Acoustic Mist Ionization for Ultra-High Throughput Mass Spectrometry

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Introduction

The most popular method for high throughput screening employs optical readers. Optically active chromophores or fluorophores are attached to the analytes of interest and their absorbance or fluorescence is detected. While optical readers offer speed and sensitivity, the technology has limitations in selectivity and cost. Lack of selectivity presents the potential for generating false positives. The cost of reagents alone for a 300,000 compound screen can easily exceed $50,000.

Value of MS for Drug Discovery Screening

An alternative method for detection is Mass Spectrometry (MS). MS offers both high sensitivity and high selectivity. Today's state-of-the-art high resolution systems deliver accurate mass capability of <1 ppm, allowing the accurate identification and quantification of analytes in complex mixtures. However, current MS systems typically rely on HPLC, UPLC or automated Solid Phase Extraction (SPE) to deliver the sample to the mass spectrometer. Being a relatively slow technique limits their adoption in the area of high throughput screening. A comparison of their relative throughput is shown in the table below. Times are given for a single sample, analysis of a 384-well microplate and prosecution of a 50,000 compound screen.

Technique	Analysis Time	384-well Plate	50k Screen
Capillary ID	5 minutes	1.33 days	175 days
UHPLC/MS	1 minute	6.4 hours	35 days
Auto SPE/MS	10 seconds	64 minutes	6 days

TABLE 1: Comparison of throughput for common MS inlets. Times are given for a single sample, analysis of a 384-well microplate and prosecution of a 50,000 compound screen.

Enter Acoustic Droplet Ejection

Unlike pipetting, Acoustic Droplet Ejection (ADE) transfers liquid without contacting the sample. Although ADE sounds complex, the process is actually very simple. An acoustic transducer focuses ultrasonic waves into the bottom of a microplate well causing the liquid at the surface to eject tiny droplets (~2.5nL) from the well. In the Labcyte Echo series of Liquid Handlers, an inverted microplate is positioned over the source well, captures the droplets and retains them due to surface tension. An X & Y stage moves each plate independently so that liquid from any well of a source plate can be transferred to any well of a destination plate in a precise, fast, low cost and no cross-contamination process.

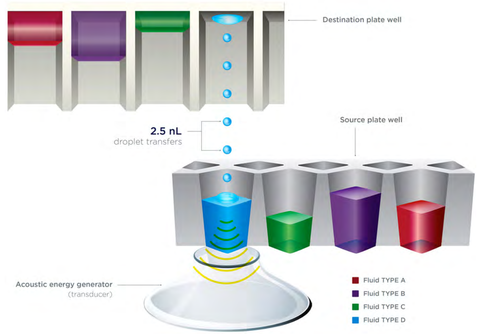

FIGURE 1: Echo Liquid Handlers have a transducer that emits low energy sound waves to eject 25 nL droplets from a source plate to an inverted destination plate above.

Acoustic Mist Ionization MS

At Labcyte, we have demonstrated how acoustic parameters can be adjusted to create a mist of tiny (1-10mm) droplets. By positioning a sampling nozzle directly over the well, we can draw the mist into a heated transfer line connected to a mass spectrometer. The sampling nozzle has a high voltage applied of the opposite polarity to the ionization mode. This causes charge separation within the well such that the ejected mist contains charged droplets of the desired polarity. The droplets are de-solvated, subsequently forming ions which then enter the mass spectrometer where they are identified and quantified. The whole process of positioning the plate, ejecting the mist and detecting the analytes can be accomplished at a rate of **3 samples per second. A 384-well plate can be read in under 3 minutes and a 50k compound screen prosecuted in 4.6 hours**. Because there is no direct contact with the sample, carryover and cross-contamination are eliminated.

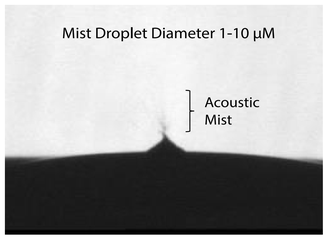

FIGURE 2: Image of acoustic mist being released. Using an acoustic transducer, droplets of similar dimensions may be produced directly from a microtitre plate well. When an electric field is applied during ejection, these droplets become charged and can be used as an ionization source for mass spectrum analysis.

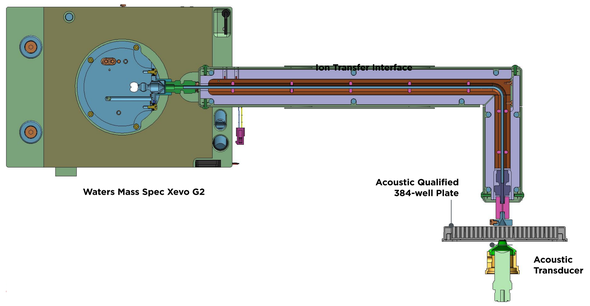

FIGURE 3: A schematic of the latest ion transfer tube assembly, used for directing charged material from the 384-well microtitre plate to the mass spectrometer.

High Throughput Screening Experiment

Our collaboration with AstraZeneca and Waters has given us the opportunity to develop the acoustic MS system and test it in a drug discovery environment with real life samples. Using a prototype acoustic MS, AZ were able to demonstrate a reduction in development time for one assay down to 2 weeks. As proof of the system's high throughput capability, a 240,000 compound screen was completed in less than 66 hours with Z' of >0.65. Because there was no direct contact with the samples and detection is label-free, there was no evidence of cross-talk, carry-over or interferences. AZ has also used the system to triage 'hit' wells for false positives.

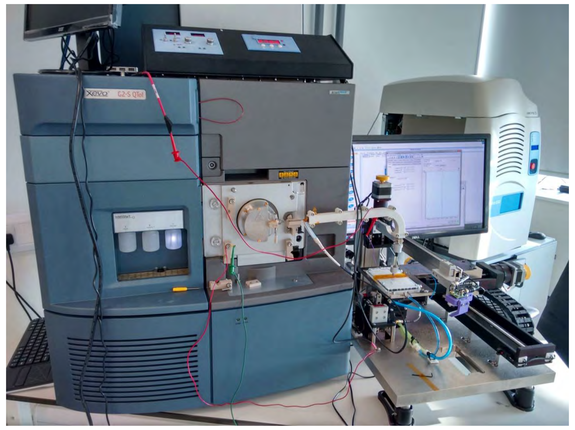

FIGURE 4: Acoustic MS prototype system using Waters Xevo G2 QTof at AstraZeneca's Alderley Park site.

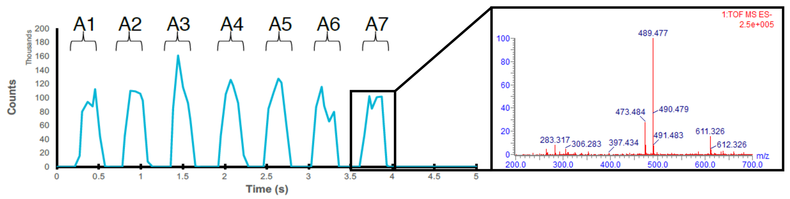

FIGURE 5: Acoustic-MS software captures the signal intensity for each well (A1 – A7) of a 384-well plate. The mass spectrum on the right is taken from well A7.

Lipodomic and Metabolic Profiling Using AMI-MS

The system was tested to determine its suitability for lipidomic and metabolic profiling. MCF7 (breast cancer) cells were first profiled in positive and negative ion modes. Selected measurable disease-relevant metabolites and lipids are listed below. The cells were then treated with glutathione-depleting compounds and changes in metabolites and lipids were followed over time.

Selected metabolites and lipids
L-Glutamine / L-Glutamate
L-Asparagine / L-Aspartate
Citric acid
(Glycero) Phosphocholine
Hexose phosphates
Inositol cyclic phosphate
AMP, ADP, ATP
UDP-glucose
Oxidised & reduced gluthathione
Free fatty acids
Cholesterol sulfate
Sphingomyelins (SM)
Glycerophosphates (PA)
Glycerophosphoserines (PS)
Glycerophosphoinositols (PI)
Glycerophosphocholines (PC)
Glycerophosphoethanolamines (PE)

TABLE 2: Selected metabolites and lipids that have been profiled using Acoustic Mist Ionization-MS.

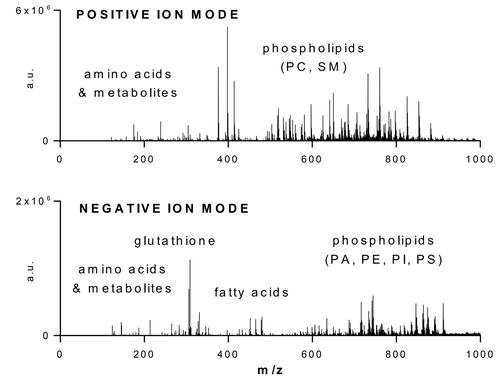

FIGURE 6: The mass spectra above show suitability for lipidomic and metabolic profiling in both positive and negative ion modes.

Reduction of glutathione levels resulted in a number of changes in the metabolome and lipidome of the treated cells: depletion of ATP, activation of glycolysis and pentose phosphate pathway, reduction in fatty acid synthesis due to lack of NADPH. This workflow will be extremely useful for cell-based screening and mechanistic studies.

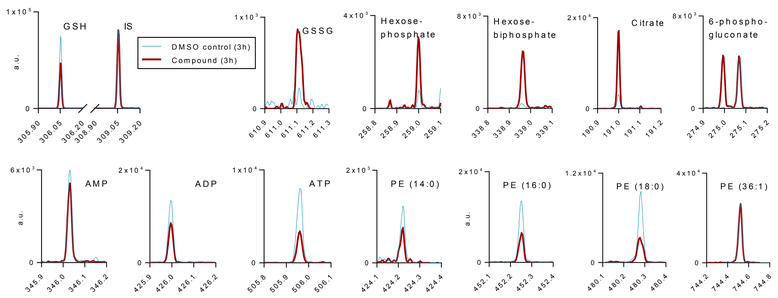

FIGURE 7: Mass spectra for select metabolites show a change in abundance when treated with glutathione-depleting compounds.

Summary

- Acoustic Mist Ionization can deliver high quality MS data at 3 samples/second
- We were able to obtain baseline spectra for the MCF7 cells
- Analytes observed included amino acids, phospholipids, fatty acids and various small molecule metabolites
- Changes in the metabolome and lipidome due to glutathione level reduction could be observed
- Line of sight expected to be 200,000 samples per day

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