ACOUSTIC MIST IONISATION (AMI) A RAPID APPROACH FOR THE DEVELOPMENT OF MASS SPECTROMETRY LIBRARIES

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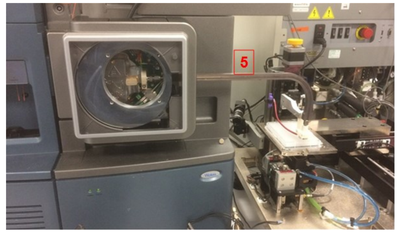
OVERVIEW

- **Investigations into the use of ambient acoustic mist ionisation (AMI) as an approach to build non-targeted mass spectrometry libraries.**
- **AMI coupled with Q-ToF MS has rapidly determined the appropriate polarity of ionisation and fragment ions, for a series of analytes relevant in forensic toxicology and food additive compounds, supporting the feasibility of such an approach.**
- **Compatibility of MS, MS/MS, data independent analyses (DIA) including MS^E and SONAR combined with AMI have been investigated.**
- **A data processing strategy has been employed that will enable a complete plate of analytes to be acquired in one data file and detected as individual analytes.**
- **The acquisition strategy employed is rapid; a subset of 30 forensic toxicology analytes (3 AMI ejections) were acquired in 60 seconds and processed in 7 seconds.**

INTRODUCTION

Ambient ionisation comprises mass spectrometric techniques which allow the direct analysis of sample surfaces with little or no sample pre-treatment e.g. Direct Infusion (DI), Direct Analysis in Real Time (DART) or Laser Diode Thermal Desorption (LDTD). Development of such novel analysis techniques has resulted in many applications being developed. We present direct acoustic ionisation mass spectrometry to deliver a proof of concept high-throughput strategy to develop mass spectrometry libraries.

A modified Echo (Labcyte, CA, USA) acoustic liquid handler has been used to eject microdroplet sprays of electrospray amenable analytes directly into a Q-ToF platform MS system.(1) A custom XY-stage has been integrated to move the plate and position wells over the piezoelectric transducer for acoustic sampling. The microdroplets may be charged via application of an electric field and are collected at the entrance aperture of a transfer tube interface. Data for each class of analyte were acquired into a single acquisition file and single component results extracted using an analyte eject time dependent targeted processing strategy. The Echo-MS was used at varying speeds with minimal sample consumption, using MS, and data independent MS^E/SONAR in positive or negative ionisation modes. Investigations into LCMS and AMI electrospray strategies to build MS libraries have been compared for two classes of analytes (a series of analytes that are relevant in forensic toxicology and food additives).



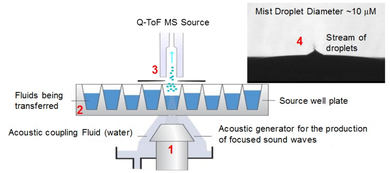


Figure 1. Acoustic mist ionisation mass spectrometry (AMI-MS). An externalised acoustic transducer (1) from an echo dispenser emits sound waves into a liquid sample in a 384-well plate (2) located on a moving XY-stage. High voltage is applied to a charging cone (3) suspended directly above the transducer, inducing charge separation in the sample. A mound (4) is formed on the meniscus and µm-sized charged droplets are sprayed off directly through an insulating piece (5) into a heated transfer tube leading to the source of a mass spectrometer.

METHODS

AMI: MS System: Waters Xevo G2-XS Q-ToF coupled with AMI source (Labcyte)
MS Acquisition modes: MS, MS/MS, MS^E, SONAR
AMI Ejections Per Sample: 1, 3 and 10
SONAR: Quad window 10 Da
Collision energy: 25 eV

UPLC-MS: MS System: Waters Synapt G2-*Si*
ESI positive. Desolvation Temperature: 550 °C. Acquisition Modes: HDMS^E. Mass Range: 50-1200 Da. Acquisition rate: 10 spectra/second. Capillary Voltage: 1 kV. Cone Voltage: 20 V. Drift Gas: N_2. Collision Energy Ramp: 10-45 eV. IMS Wave Velocity Range: 650 m/s. IMS Wave Height: 40 V. IMS Gas Flow: 90 mL/min. Lockmass: leucine enkephalin. Collision cross-section (CCS) calibrant: IMS Q-ToF calibration kit.

LC System: Waters ACQUITY UPLC I-Class
Column: Waters ACQUITY UPLC BEH C18 (50 mm x 2.1 mm, 1.7 µm)
Column temperature: 45 °C Flow: 0.4 mL/min
Mobile phase: (A) water (0.1% formic Acid) and (B) acetonitrile (0.1% formic Acid).

UPLC gradient: Reverse phase separations (0.45 mL/min) at 45 °C were performed using the gradient: 0–0.14 min isocratic at (99:1 (A:B)); 0.42 min (85:15); 0.83 min (50:50) 1.25 min (95:5) 1.26 min (99:1) 2.5 min (99:1.0). Injection volumes of 10 µL were employed.

DATA PROCESSING.

All data processing was performed using UNIFI 1.9.2.

RESULTS AND DISCUSSION

The non-targeted screening strategy employed using AMI was compared with a conventional non-targeted rapid gradient approach, developed using UPLC-MS, which enabled polarity of ionisation, precursor, product ion(s) and collision cross-section (CCS) values to be determined. With the aim of providing greater time efficiency in the production of mass spectrometry libraries, a proof of concept study was performed using the integrated Echo-MS platform described in Figure 1, where non-targeted MS libraries may be developed by dispensing sample directly from the micro plate into the mass spectrometer.

A variety of MS parameters have been investigated. The analysis time to determine optimum analyte ionisation mode and analyte fragment ions has been compared to that of a conventional rapid gradient ESI library building strategy. The impact of sampling rate on the MS strategy employed to generate fragment ions using MS, MS^E, SONAR have been investigated.

A set of 50 "unknown" forensic toxicology analytes were provided for AMI screening. The results are presented in Figure 2, where the AMI TIC generated is presented. Initial exploratory positive comprised AMI screening of forensic toxicology compounds using 10 AMI ejections, with blanks and leucine enkephalin lockmass acquired between analyte well ejections.

The analytes, lockmass and blanks were acquired in both positive and negative ion modes, in 10.5 min per ionisation mode, giving an average analysis time of 0.2 min for each respective analyte (12 times faster than the 2.5 min UPLC gradient strategy developed). Data for each analyte were acquired into a single acquisition file and single component results extracted using an analyte eject time dependent targeted processing strategy, which can be treated as an effective retention time. Once the data was acquired, empirical information with respect to each analyte was provided, enabling all analytes to be detected in a single acquisition data file.

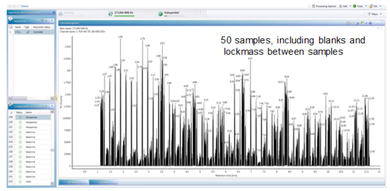


Figure 2. Example of AMI total ion current (TIC) generated from ejections of individual samples in a 384-well plate. Initial exploratory positive AMI screening of forensic toxicology compounds using 10 AMI ejections, with blanks and leucine enkephalin lockmass acquired between analyte wells.

The rapid acquisition strategy produces small data file sizes, which results in rapid data processing times, (data file comprising 50 analytes processed in 37 sec). The data generated was confirmed to be representative of that acquired using a well characterised forensic toxicology method.(2,3) Based upon the success, the quality of mass spectrometry data generated with decreasing AMI ejection number was explored.

Figure 3 presents the positive AMI TIC generated from individual forensic toxicology analytes where 3 ejections per analyte well were performed; analysis time per analyte was approximately 2.1 sec and the quality of mass spectrometry data acquired was not compromised. Inset in Figure 3 is the AMI TIC for 1 ejection, taking approximately 0.8 sec per analyte can be seen, sufficient spectral quality was achieved for 90% of the analytes analysed compared to using 3 AMI ejections.

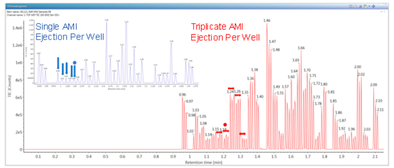


Figure 3. Positive AMI TIC generated from individual forensic toxicology compounds using single and triplicate ejections from a 384-well plate (atenolol highlighted).

The quality of MS^E data may suffer where analytes are provided in complex matrices or dimethyl sulfoxide (DMSO) as in the case of many commercially available libraries, hence it was decided to explore the use of SONAR mass spectrometry acquisition strategy.

SONAR is a relatively new mode of DIA discovery, whereby the quadrupole is scanned repetitively, isolating specific mass regions over alternating low and elevated-energy scans prior to oa-ToF.

The data produced is of a similar specificity to ion mobility data. The alternate MS scans comprise precursor/CID product ions respectively, with the product ions derived from the specific low-energy region scanned. Unlike MS^E where fragment ions are derived from the full MS mass range. Hence the feasibility of combining SONAR and AMI was explored to provide enhanced specificity where sample and matrix complexity occurs. Figure 5 presents the AMI SONAR MS^E acquired data for atenolol, showing the low and high energy scans for the precursor ion *m/z* 267 and its product ions. To generate this data, it was necessary to increase the number of AMI ejections; this illustrates the potential and flexibility with which AMI can be utilised and controlled to generate high quality spectra using different designs of MS experiments.

Figure 4. Positive AMI extracted mass chromatogram for atenolol and corresponding MS^E precursor/fragment ion MS spectra.

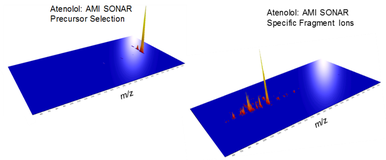


Figure 5. Positive mode AMI SONAR MS^E precursor/product ions of atenolol.

The utility and the potential to rapidly generate mass spectrometry data using AMI is illustrated in Figures 6 and 7, where ionisation profiles of food additives (examples sweeteners/food colourings) are shown. When comparing AMI MS and UPLC MS, evidence of the sodiated adducts is clear in the AMI data. UPLC conditions incorporating formic acid reduce the formation of sodiated adducts. The data illustrates a potential, to further consider the sample preparation prior to AMI in order reduce adducting or enhance ionisation efficiency.

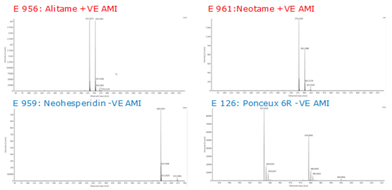


Figure 6. Positive and negative AMI MS spectra obtained for screening of food additive standard compounds (example for E 961, E 956, E 959 and E 926 presented).

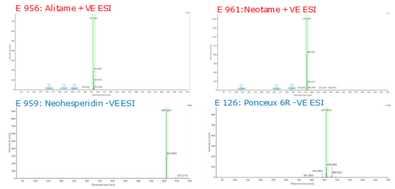


Figure 7. Positive and negative UPLC ESI MS spectra obtained for screening of food additive standard compounds (example for E 961, E 956, E 959 and E 926 presented).

CONCLUSION

- **By leveraging the speed, accuracy, and precision of the acoustic mist ionisation technology with MS detection we have illustrated that the developed platform can be used for a non-targeted MS library building strategy.**
- **The proof of concept of a non-targeted AMI screening strategy uses high speed, low sample consumption and low solvent consumption, which importantly, simultaneously provides cost reduction with environmental benefits.**
- **Using this approach, we were able to compare the results of a "blind test", where analyte identity was only provided after both LC/MS ESI and AMI ESI were performed, illustrating the comparability of AMI and ESI.**
- **Ambient AMI and ESI have been shown to be comparable.**

References

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