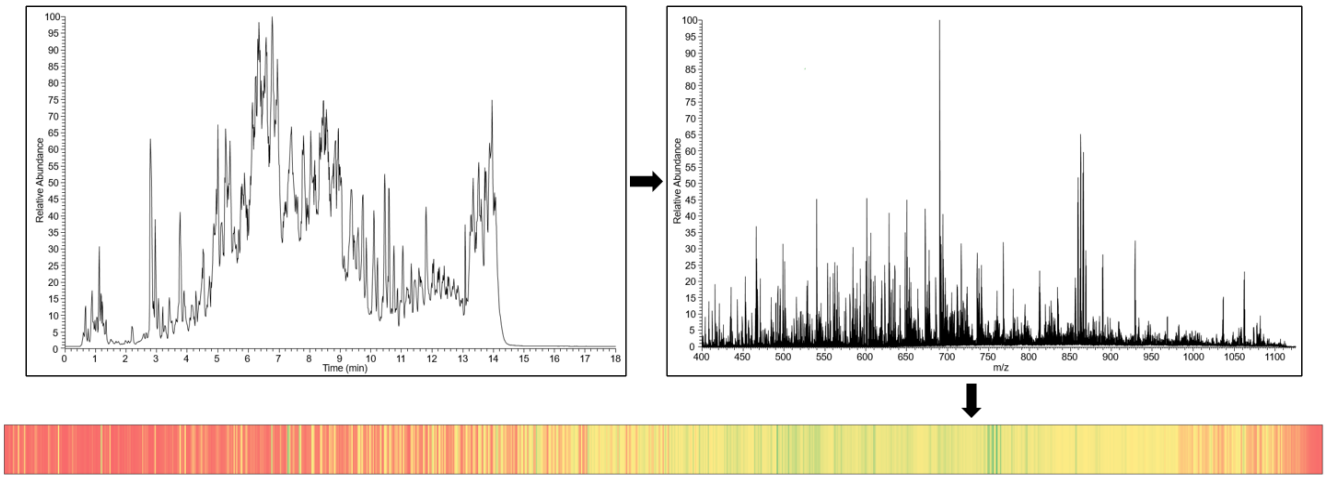
TRISKELION

Molecular Barcode™

Molecular Barcode is a novel approach to visualize the molecular composition (similarity) of food products and other complex matrices, based on LC-HRMS data. In a convergent (data) analytical workflow, Molecular Barcode is also a suitable starting point to facilitate the design of targeted methods, either to discriminate between samples or to find a generic target.



Approach

Based on a previously published (data) analytical approach for non-targeted to targeted liquid chromatography - mass spectrometry (LC-MS) [1], Molecular Barcode was developed [2,3]. After dissolving the products of interest or extraction of the biological matrices, samples are subjected to LC and data-dependent MS/MS using an Orbitrap MS (Thermo Scientific). Chromatograms are converted to MS spectra in the relevant time range and the MS data are exported as intensities per nominal m/z value in the relevant m/z range. The summed intensity data per nominal m/z is transposed and column widths are set to 1 pixel. The Molecular Barcode is completed by applying a color range to the dataset, going from red (low intensity), via yellow to green (high intensity). In example 1, protein hydrolysate sample X, from an unknown animal species with unknown processing, was compared to references A-E, see Figure 1. In example 2, five batches of a plant extract, in development as a plant protection product in Europe, were compared in negative and positive mode, see Figures 2-4.

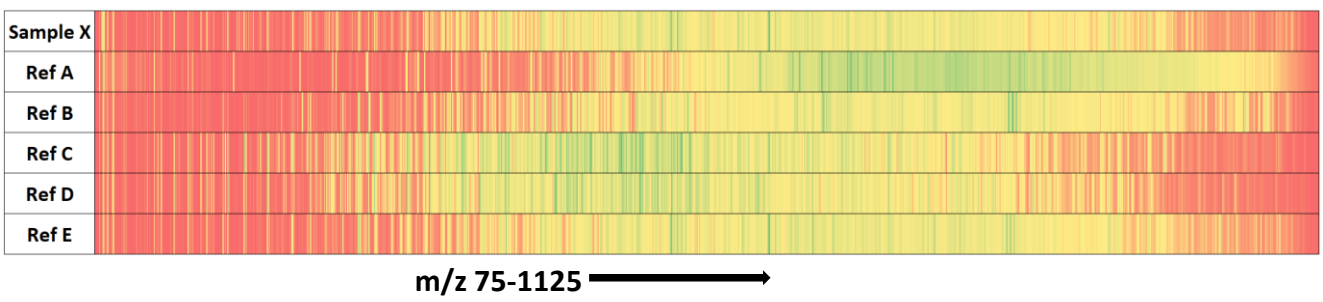


Figure 1: Protein hydrolysate sample X has the highest similarity to reference E (with known processing and animal species). The nature of sample X could thus be clarified based only on the Molecular Barcode. In extensively hydrolyzed samples most of the ions are singly charged after electrospray ionization. The range of summed intensities per m/z then resembles the molecular weight distribution and represents an output similar to an SDS-PAGE gel, but then for smaller molecules. The barcodes also indicate the specificity of the treatment. Whereas references B to E were prepared using enzymes, reference A was prepared using acid hydrolysis, which explains the more smeared appearance. Interesting differences between barcodes can be further investigated on the molecular level using the underlying high resolution MS/MS data [4].

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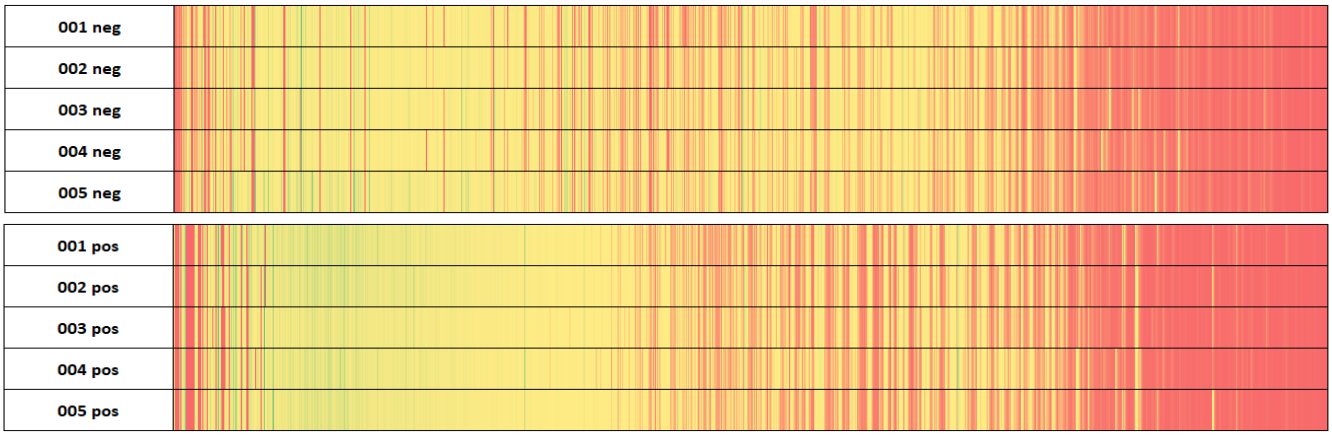

Figure 2: Molecular Barcode (m/z 75-1125) of five batches of a plant extract in negative mode (top) and in positive mode (bottom). It appeared that the 5 batches were very similar in both polarity modes, but minor differences can be observed.

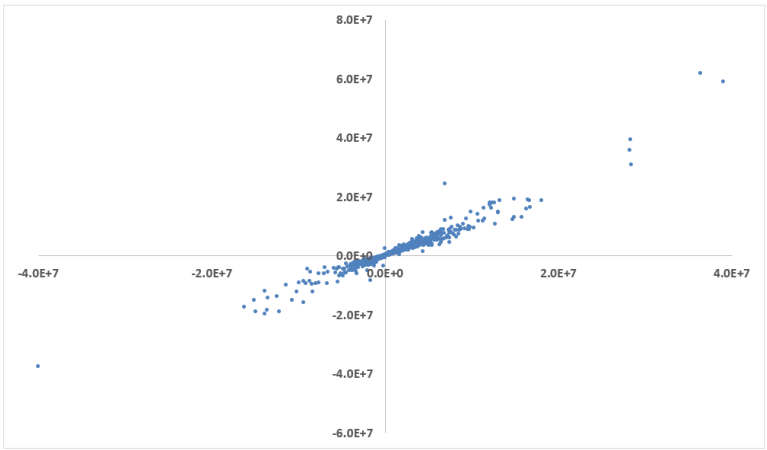

Figure 3: Summed intensities per nominal m/z of plant extracts in the m/z 75-1125 range, in negative mode (converted to negative intensities) and positive mode, batch 002 (x-axis) versus 003 (y-axis).

	001 posneg	002 posneg	003 posneg	004 posneg	005 posneg
001 posneg	1.000	0.959	0.913	0.950	0.919
002 posneg	0.959	1.000	0.969	0.958	0.944
003 posneg	0.913	0.969	1.000	0.946	0.928
004 posneg	0.950	0.958	0.946	1.000	0.928
005 posneg	0.919	0.944	0.928	0.928	1.000

Figure 4: Correlation coefficient matrix [1,3] of the five plant extract batches of the combined negative and positive data, summarizing the similarity between these complex samples in 1 number.

Applications

● Molecular Barcode has been applied to:

1) Investigate the similarity between milk protein hydrolysates and between collagen hydrolysates, to compare processing conditions (e.g. different enzymes or chemical hydrolysis) and to assess inter-batch variation.

2) Assess the similarity of food products. After selecting a sufficiently generic extraction method, different food products can be compared, such as soy-, dairy- and animal tissue-based products [3].

3) Perform analysis of inter-batch variation of a plant protection product having a biological origin (plant extract) and obtain fingerprints of the extracts.

● Possible future applications: applicability to other data types (besides LC-MS) and in pre-assessment of non-intentionally added substances (NIAS).

References

[1] *Analytical and Bioanalytical Chemistry* 2020; **412**: 973–982.
[2] *ResearchGate* 2023; DOI: 10.13140/RG.2.2.35602.32960
[3] *Food Chemistry* 2024; **442**: 138466.
[4] *ResearchGate* 2024; DOI: 10.13140/RG.2.2.34412.17284

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