

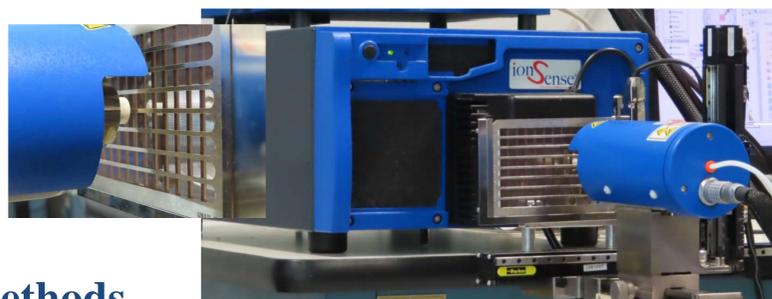
Post-Acquisition Data Analysis Program for Automating Data Processing in High-throughput Experimentation with DART-MS

Rob Goguen, Brittany Laramée, Frederick Li, Paul Liang, and Brian D. Musselman – IonSense, Inc., Saugus, MA 01906

Overview

In recent years the organic synthesis and drug discovery communities are shifting towards screening and optimizing reaction conditions in arrays of 96 or 384 to meet stringent timelines. As analytical methods, such as DART-MS, are becoming faster, the limitation on throughput falls on the time the MS software needs to initialize and close individual files for each sample. All samples therefore need to be acquired in a single data file, which consequently complicates the data analysis. Therefore, it is critical to have software programs that can automate the data processing process and segment the samples into individual files. A post-acquisition software program was developed to automate the parsing of the data and provide heat map views and statistical analysis.

DART-SVP ionization source for high-throughput analysis



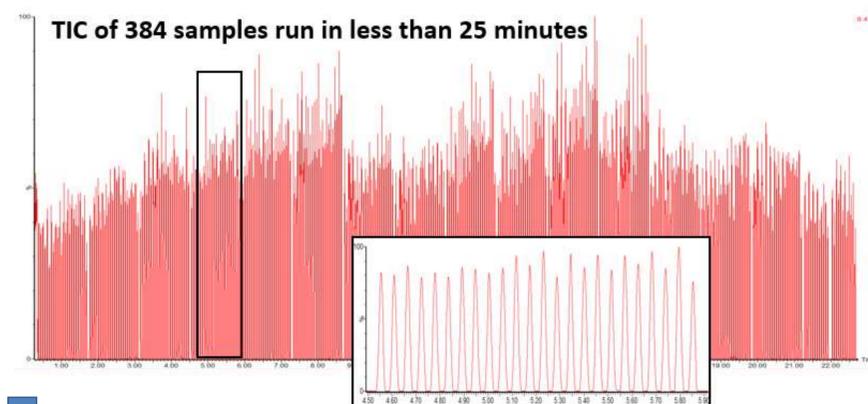
Methods

A DART-SVP high-throughput ionization source was interfaced to a variety of MS systems to generate the datafiles loaded into AnalyzerPro XD software. The DART-HT source & software are compatible on Thermo, Waters, Agilent, Bruker, JEOL, and Shimadzu.

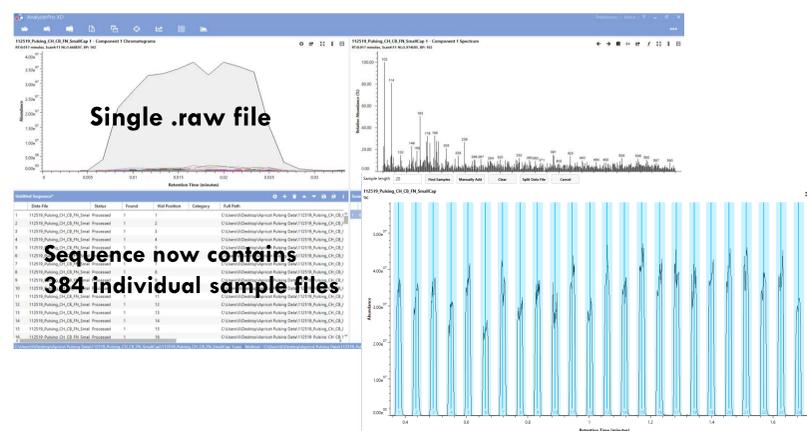
1. A data file containing 96 or 384 samples is first loaded to the software
2. The total ion chromatogram (TIC) is then displayed and the starting and end points are designated by the user. The software's automatic peak detection will then identify all peaks within those two points and parse them into individual data files.
3. Each individual data file is then automatically integrated and the extracted ion chromatogram (EIC) of all mass-to-charge ratios are provided. Target masses can be entered to generate a heat map of the well plate. Statistical analyses on each sample can also be performed in the software.

Experimental Analysis Workflow:

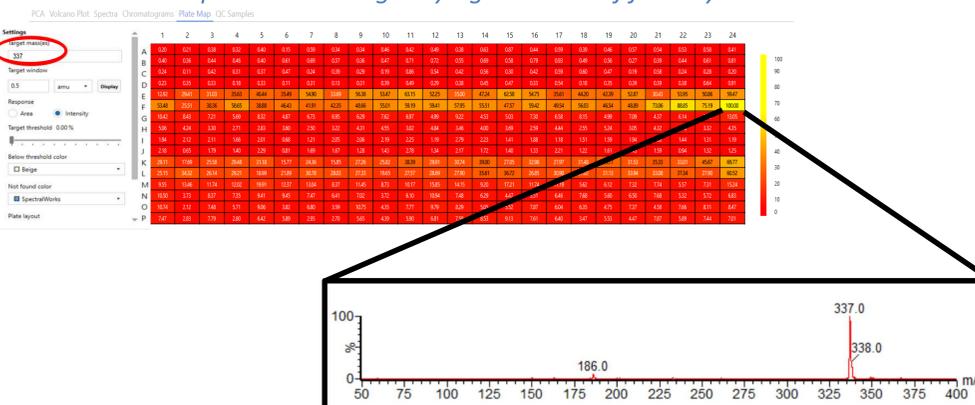
Load a data file generated by DART-MS with 96/384 samples into AnalyzerPro XD



The software's automatic peak detection will then identify all peaks within two points on the datafile and parse them into individual data files.



Target masses can be entered to generate a heat map of the well plate. A 384 well plate containing varying amounts of fentanyl in several rows.



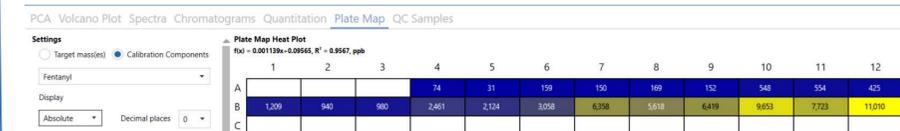
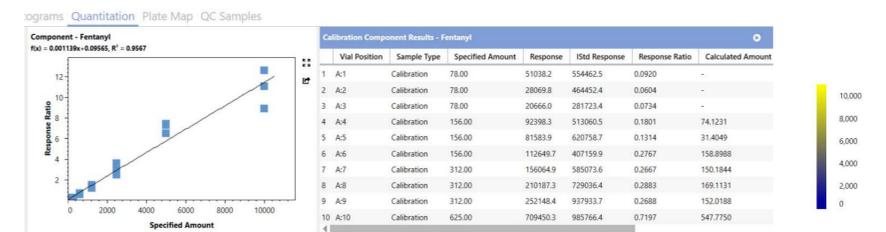
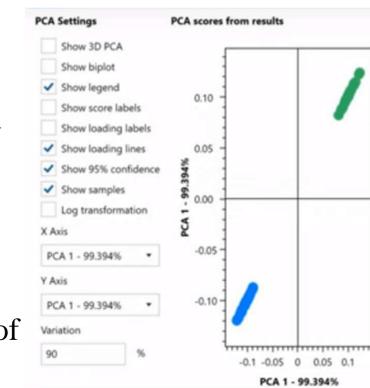
Other Features of AnalyzerPro XD software with DART-MS

PCA / statistical analysis

- Parsed data files can be processed to automatically generate statistical PCA models

Quantitation

- Calibration curves using known quantities of target compounds for quantitation can be generated as part of the post acquisition processing
- Samples can be processed to generate heat maps for absolute quantities of target compounds in each well



Conclusion

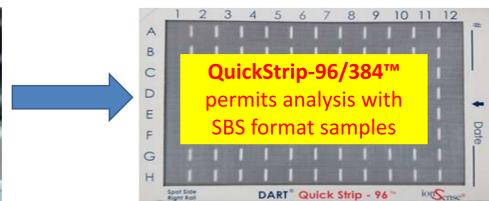
- Automatic peak detection in AnalyzerPro XD allows for parsing of large quantities of samples in a single data file to be separated into separate data files for post acquisition processing.
- Analysis of 384 samples with DART-MS takes less than 25 minutes. Following analysis with parsing by AnalyzerPro XD software for post acquisition processing, detecting targeted compounds in heat mapping format of the 384 well plate takes ~ 2 minutes. This leads to **total analysis and sample processing time for 384 samples screening to be less than 30 minutes.**
- AnalyzerPro XD also offers statistical analysis and facilitated quantitation for post acquisition sample processing

Rapid Analysis of Products from High Throughput Experimentation Utilizing a Novel Pulsed Gas Control System for an Ambient Ionization Source

Brittany Laramée, Scott Oro, Fred Li, Paul Liang and Brian Musselman – IonSense, Inc., Saugus, MA, USA

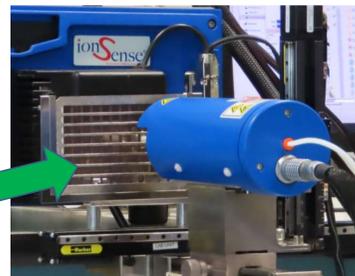
Abstract

High Throughput Experimentation involves the use of small-scale samples to minimize chemical use while permitting synthesis route optimization, biocatalysts performance evaluation and bioanalytical assays while using very low volumes of chemicals, catalyst and reagents. HTE is conducted using microtiter plates potentially generating thousands of samples per cycle. Using nanoliter pipetting and a new pulsed ionizing gas system incorporated into the controller of a direct analysis in real time (DART) source an analysis rate of >20 per minute is demonstrated. Incorporating this pulsed gas method yields data with improved sensitivity, signal-to-noise, and permits simplified peak detection for targeted analytes.



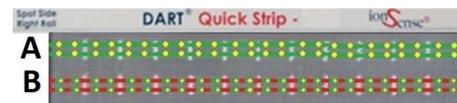
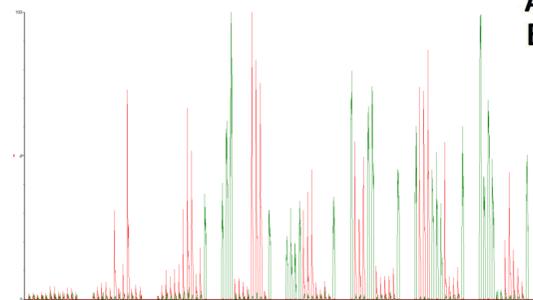
SPT LabTech Mosquito XL

DART-HT on WATERS QDa



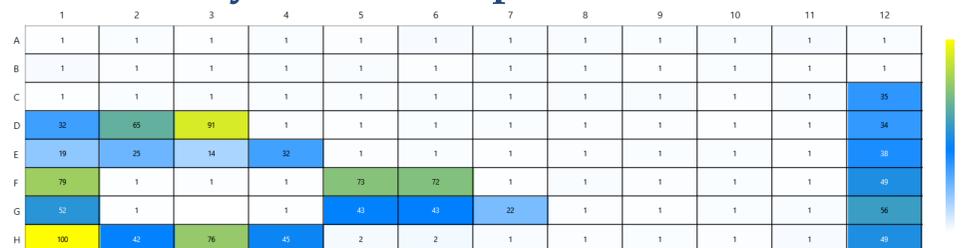
Experiment: What is Pulse Gas

Schemes for sample movement using continuous gas (A) or Pulse Gas (B) DART.



Analysis of 96 samples where starting material EIC (red) is shown relative to Product EIC (green). No ions are generated between samples enabling more efficient peak detection

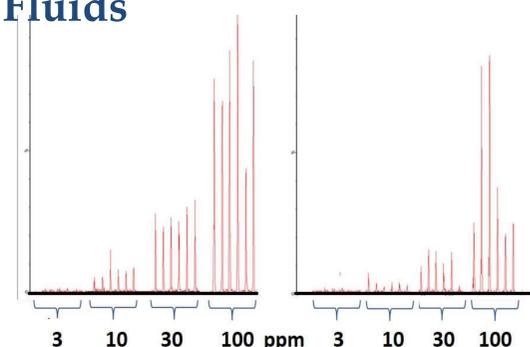
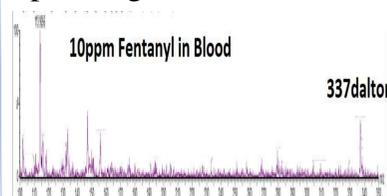
Data Analysis: Route Optimization



Due to time constraints the MS data system cannot collect this data as individual files and manual processing would require days. Here the user selects the mass values of the starting material and product. The Yellow highlights the wells with highest concentration of product. SpectralWorks' AnalyzerPro XD used for data automated data reduction

Experiment: Viscous Fluids

Analysis of blood and plasma using a single quadrupole MS operating in scan mode.



Experiment: Biocatalysis Performance

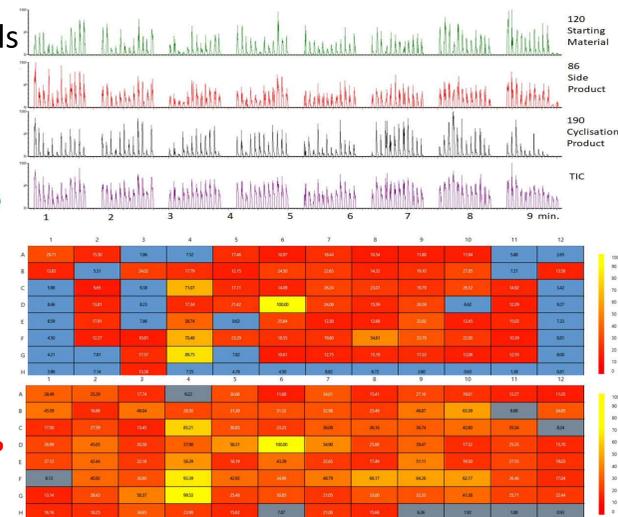
Optimization of the biocatalysts performance is demonstrated by testing the cyclization of a chloroacetamide to afford a gamma lactam with visible light irradiation, completed by using very low volumes of chemicals, catalyst and reagents. For this discussion HTE is conducted using a 96 well microtiter plate with varying reactant concentration and enzyme.

DART-MS 96 wells EIC and TIC



MOST product P
LEAST starting Material SM

MOST Product P
LEAST Side Product SP



Heatmap permits rapid decision-making facilitating progress

Conclusions:

- Utilizing a pulse gas DART source permit higher throughput ambient ionization of samples containing high concentrations of chemicals vs solvent ideal for HTE
- Analysis of 6 -10 samples per minute is enabled with limited sample preparation using low volume samples
- Analysis of samples of biological origin is facilitated with small molecule detection using scanning or MRM

Authors thank Prof. **Todd Hyster**, and **Bryce Nichols**, Princeton U. for generous use of results from their samples. See Biegasiewicz et al., Science 364, 1166–1169 (2019) for details on their Biocatalysis efforts.

Skip Sample Preparation and Facilitate Analysis of Food by Screening with the 24-Pin Sampler with DART-MS

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Overview

Sample preparation techniques can be complicated and often the most time-consuming steps in analysis of a variety of food samples. Directly ionizing samples using DART-MS can eliminate this preparation necessity. Combining a DART source with a versatile sample introduction module called the 24-Pin Sampler was used to improve throughput and facilitate sampling of several different food matrices. Forgoing most sample preparation, the 24-Pin Sampler directly dips into matrices like meat, olive oil, as well as into 384/96 well plates before being analyzed directly by DART-MS. This sampling is useful in obtaining replicates for characterization of food varieties by statistical analysis, screening for specific adulterants, and provides higher-throughput analysis of 24 samples in less than a minute.



Methods

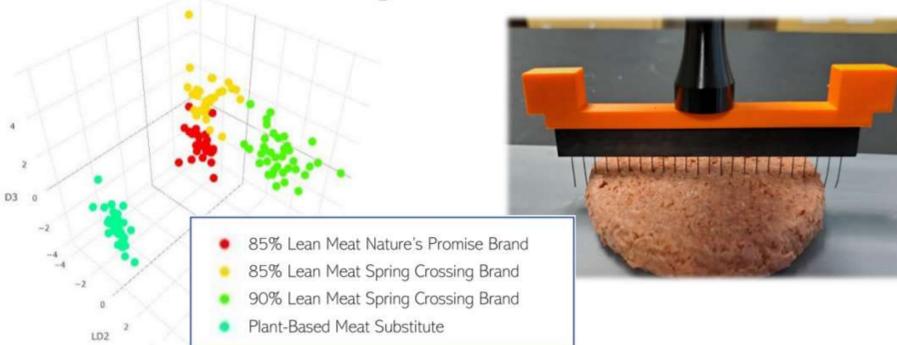
A DART-SVP ionization source is interfaced to a Waters QDa mass spectrometer. The 24-Pin Sampler was directly dipped into various food matrices and placed onto an automated linear rail for analysis.

- 1) Meat varieties directly sampled with the 24-Pin Sampler creating 24 data files in one run for statistical processing into PCA-LDA plots by LiveID software
- 2) EVOO and cheap vegetable oils were sampled with the 24-Pin Sampler, with cheap oils spiked into EVOO between 5-50%. LiveID created PCA-LDA plots to discriminate between adulterated oils.
- 3) Black cohosh supplement extracts are spiked with PDE-5 inhibitor sildenafil, and displayed in a 96 well plate. 24-Pin Sampler is dipped into the well plate for DART analysis. These data files are parsed in AnalyzerPro XD, and target adulterant compounds were displayed with heat mapping when detected.

Experimental:

Characterizing Beef & Plant-Based Meat Varieties with PCA-LDA

- The 24-Pin Sampler was directly inserted into the frozen patty followed by 1 minute DART analysis of 24 samples. The sampler was washed and this was repeated for all 4 meat varieties



- PCA-LDA plot distinguishing meat varieties (organic vs non-organic, brand, and by meat vs plant-based) show **correctness score of 82.50%**

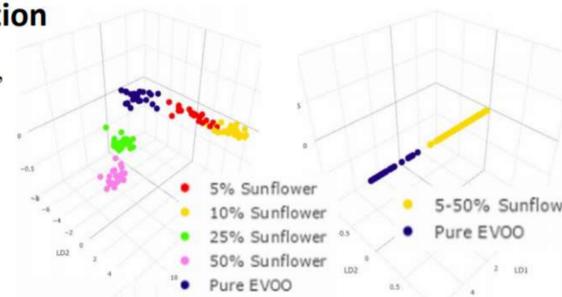
Detecting Sunflower Oil & Sesame Oil Adulteration in Pure EVOO

- Both Sesame and sunflower oil were spiked into pure EVOO at levels between 5-50%
- The 24-Pin Sampler was directly dipped into samples before analysis with no sample prep



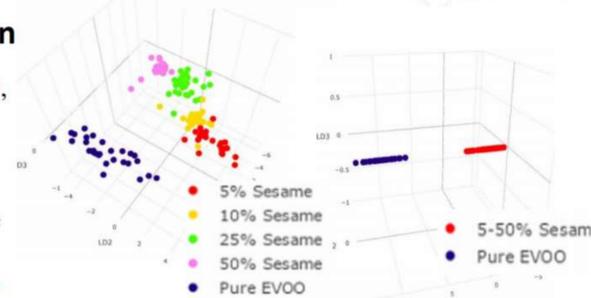
Sunflower Oil Adulteration

- PCA-LDA plot with 5, 10, 25, 50% & pure EVOO groups show **correctness score of 81.43%**
- LDA plot with 5-50% & Pure EVOO groups show **correctness score of 92.14%**



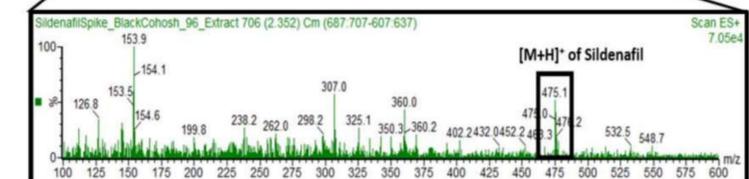
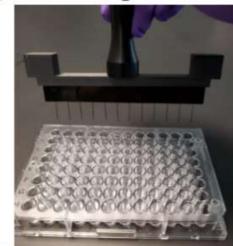
Sesame Oil Adulteration

- PCA-LDA plot with 5, 10, 25, 50% & pure EVOO groups show **correctness score of 88.15%**
- LDA plot with 5-50% & Pure EVOO groups show **correctness score of 97.04%**



High-Throughput Screening for Contaminants in Black Cohosh Supplement Extract

- Black cohosh supplement was dispensed into a 96 well plate, and a few wells were spiked with 1 or 10 ppm solutions of the PDE-5 inhibitor sildenafil.
- The 12-Pin Sampler for 96 well plates (equivalent to 24-Pin Sampler for 384 well plates) was dipped directly into the plate followed by DART-MS analysis of the pins
- The data files generated were parsed with AnalyzerPro XD. The target mass 475 m/z of sildenafil was searched and displayed in the well-plate format with heat mapping when the contaminant was detected



Conclusion

- The 24-Pin Sampler with DART-MS analysis is a versatile tool for screening food matrices with little to no sample preparation. The metal pins are formatted to fit in 96/384 well plates, and consistently pick up viscous liquid like oil or solid residue from burgers
- The 24 pins help quickly create sample replicates for building statistical models to help in characterization and authentication in food such as meat varieties and vegetable oils.
- The 12 or 24-Pin Sampler is formatted to directly dip into 96/384 well plates for screening. Combining this with the targeted heat mapping of contaminants in AnalyzerPro XD allows for a quick and easy screening technique.