Developing 2D mzCompare for single GC×GC-TOFMS chromatograms: Substantial resolution enhancement in the context of statistical overlap theory

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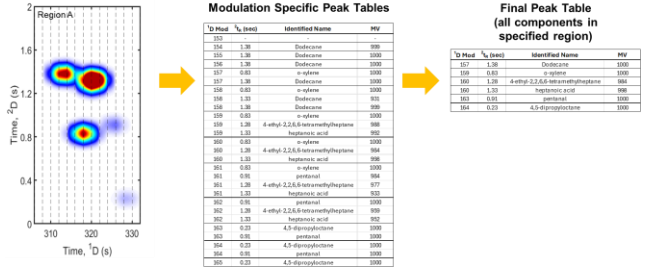
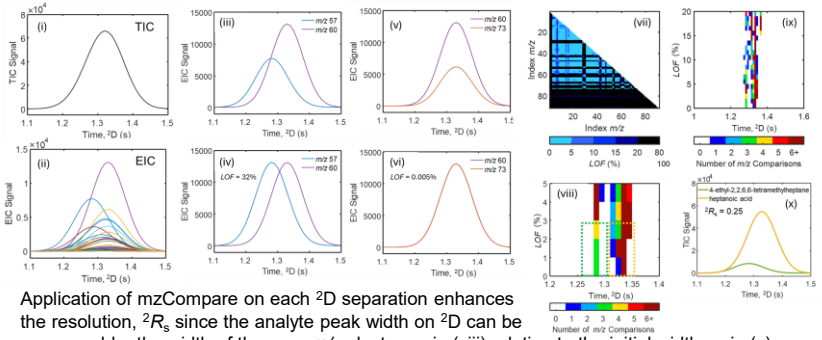
Overview

Accurate identification of all detectable analyte components in a single comprehensive two-dimensional (2D) gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS) chromatogram is a fundamental interest in the field. Herein, we developed a new algorithmic software approach called 2D mzCompare to generate accurate peak tables for GC×GC-TOFMS. Extending from our original method for one-dimensional GC-MS data, the 2D mzCompare algorithm discovers selective mass channels (*m/z*) for each analyte to resolve overlapping peaks and improve analyte identification, leveraging the similarity in retention time and peak shape across *m/z* of the same analyte. The 2D mzCompare algorithm calculates the peak shape similarity between *m/z* at every modulation via lack-of-fit (*LOF*), followed by clustering and focusing steps, to generate a final peak table. To evaluate this software, we simulated realistic GC×GC-TOFMS data in the context of the statistical overlap theory (SOT), so the exact number and identities of analytes are known *a priori*. Utilizing an in-house mass spectrum library of similar compounds, GC×GC-TOFMS chromatograms were simulated with varying degrees of chromatographic saturation (α_{2D}). First, we provide a new algorithmic approach, 2D mzCompare, to resolve overlapped analytes in GC×GC-TOFMS data, and second, we validate the accuracy of the software performance using SOT.



1D mzCompare Algorithm

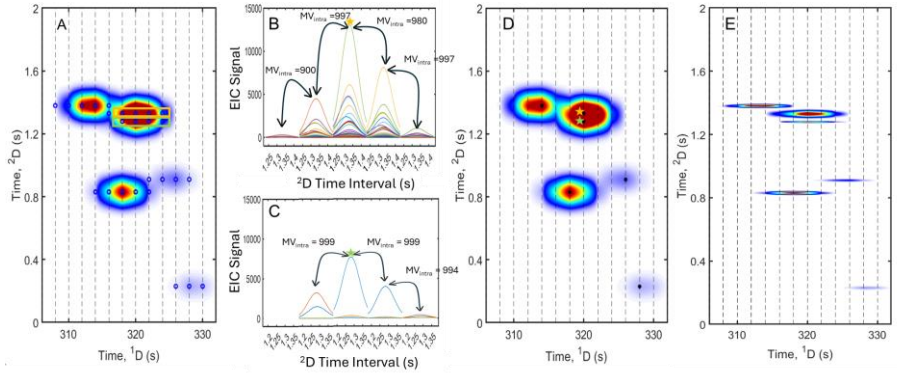


Application of mzCompare on each ^{2}D separation enhances the resolution, ^2R_s since the analyte peak width on ^{2}D can be expressed by the width of the pure *m/z* cluster as in (viii) relative to the initial width as in (x),

$$R_S = \frac{(t_{R_2} - t_{R_1})}{(w_{b_2} + w_{b_1})/2} \qquad R_{s,mzCompare} = R_S\left(\frac{w_{b_2} + w_{b_1}}{w_{b,cluster_2} + w_{b,cluster_1}}\right)$$

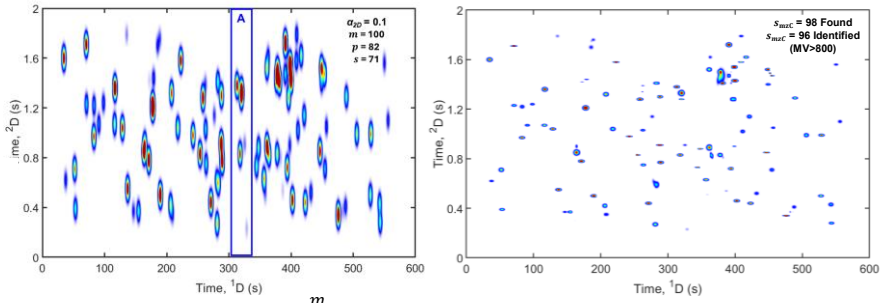
where $^2w_{b,cluster_2}$ and $^2w_{b,cluster_1}$ are pure *m/z* cluster ^{2}D "peak widths" in (viii).

2D mzCompare Algorithm: Clustering and Focusing



The ^2t_R **locations for each analyte** (**blue circles** in A) are obtained from applying mzCompare at each modulation. These are clustered across adjacent modulations via intra-mass spectrum comparison, and when the MV exceeds 800 and falls within the ±2-pixel cluster window, a **final singlet component location** is obtained (**black dots** in D).

2D mzCompare Results at α_{2D} = 0.1



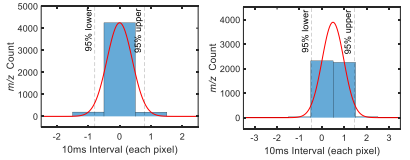
Saturation factor (α_{2D}) $\alpha_{2D} = \frac{m}{n_{c,2D}}$

m = number of analytes in simulation

$n_{c,2D}$ = ideal peak capacity of a 2D chromatogram

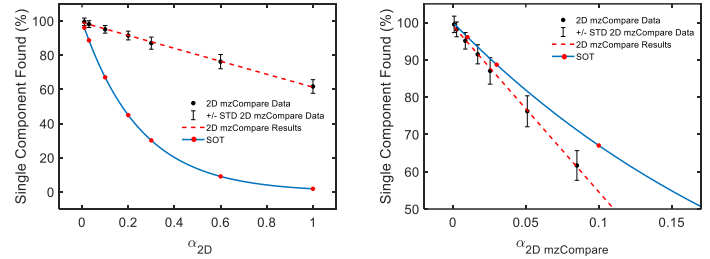
Number of peaks (*p*) $p = m\frac{4\alpha_{2D}e^{-4\alpha_{2D}}}{1 - e^{-4\alpha_{2D}}}$

Number of singlets (*s*) $s = me^{-4\alpha_{2D}}$



Cumulative distribution for pure *m/z* cluster plots from 100 singlets with *LOF* below 3%. The exponential decay peak area distribution was randomly assigned to 100 singlets. These cumulative histograms quantify ^{2}D analyte peak widths, defined by the 95% inclusion after applying 2D mzCompare. (Left) In-phase with a 95% inclusion width of 15.7 ms. (Right) Out-of-phase with a 95% inclusion width of 19.0 ms.

Evaluation Against Statistical Overlap Theory



The 2D peak capacity and saturation factor will be enhanced by applying 2D mzCompare,

$$n_{c,2D\ mzCompare} = n_{c,2D}\left(\frac{^2w_b}{^2w_{b,cluster}}\right) \qquad \alpha_{2D\ mzCompare} = \alpha_{2D}\left(\frac{^2w_{b,cluster}}{^2w_b}\right)$$

where ^2w_b and $^2w_{b,cluster}$ represent an *average* ^{2}D peak width for the single component analyte peaks before and after applying 2D mzCompare, respectively

"global" estimate of the *enhancement ratio* given by $\frac{^2w_{b,cluster}}{^2w_b} \approx \frac{17ms}{200ms} \approx 0.085$

~12-fold enhancement

Saturation factor (α_{2D})		0.01	0.03	0.1	0.2	0.3	0.6	1.0
Singlet Components Found (%)	Average	100	98	95	91	87	76	62
	Std	2.2	2.0	2.2	2.6	3.5	4.2	4.0
Singlet Components Found and Identified (MV>800) (%)	Average	100	98	95	90	85	71	54
	Std	2.2	2.0	2.4	2.7	3.4	4.1	3.2

Conclusions and Future Work

An extended algorithm, 2D mzCompare designed for intra-chromatogram comparison to enable rigorous analyte discovery and identification, was developed for GC×GC-TOFMS data. Within the context of SOT, 2D mzCompare increases separation resolution by computationally minimizing the ^{2}D peak widths, enhancing 2D peak capacity and reducing the saturation factor. At low saturation factors (α_{2D}= 0.01, 0.03, and 0.1), over 95% of the simulated components are found to be mathematically resolved singlets (pure analyte components) by 2D mzCompare, while approximately 62% were found at α_{2D} = 1, exceeding predictions made by SOT. Using optimized 2D mzCompare parameters, improvements in ^2R_s and α_{2D} are about 12-fold, empirically validating SOT expectations. Furthermore, 2D mzCompare can be used as a preprocessing tool to determine or validate the "rank" (the number of analyte components) in overlapped regions of GC×GC-TOFMS chromatograms when combined with chemometric methods such as MCR-ALS or PARAFAC. Future studies will focus on applying the algorithm to real sample datasets.

References

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