds found and the calculated concentration in the blank and spiked sample. At a level of 10 ng/g more than 91% of the fortified compounds were detected (including checked for retention time). The number of false negatives for baby food is 31 (356-325). Compounds found in the blank sample, having a concentration > 10 ng/g where checked in ChromaTOF, they could not be confirmed manually, these compounds are false positives.

Match 850	Number of compounds found in blank samples						Number of compounds found fortified sample = 356 compounds conc. 10 ng/ml
	found library 463	quantified spike 356	quantified conc. >1 ng/ml	quantified conc. >2 ng/ml	quantified conc. >3 ng/ml	quantified conc. >10 ng/ml	
Baby food - fruit	26	17	8	5	1	0	325 (91%)
Wheat	39	29	14	10	7	3	335 (94%)
Fish oil	52	38	10	6	4	4	329 (92%)

Table 2 shows the average concentrations against calibration solution in solvent. A matrix effect is visible.

	Concentration results - Match 850			
	Baby food - fruit	Wheat	Fish oil	Solvent standard
Average conc.	15	15	19	10
Stand. Dev.	22	19	78	6
Median	13	13	12	9
Minimal	2	0	3	2
Maximal	359	266	1311	109



Concentration distribution based on the relative response factor for fortified samples at 10 ng/ml.

Advantages developed module

- Flexibility in search criteria
- Uses 1D&2D retention time data
- Reduced analyst review time
- Well suited for target screening
- Fast and accurate quantitative result