Supplementary Material

Automated label-free quantification of metabolites from LC-MS data

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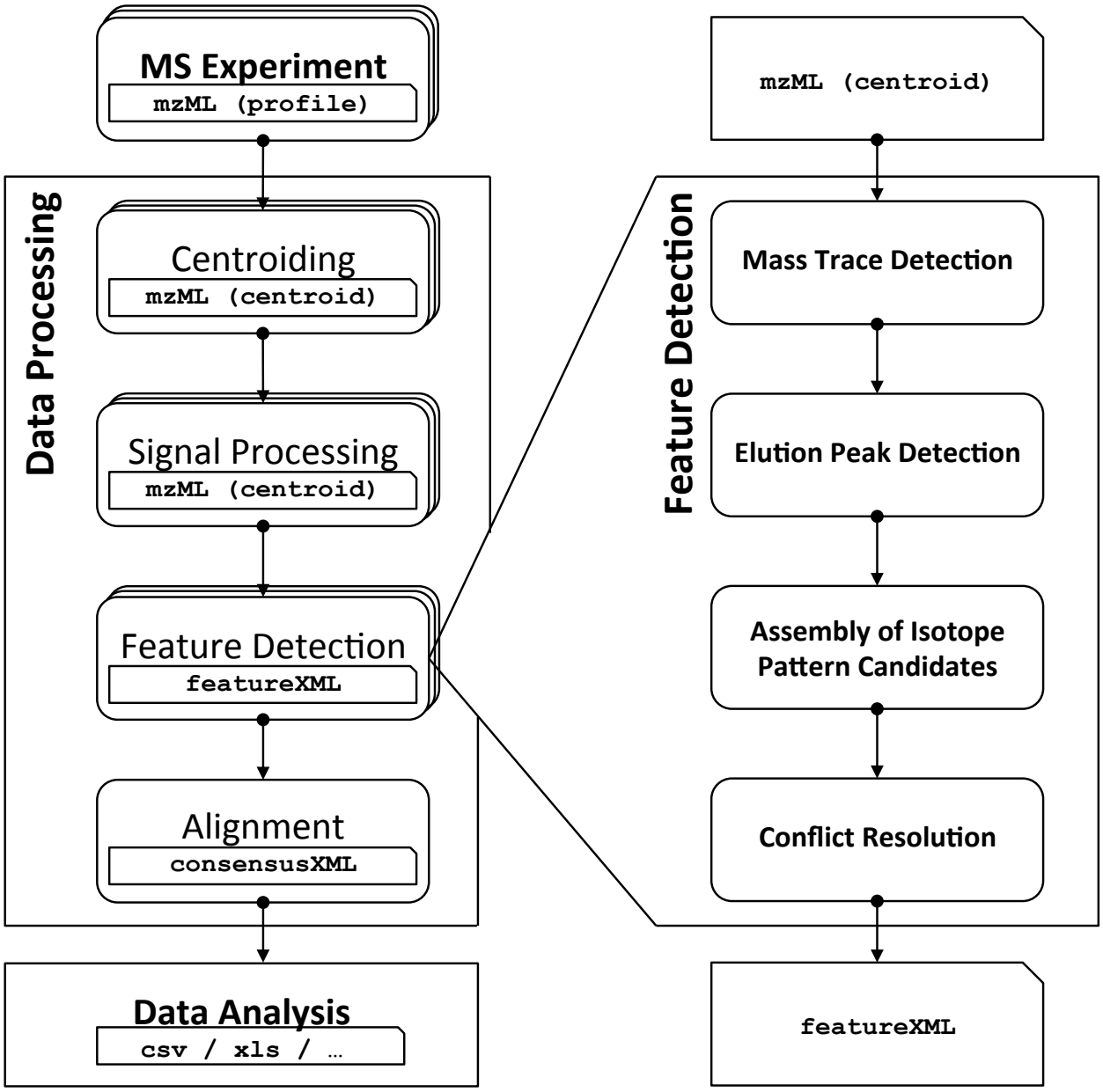
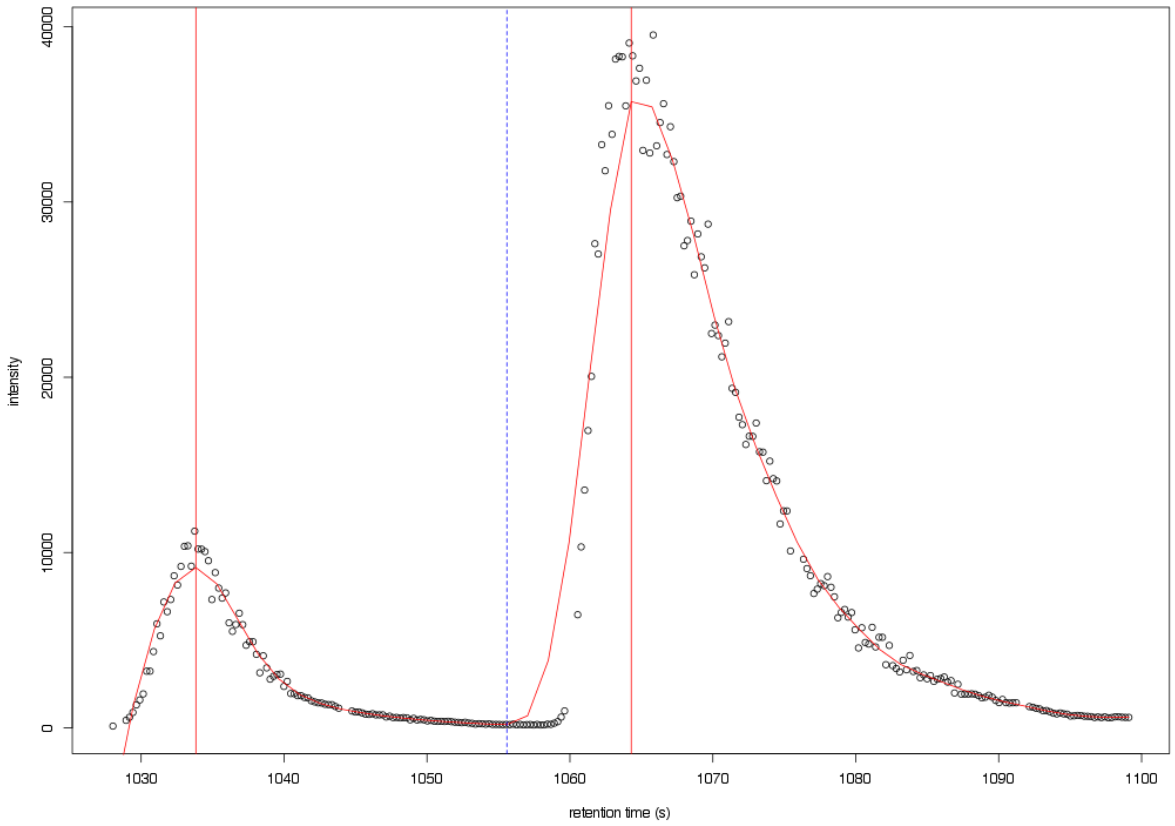
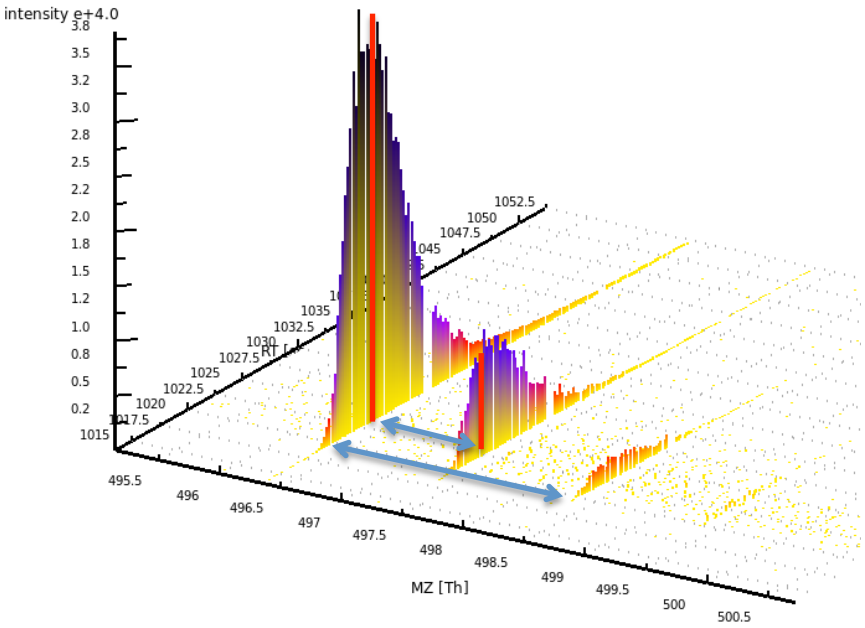
List of figures and tables



S 1: General outline of a computational pipeline employed for metabolomics data analysis. The first three steps centroiding, signal processing (e.g., mass recalibration), and feature detection are applied to all MS experiments individually. Each processed experiment results in a condensed representation (featureXML), respectively. In the final alignment step, all featureXML files are merged to a single consensusXML file, which may be exported to comma-separated value (CSV) format for further analysis.



S 2: Separation of chromatographic peaks within the same mass trace. A smoothed multimodal mass trace (red line) is scanned for time points corresponding to local intensity maxima. Two vertical red lines indicate apex peaks that show higher intensity than at least k/2 neighboring peaks. The split into two separate mass traces is done at the time point with minimum intensity between these apeces (dashed blue line).



S 3: Characteristics of a metabolite isotope pattern measured in LC-MS. In a typical isotope pattern, mass traces belonging to the monoisotopic and higher isotopic ions can be observed. The relationships between these mass traces are characteristic for any naturally occurring isotope pattern: The m/z distances between each satellite and the monoisotopic trace (blue arrows), the coelution of trace profiles (apeces indicated as red lines), and the intensity ratios between satellite traces and monoisotopic trace.

S 4: User-adjustable parameters of the FeatureFinderMetabo tool.

Parameter	Description	Default value
mass_error_ppm	Allowed mass error deviation in ppm (mass trace detection stage)	20
chrom_fwhm	A chromatographic peak's expected full-width-at-half-maximum in seconds (chromatographic peak separation)	5
noise_threshold_int	Intensity threshold below which signals are regarded as noise	10
chrom_peak_snr	The minimum signal-to-noise a mass trace should have.	3

S 5: Parameter settings of MSSimulator. The first nine parameters are common to all simulated datasets. Regarding the detector noise, m/z variation, and elution profile distortion experiments, further settings for noise-specific parameters are shown.

MSSimulator parameter name	detector noise	m/z variation	elution profile distortion
RT:total_gradient_time [s]	1500		
RT:sampling_rate [s]	0.25		
RT:scan_window:min [s]	0		
RT:scan_window:max [s]	1500		
RawSignal:resolution:value	20000		
RawSignal:resolution:type	constant		
RawSignal:variation:intensity:scale	1		
Ionization:mz:lower_measurement_limit [Da]	100		
Ionization:mz:upper_measurement_limit [Da]	1000		
RawSignal:noise:detector:stddev [counts]	0, 3, 5, 10, 12, 15, 20, 25, 30, 40, 50, 70, 100	0	0
RawSignal:variation:mz:error_stddev [ppm]	0	0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50	0
RT:column_condition:distortion	0	0	0, 10

S 6: Detection parameters applied for MS measurements in positive ESI mode.

Detection parameters	Positive ESI
Capillary voltage (kV)	3.10
Sample cone (V)	30.00
Extraction cone	4.00
Source temperature	120
Desolvation temperature (ºC)	300
Desolvation flow (L/h)	800
Cone (L/h)	50
Detector (V)	1800

Recall, precision, and robustness of the algorithm

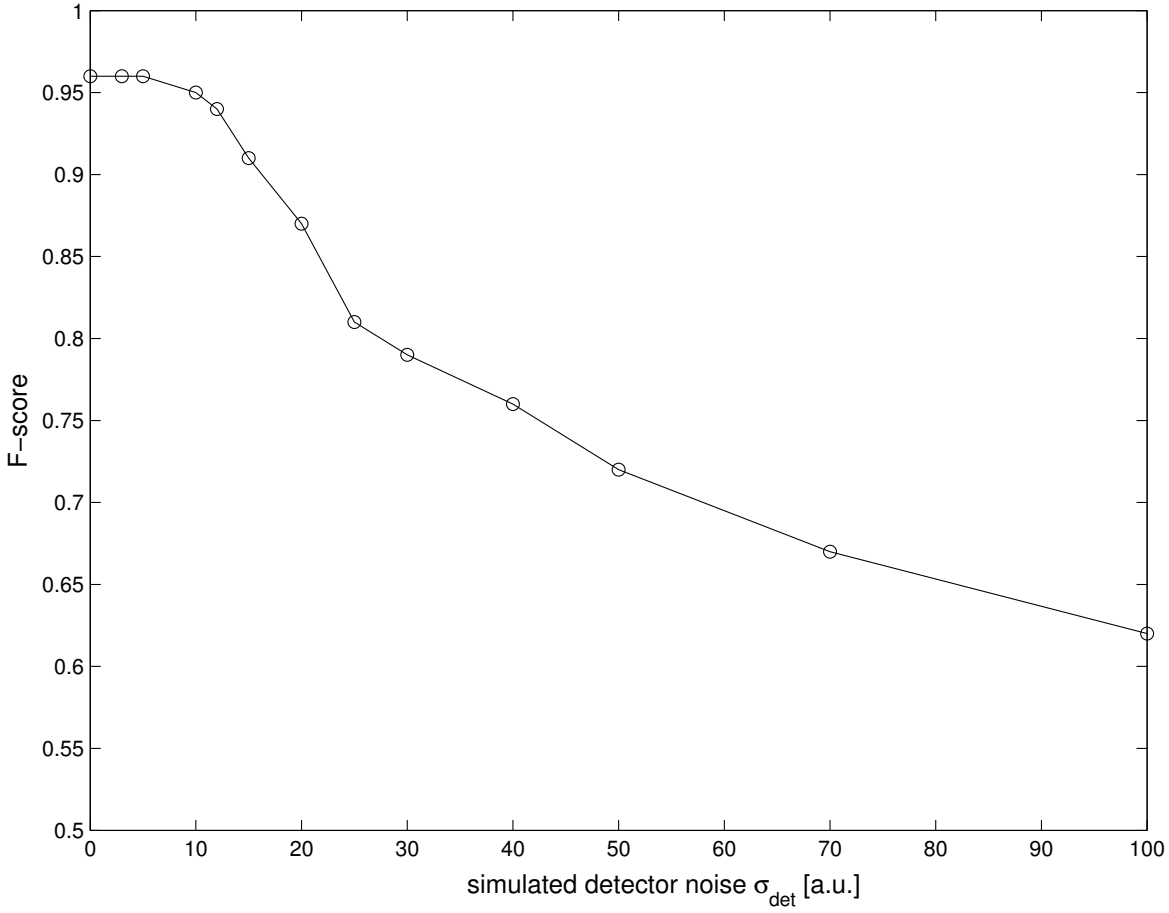
In this section, we give further details about the benchmark results based on the simulated datasets. The parameter names used in the text correspond to those used in the algorithm.

In the first experiment, we examined the influence of the noise_threshold_int parameter by adjusting it to each simulated detector noise level ($\text{noise\_threshold\_int} = \sigma_{\text{det}}$). For low detector noise levels, our algorithm achieves its maximum F-score of 0.96 (see Figure S 7). Above $\sigma_{det} = 5$, the performance decreases moderately until reaching 0.62 on the highest noise level $\sigma_{\text{det}} = 100$. When filtering out mass spectrometric peaks by increasing noise_threshold_int , potential feature candidates are not considered and, thus, the observed decline of the F-score results primarily from the decrease of the feature recall rate.

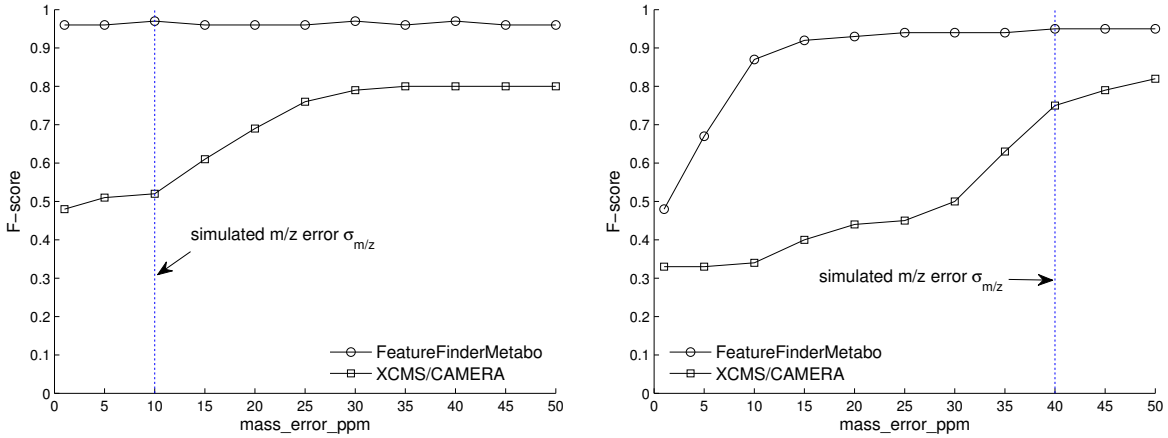
We studied the impact of under- and overestimating the mass error inherent in the data by considering two datasets each simulated with a specific mass error and varying configurations of the mass_error_ppm parameter (see Figure S 8). For a simulated m/z error $\sigma_{m/z}$ of 10 ppm (Figure S 8, left plot), the algorithm achieves an F-score of 0.97 when mass_error_ppm is set accordingly. In comparison, we observe a similar performance for a simulated m/z error $\sigma_{m/z}$ of 40 ppm (Figure S 8, right plot). The performance plots in Figure S 8 suggest that the algorithm's performance is insensitive to moderate underestimation of the mass_error_ppm parameter. In case of overestimation, the performance remains stable on a high level comparable to the F-score yielded by the optimal settings due to the dynamic re-estimation of mass_error_ppm .

Analogously, we assessed the robustness of the chrom_fwhm parameter for elution profiles simulated with heavy distortion (see Figure S 9). Based on the distribution of all simulated elution profiles, we estimated an average chromatographic peak width of eight seconds. The algorithm achieves its maximum performance of 0.9 for $\text{chrom\_fwhm} = 8\,\text{s}$ and remains stable for higher settings. For settings below eight seconds, the algorithm's performance decreases slightly until approximately four seconds and more steeply below this mark. This drop in

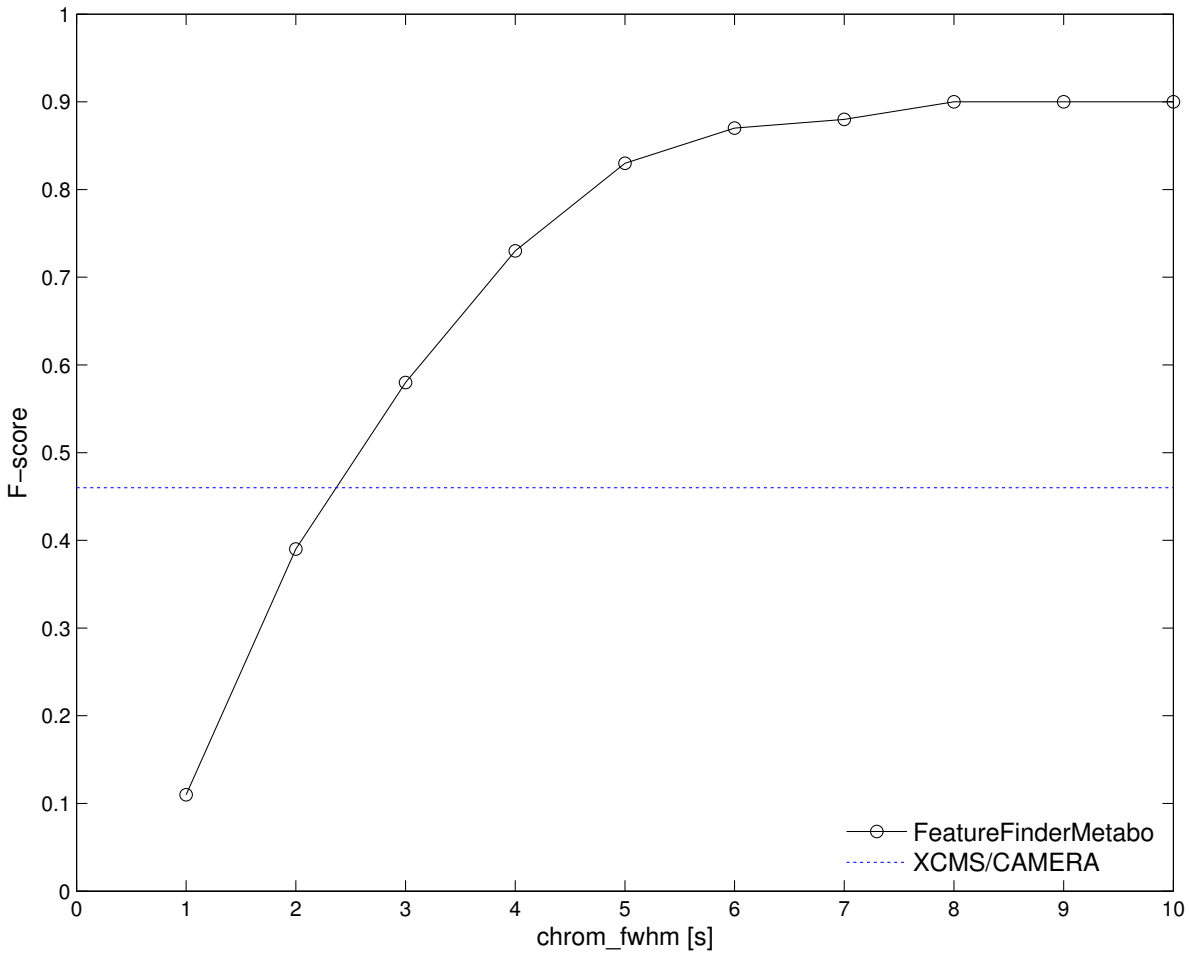
performance is due to the loss of feature precision rate since very low settings of chrom_fwhm lead to insufficient smoothing of the distorted elution profiles and thus to their fragmentation. However, the performance plots reveal that the F-score remains insensitive to sharp changes within a rather broad margin of values lower than the optimal chrom_fwhm setting (between four and eight seconds). This suggests that the algorithm allows for good performance in a robust manner even if chrom_fwhm is moderately underestimated.



S 7: Feature finding performance with respect to varying degrees of simulated detector noise. For each simulated detector noise setting, the noise_threshold_int parameter was chosen accordingly as an optimal setting. Additionally, only mass traces with a signal-to-noise ratio of at least 10 were accepted.



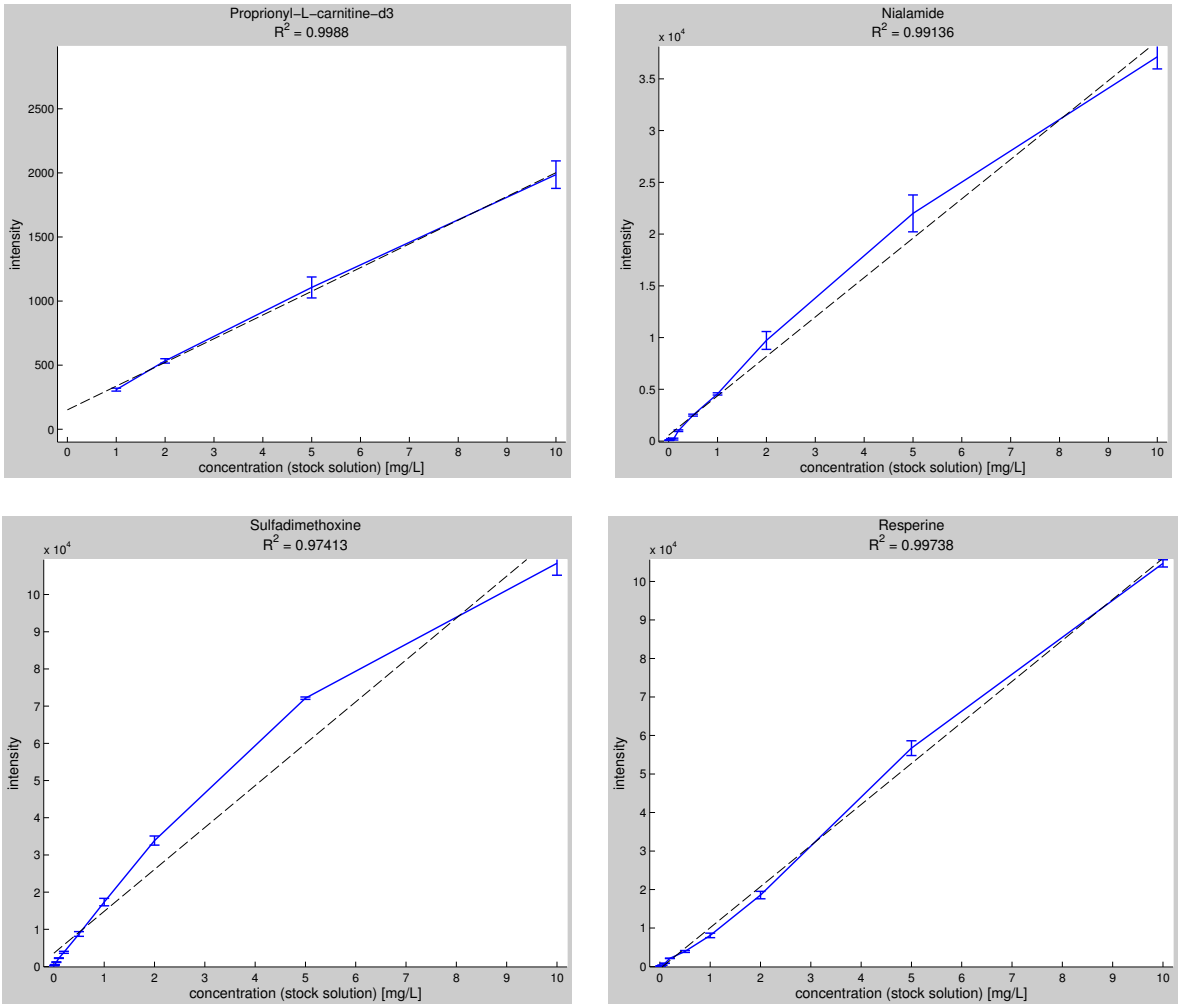
S 8: Feature finding performance comparison with respect to varying settings of the mass_error_ppm parameter. Feature detection runs were performed on datasets with a simulated m/z error $\boldsymbol{\sigma_{m/z}} = \mathbf{10}$ ppm (left plot) and $\boldsymbol{\sigma_{m/z}} = \mathbf{40}$ ppm (right plot). Our algorithm's mass_error_ppm parameter and the corresponding parameter in XCMS/CAMERA were varied between 1 and 50 ppm, respectively.



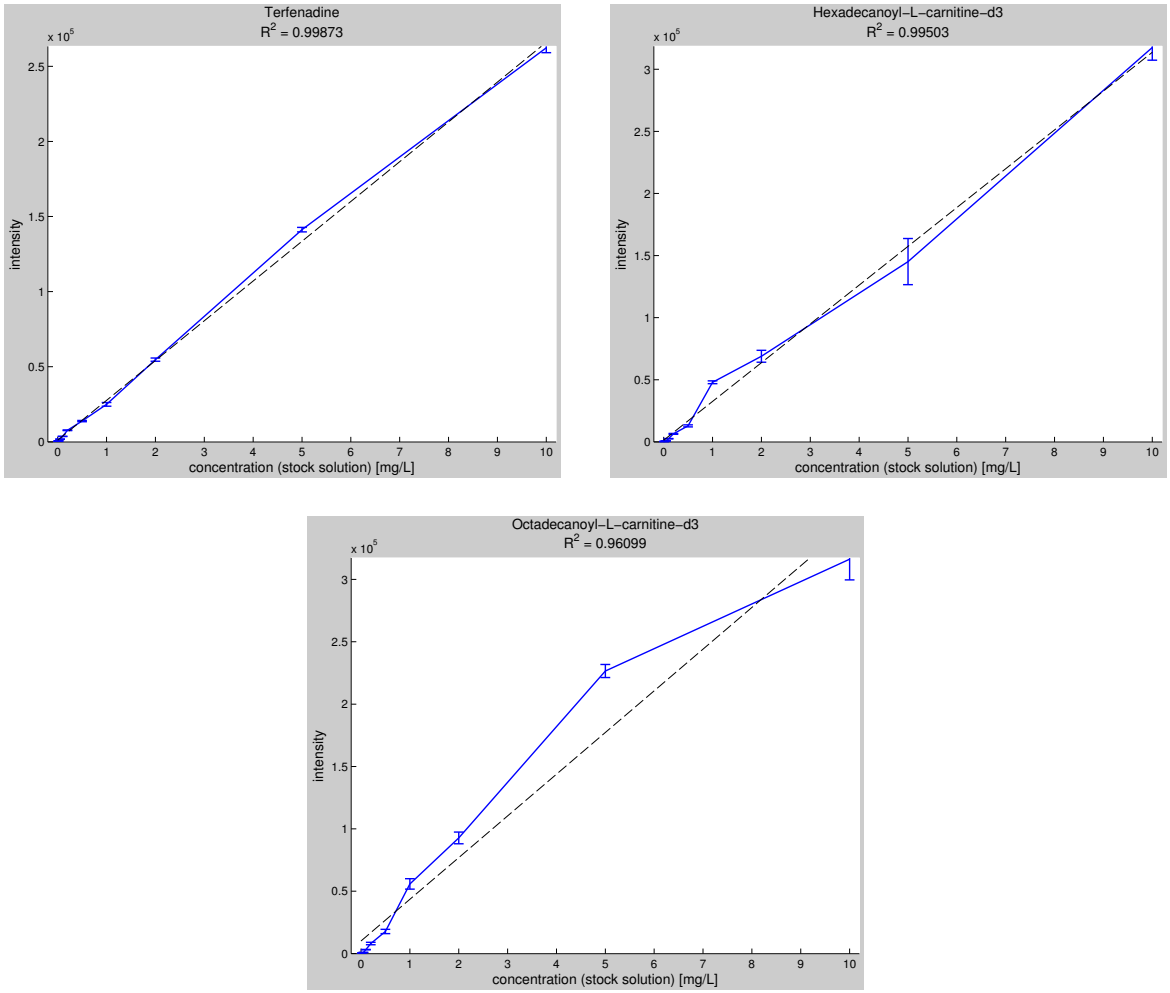
S 9: Feature finding performance with respect to varying settings of the chrom_fwhm parameter. Feature detection runs were performed on a dataset with elution profiles simulated with heavy distortion. The chrom_fwhm parameter was varied between 1 and 10 seconds. Since XCMS/CAMERA does not offer a smoothing parameter corresponding to chrom_fwhm, we conducted a single run with the parameter *peakwidth* set to the interval [3, 30] seconds, allowing only chromatographic peaks within this range. The set of chromatographic peaks resulting from our algorithm were also filtered by this criterion.

S 10: Feature intensities for spiked-in compounds. The first row contains the Pearson correlations between the concentrations and the corresponding feature intensities for each standard compound.

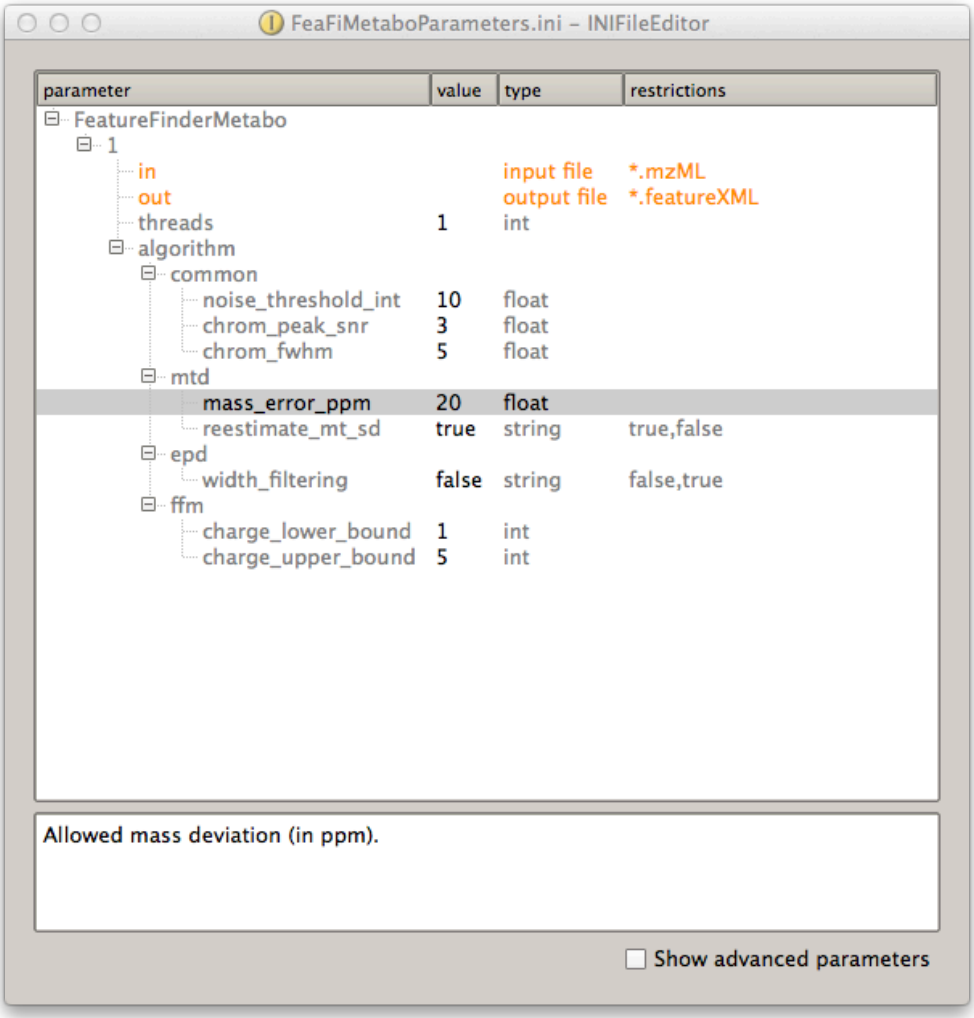
Compound	C3-Carnitine	Nialamide	Sulfadimethoxine	Reserpine	Terfenadine	C16-Carnitine (Palmitoylcarnitine)	C18-Carnitine (Stearoylcarnitine)
Correlation	0.9994	0.9957	0.987	0.9987	0.9994	0.9975	0.9803
Stock Solution Concentration [mg/L]	intensity mean +/- standard deviation						
0	not detected	not detected	not detected	not detected	not detected	not detected	not detected
0.01	not detected	6.3×10^{1} +/- 0 (*)	2.5×10^{2} +/- 2.7×10^{1}	8.0×10^{1} +/- 8.3	3.8×10^{2} +/- 4.3×10^{1}	2.2×10^{2} +/- 0.0 (*)	not detected
0.02	not detected	1.1×10^{2} +/- 1.1×10^{1}	4.8×10^{2} +/- 7.2×10^{1}	1.4×10^{2} +/- 9.3	6.4×10^{2} +/- 8.4×10^{1}	3.6×10^{2} +/- 3.4	4.5×10^{2} +/- 7.3
0.05	not detected	2.5×10^{2} +/- 1.1×10^{1}	1.2×10^{3} +/- 9.7×10^{1}	3.6×10^{2} +/- 3.8×10^{1}	1.8×10^{3} +/- 1.5×10^{2}	6.9×10^{2} +/- 8.8	9.1×10^{2} +/- 1.9×10^{1}
0.1	not detected	2.0×10^{2} +/- 1.2×10^{2} (**)	2.3×10^{3} +/- 9.5×10^{1}	8.3×10^{2} +/- 2.5×10^{1}	3.6×10^{3} +/- 1.3×10^{2}	2.5×10^{3} +/- 1.9×10^{1}	3.2×10^{3} +/- 1.8×10^{2}
0.2	not detected	1.0×10^{3} +/- 9.9×10^{1}	3.8×10^{3} +/- 3.3×10^{2}	2.1×10^{3} +/- 6.7×10^{1}	7.7×10^{3} +/- 2.7×10^{2}	6.4×10^{3} +/- 4.7×10^{1}	8.0×10^{3} +/- 1.2×10^{2}
0.5	not detected	2.5×10^{3} +/- 1.1×10^{2}	8.7×10^{3} +/- 7.8×10^{2}	3.9×10^{3} +/- 3.0×10^{2}	1.4×10^{4} +/- 5.0×10^{2}	1.3×10^{4} +/- 1.0×10^{2}	1.8×10^{4} +/- 2.1×10^{2}
1	3.1×10^{2} +/- 1.4×10^{1}	4.5×10^{3} +/- 1.3×10^{2}	1.7×10^{4} +/- 1.2×10^{3}	8.1×10^{3} +/- 7.2×10^{2}	2.5×10^{4} +/- 1.5×10^{3}	4.8×10^{4} +/- 1.4×10^{2}	5.6×10^{4} +/- 5.1×10^{2}
2	5.3×10^{2} +/- 2.1×10^{1}	9.7×10^{3} +/- 1.1×10^{3}	3.4×10^{4} +/- 1.5×10^{3}	1.9×10^{4} +/- 1.2×10^{3}	5.5×10^{4} +/- 1.3×10^{3}	6.9×10^{4} +/- 5.9×10^{2}	9.3×10^{4} +/- 5.7×10^{3}
5	1.1×10^{3} +/- 1.0×10^{2}	2.2×10^{4} +/- 2.2×10^{3}	7.2×10^{4} +/- 4.1×10^{2}	5.7×10^{4} +/- 2.3×10^{3}	1.4×10^{5} +/- 1.8×10^{3}	1.5×10^{5} +/- 2.3×10^{3}	2.3×10^{5} +/- 6.4×10^{3}
10	2.0×10^{3} +/- 1.3×10^{2}	3.7×10^{4} +/- 1.4×10^{3}	1.1×10^{5} +/- 4.0×10^{3}	1.0×10^{5} +/- 1.1×10^{3}	2.6×10^{5} +/- 4.0×10^{3}	3.2×10^{5} +/- 1.2×10^{3}	3.2×10^{5} +/- 2.0×10^{3}



S 11: Relationship between stock solution concentrations and feature intensities for Propionyl-L-carnitine-d3, Nialamide, Sulfadimethoxine, and Reserpine.



S 12: Relationship between stock solution concentrations and feature intensities for Terfenadine, Hexadecanoyl-L-carnitine-d3, and Octadecanoyl-L-carnitine-d3.



S 13: Snapshot of the INIFileEditor window. The parameters for the FeatureFinderMetabo tool are easily changed with the INIFileEditor tool of OpenMS.