A High Throughput Mass Spectrometry Plate Reader: Acoustic Droplet Ejection to an Open-Port Probe Sampling Interface

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INTRODUCTION

Label-free LC/MS based screening technology is routinely used in pharmaceutical industries for hit discovery and various ADME profiling applications. Although the current analysis speed of <30 seconds per sample is quite promising, it still cannot match the throughput provided by plate-reader based HTS platforms. Acoustic droplet ejection (ADE) is a droplet transfer technology capable of high speed, reproducibility and absolute accuracy. In this work, we successfully couple ADE and the standard ESI ion source of a mass spectrometer with the open-port probe (OPP) sampling interface. Screening speeds as fast as 0.4 seconds-per-sample are demonstrated with superb sensitivity, great reproducibility even without internal standard, good quantitation capability, no matrix effect, and broad compound coverage.

MATERIALS AND METHODS

In this study, an Echo® acoustic liquid handler has been modified to drive a custom stage that moves a 384-well microplate to position wells over a piezoelectric transducer for acoustic sampling. The OPP sampling interface is positioned above the plate so that 2.5-nL sized droplets ejected by ADE are quantitatively captured at the sampling end of the OPP sampling interface (Figure 1A). This interface uses a vertically aligned, co-axial tube arrangement enabling solvent delivery down to the sampling end of the device through the tubing annulus with solvent aspiration up the center tube and into the standard ESI ion source of a SCIEX QTRAP 6500+ MS system (Figure 1B). Figure 1C and 1D show the representative data collected with the ADE-OPP-ESI-MS system for a full 384-well plate.

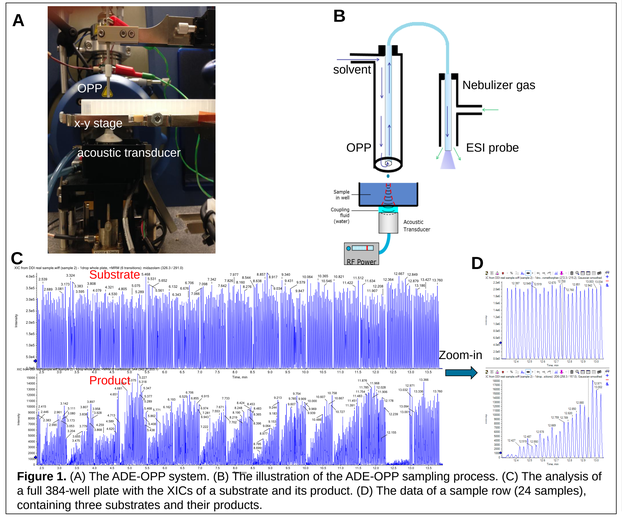

Figure 1. (A) The ADE-OPP system. (B) The illustration of the ADE-OPP sampling process. (C) The analysis of a full 384-well plate with the XICs of a substrate and its product. (D) The data of a sample row (24 samples), containing three substrates and their products.

RESULTS

The sensitivity of the ADE-OPP system was evaluated by ejecting the 10 to 1 drops ladder (25 nL down to 2.5 nL) of 25 nM propranolol. The sensitivity was about 1 amol loading. A blank injection was in between of each two sample injections. The background was very clean without any carry over.

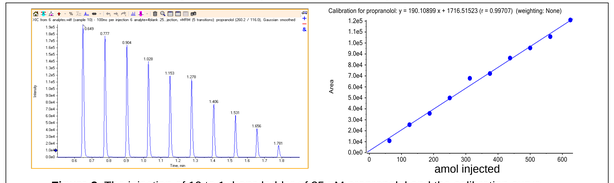

Figure 2. The injection of 10 to 1 drops ladder of 25 nM propranolol and the calibration curve.

The sensitivity and linear dynamic range (LDR) were further evaluated by ejecting a single sample drop (2.5 nL) containing the analytes with the concentration ranging from 10 nM to 10 μM. The calibration curves shown in Figure 3 indicate the LDR at least 3 orders of magnitude without internal standard.

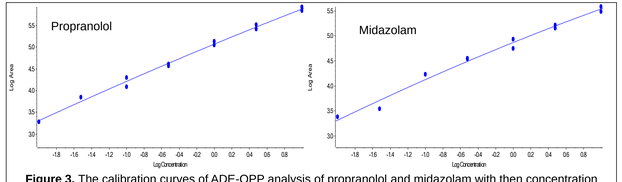

Figure 3. The calibration curves of ADE-OPP analysis of propranolol and midazolam with then concentration ranging from 10 nM to 10 μM. The sample injection volume was 2.5 nL (single drop).

The reproducibility and the analysis throughput were demonstrated with the results shown in Figure 4. The full 384-well plate containing four different compounds was analyzed in 2.8 min (~0.4 sec per sample), with the inter-well CV of 4-8% without internal standard.

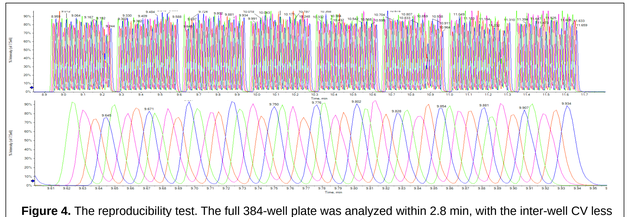

Figure 4. The reproducibility test. The full 384-well plate was analyzed within 2.8 min, with the inter-well CV less than 8% without IS.

The same analysis speed (0.4 sec per sample) was used for the HLM metabolic stability half time test. The results were cross-validated with the LC-MS approach.

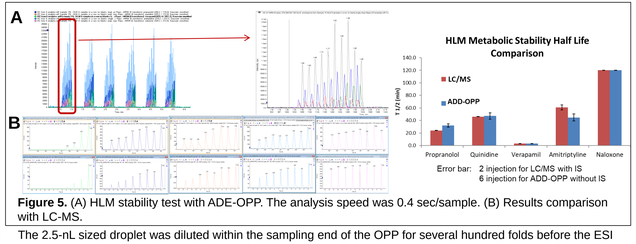

Figure 5. (A) HLM stability test with ADE-OPP. The analysis speed was 0.4 sec/sample. (B) Results comparison with LC-MS.

The 2.5-nL sized droplet was diluted within the sampling end of the OPP for several hundred folds before the ESI process, thus no matrix effect was observed. The same sensitivity was demonstrated as in the neat solution even for the samples containing the detergent, as shown in Figure 6.

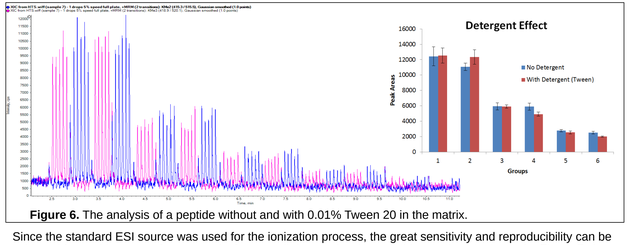

Figure 6. The analysis of a peptide without and with 0.01% Tween 20 in the matrix.

Since the standard ESI source was used for the ionization process, the great sensitivity and reproducibility can be achieved for a wide range of compounds, from small drug molecules, peptides, to the intact protein. Figure 7 shows the analysis of the intact antibody with the molecular weight about 150 K. The sample loader amount below 1 fmol can be reliably detected.

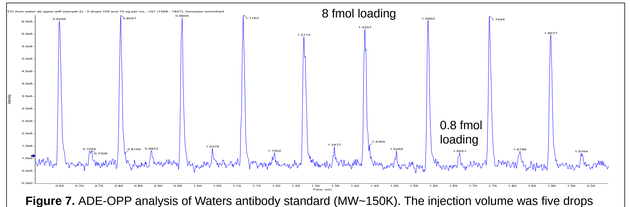

Figure 7. ADE-OPP analysis of Waters antibody standard (MW~150K). The injection volume was five drops (12.5 nL), and the concentration was 667 nM and 67 nM, respectively .

Another benefit of this ADE-OPP system is the flexible length of the data collection window. The continuous droplet injection extends signal time, allowing the wide window for tuning or quantifying more analytes simultaneously. Figure 8 shows an example of continuous ejection the droplets at 10 Hz (10 drops per second) for 20 sec. The total sample consumption amount was only 0.5 μL to get a 20 sec wide window. The analyte was the intact myoglobin. The charge envelope and the deconvolution result were shown in Figure 8 as well.

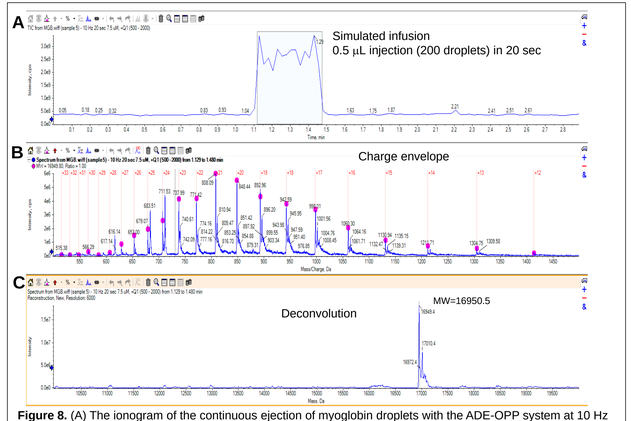

Figure 8. (A) The ionogram of the continuous ejection of myoglobin droplets with the ADE-OPP system at 10 Hz (10 drops per sec) for 20 sec. The total sample injection amount was 0.5 μL. (B) The MS spectra showing the charge envelope of the intact myoglobin. (C) The deconvolution result based on the charge envelope data. The calculated MW matches well with the theoretical value.

CONCLUSIONS

The ADE-OPP system is introduced here as an ESI-MS based high-throughput plate reader. The sample droplet acoustically ejected from the microtiter plate is quantitatively captured by OPP and delivered to the standard ESI source for analysis. High sensitivity (attomol loading for small molecules, or sub-femtomol for intact antibody) and reproducibility (<8% CV without IS) was demonstrated, showing good linearity over at least three orders of magnitude even without IS. The "classic" ESI ion source used in this setup enables detection of a broad range of analytes including small drug molecules, peptides and large proteins (e.g. antibody with MW~150 kD). The analysis speed can be as fast as 0.4 sec per sample. No matrix effect or carry-over was observed, so the system can be used for the direct analysis of unprocessed samples.

TRADEMARKS/LICENSING

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