

POSTER SUMMARY

96 Bottles of Beer: Metabolic Profiling of Spent Growth Media Using Rapid, High Throughput Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry

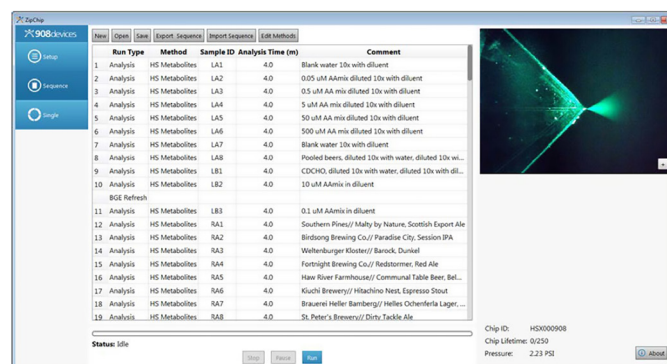
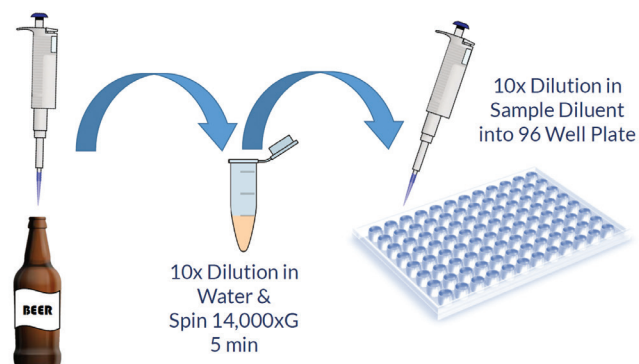
OVERVIEW

Bioreactor monitoring is a vital part of quality control and process refinement. There is an ever-growing demand for high throughput analysis methods capable of delivering metabolic profiling data rapidly to the user without the need for extensive sample processing. The presence and concentration of biogenic analytes can often indicate the progress and success or failure of the production process. Beer production is no exception to this as several metabolites have been identified as indicators of beer quality. Here we demonstrate the detection and quantitation of several biogenic amines that have been shown to affect beer flavor. Using a microfluidic capillary electrophoresis (CE)-MS platform (ZipChip®, 908 Devices, Boston MA) we can rapidly (<4 min) analyze a wide range of biologically relevant metabolites with virtually no sample preparation.

METHODS

Beer samples were stored frozen until all 96 were collected, then thawed, diluted 10x in water, centrifuged to remove particulates, diluted 10x in Metabolite Sample Diluent (908 Devices, Boston, MA) containing heavy isotope labeled amino acid internal standards, and then loaded directly into a 96 well plate. The plate was then placed into an autosampler equipped microfluidic CE-ESI devices (ZipChip Interface, 908 Devices, Boston, MA) which was coupled to an orbitrap MS (Exactive Plus EMR, Thermo Scientific) for analysis with a scan range of 70-350 m/z. A microfluidic chip with a 10 cm separation channel was used (ZipChip HSX). The chip was filled with Metabolites background electrolyte (BGE) and a field strength of 1000 V/cm was applied, yielding analysis times of 4 minutes.

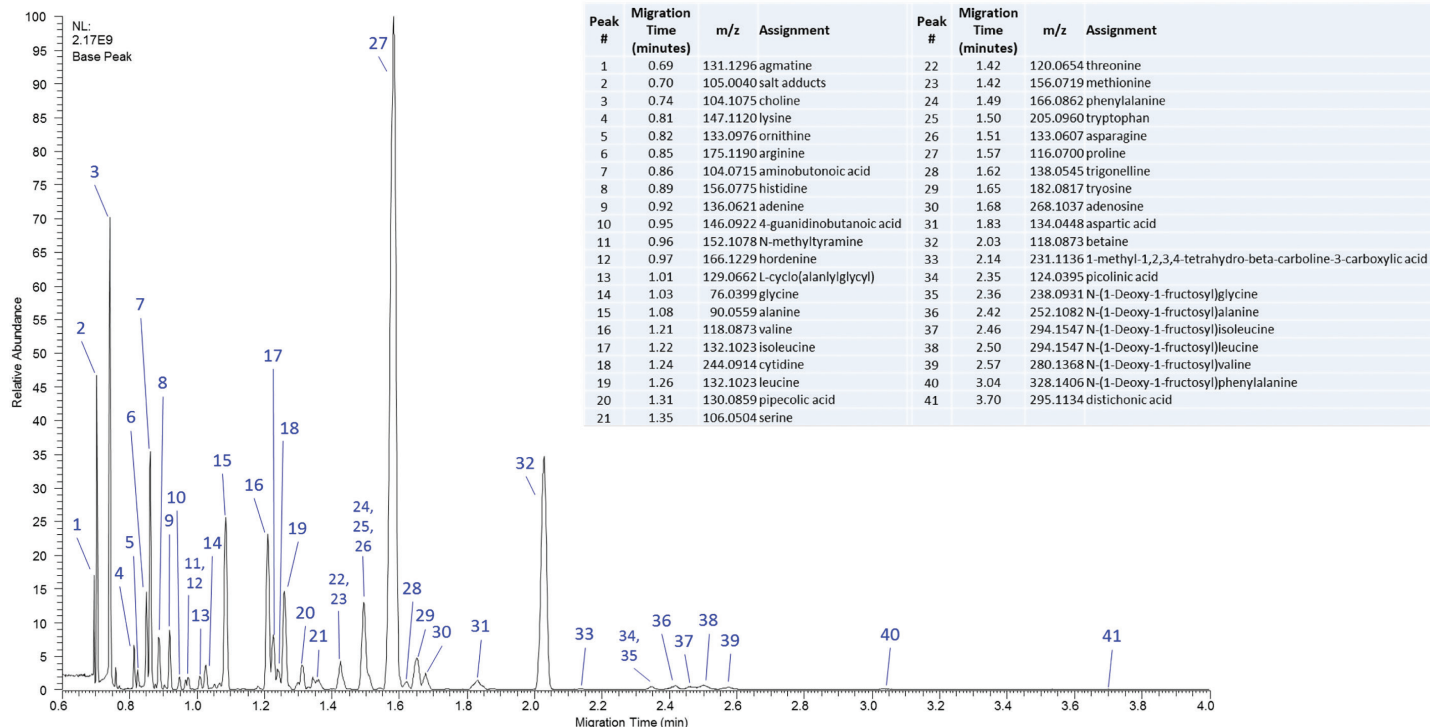
Sample preparation, analysis and data processing was all completed in less than 24 hours for the 133 ZipChip runs performed. Each analysis (5 nL injection into the separation channel) consumed 50 pL of beer. The remaining beer samples (~375 mL x 96) were disposed of in accordance with local (NC-ABC) laws via eager human waste-processing bioreactors.



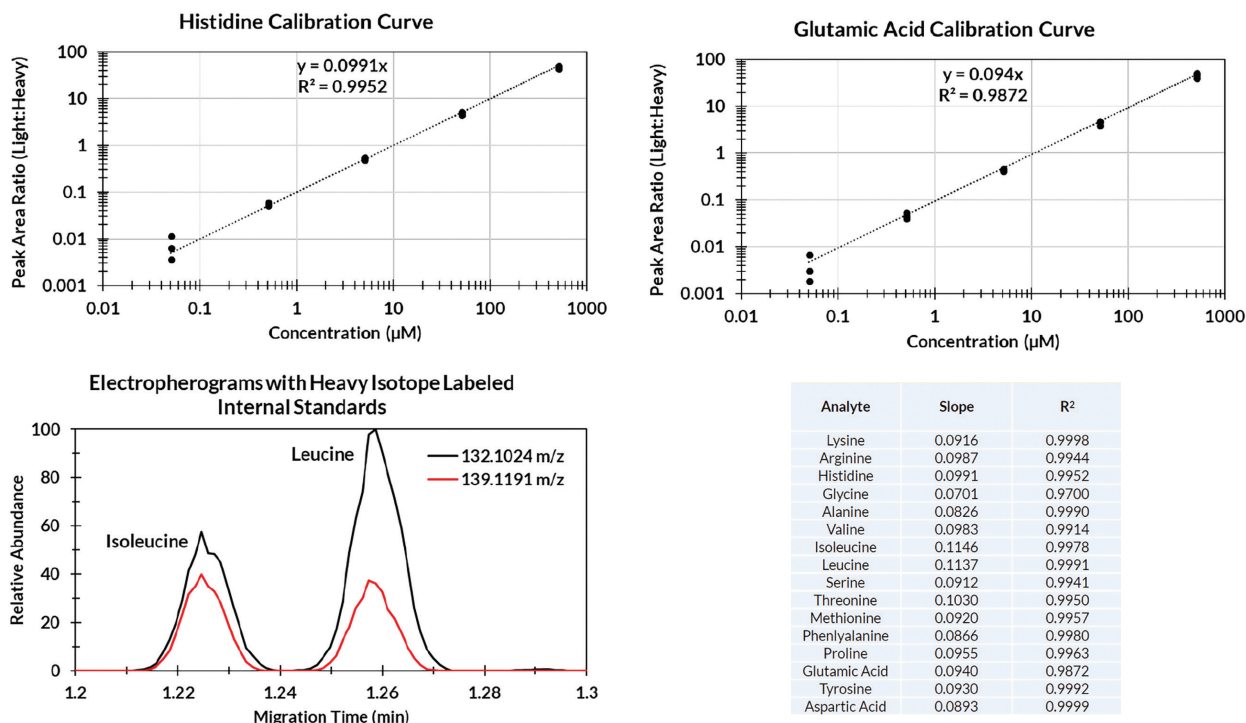
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SPENT GROWTH MEDIA ANALYSIS



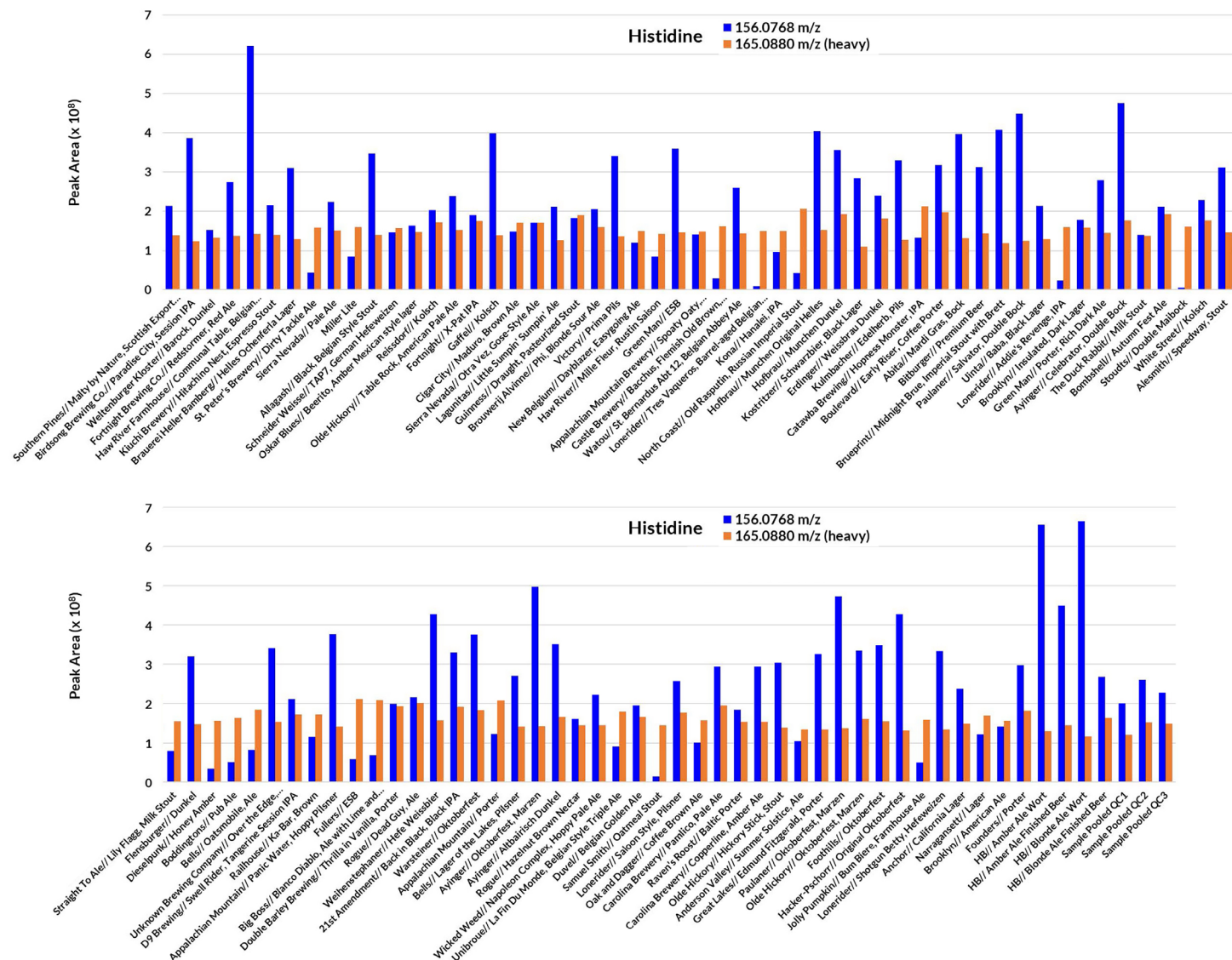
AMINO ACID QUANTITATION



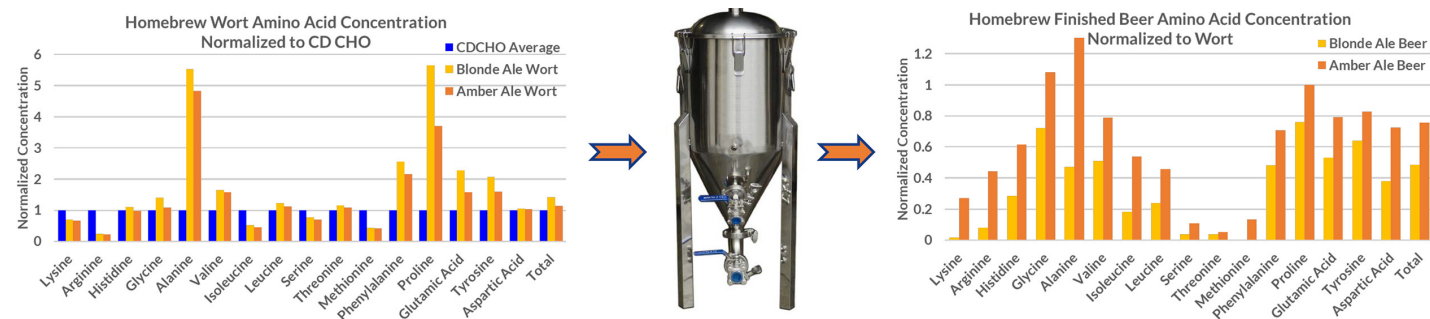
Heavy isotope labeled internal standards of the above 16 amino acids were spiked into each sample at a concentration of 1 μM. Calibration curves were plotted for each amino acid's light-to-heavy isotope peak area ratio. Histidine and Glutamic Acid calibration curves are shown at left and are representative of others produced in this experiment. Below the raw histidine peak areas are shown for each of the beer and homebrew samples for both the heavy and light isotopes.

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BIOREACTOR MONITORING : HOMEBREW ANALYSIS

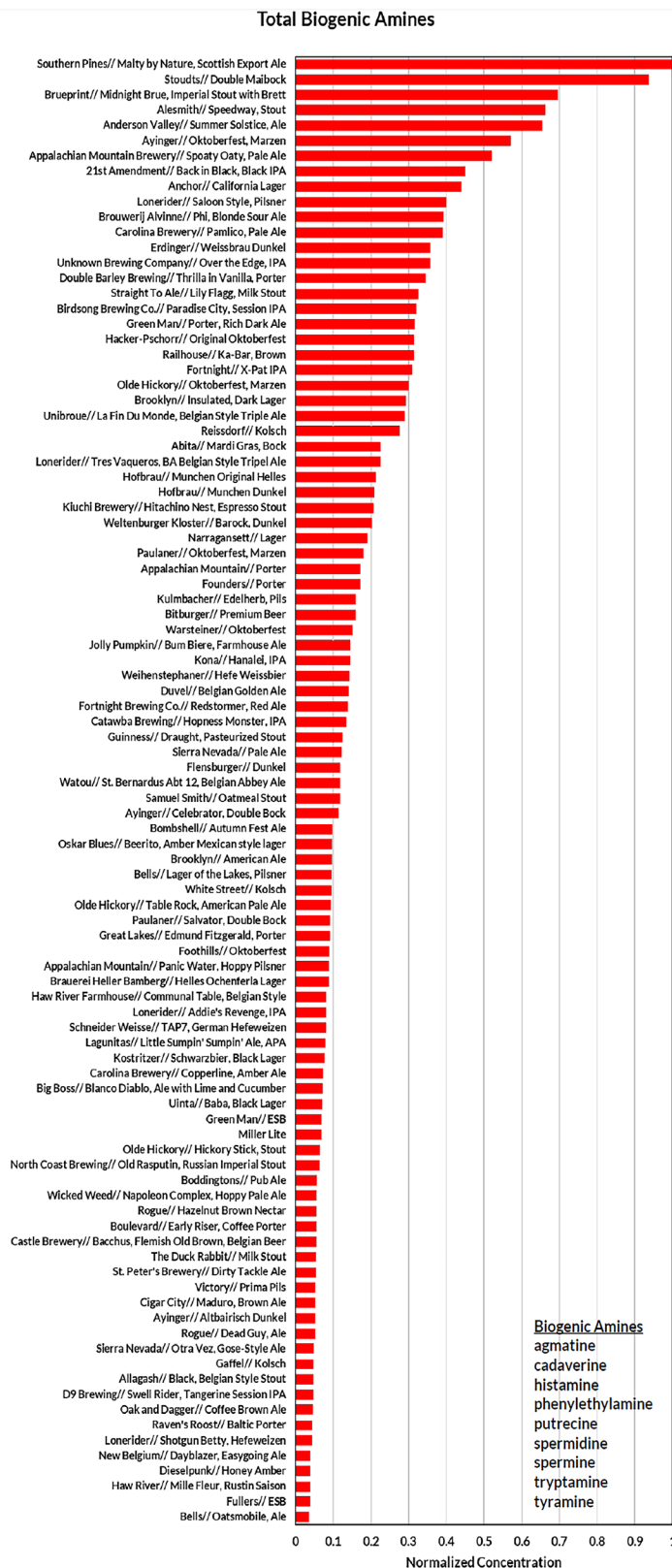
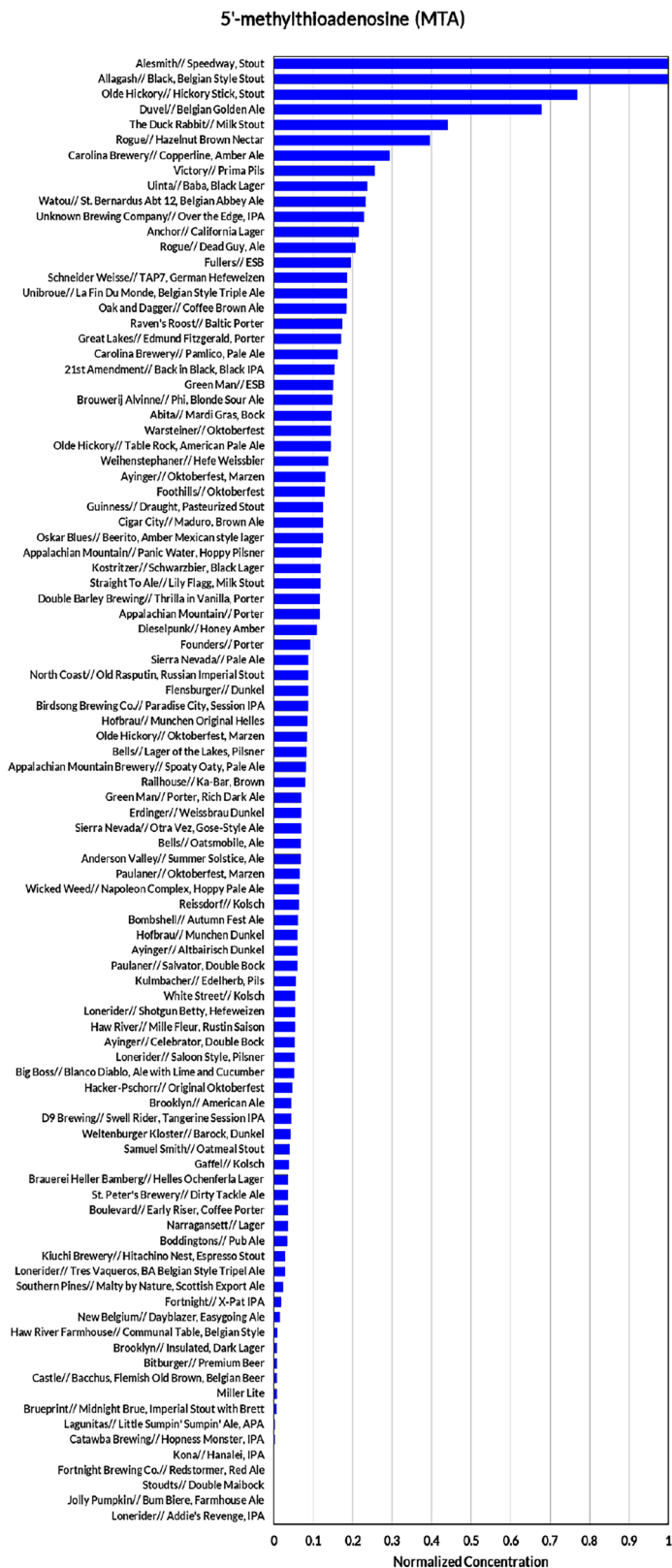


Homebrew wort was fermented w/ *Saccharomyces cerevisiae* at $\sim 5 \times 10^9$ cells/liter wort for 3 weeks at 17°C in a stainless steel conical fermentation vessel.

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METABOLITES AND TASTE



MTA and biogenic amines, products of several metabolic processes of common brewing yeast and bacterial strains as well as strains that are responsible for beer spoilage, have been shown to contribute off-flavors to beers. All beers analyzed, regardless of MTA or BA levels, were found to be delicious.

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CONCLUSIONS

The ZipChip microfluidic CE-MS system provides an easy-to-use platform for rapid bioreactor monitoring. These analyses require little, if any, sample preparation and yield high quality MS data with hundreds of quantifiable features in a 4 minute run.

- Heuberger et al., Food Chemistry 200 (2016) 301–307.
- Dandridge et al., Food Chemistry 89 (2005) 519–525.
- www.908devices.com

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ZipChip is for research use only.

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