The Only Thing Faster Than Ultra-Fast Is Instantaneous

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Abstract

True high-throughput screening of candidate compounds during drug discovery has been hindered by the limitations of traditional liquid chromatography (LC), such as method development, sample preparation and sample throughput that prevents it from being more widely applicable. Acoustic Ejection Mass Spectrometry (AEMS) leveraging Acoustic Droplet Ejection (ADE) technology combined with an Open Port Interface (OPI) allows sample analyses at speeds up to 50 times faster than conventional LC-mass spectrometry.

Need to Speed Up High-Throughput Drug Discovery

Drug discovery today involves evaluation of massive compound libraries of thousands, or even millions, of potential drug candidates. High-throughput screening (HTS) to assess the pharmacokinetic (PK) properties of molecules is essential for lead candidate selection in a practical timeframe. Analyses are typically performed using plate reading technology because of the speed, but have inherent development costs and specificity issues. Liquid chromatography (LC), or high-performance LC (HPLC), for separation and mass spectrometry (MS) for detection have the specificity and information rich data, but currently lack the speed for wider applicability.

When plate reading technology is used, the output is typically based on a reaction of the compound that generates an absorption or emission. This is a simple end point, but lacks specificity and can become complicated to develop and troubleshoot. Often, secondary screens are needed to evaluate false positives. LC-MS/MS methodologies currently have high specificity but low throughput due to sample preparation and separation workflows, which are rate limiting bottlenecks for HTS. There is a need for simple ultra-high throughput sampling that directly measures product and substrate, enables HTS screening with complex biology and delivers unequivocal endpoints, to reduce ambiguity and enable better decision-making on progression of a therapeutic along the development pipeline.

Simplification Through LC Elimination

Acoustic Ejection Mass Spectrometry (AEMS) combines both Acoustic Droplet Ejection (ADE) technology and Open Port Interface (OPI) technology [1] to enable direct contactless sampling into an electrospray ionisation mass spectrometer (ESI-MS). It has the potential to eliminate the limitations associated with LC-based sample introduction. At the same time, the technology provides access to ultra-fast sampling at the same speeds as the incumbent plate reader technology in HTS, but with the specificity and information-rich data of MS.

With AEMS, nanolitre-sized sample droplets are ejected via the application of sound waves focused at the bottom of the sample well. The ejected samples from the microwell plate are captured into the vortex of the OPI. The sample is then diluted with carrier solvent and delivered directly to the electrospray source, ionised via conventional electrospray and detected by the mass spectrometer (Figure 1). No injection needles or loops are required. Sample introduction into the MS is achieved through a contactless interaction of the ADE with the OPI, virtually eliminating sample-to-sample carryover, even when moving between concentration extremes. Matrix suppression effects are also essentially eliminated due to dilution effects that occur within the OPI, allowing for clean and sensitive analyses for most molecules in drug discovery.

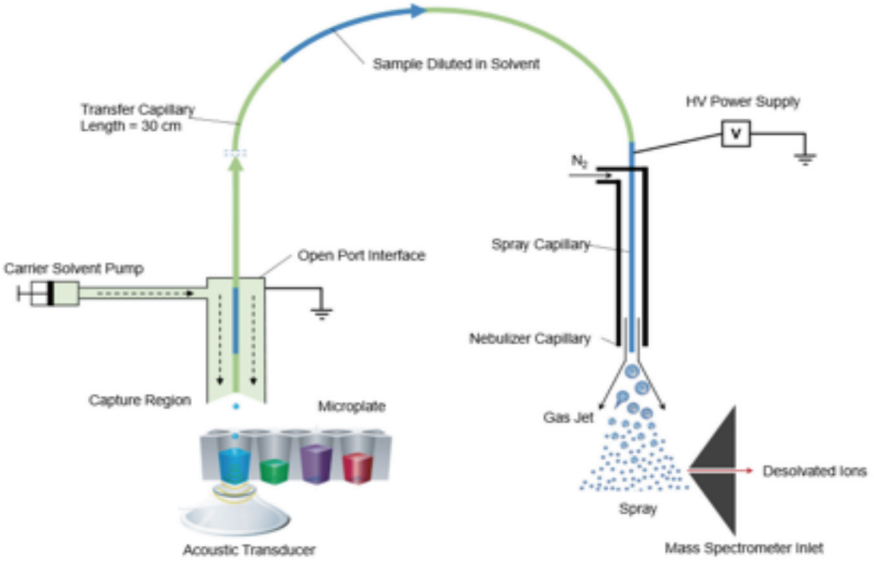

Figure 1. Acoustic Ejection Mass Spectrometry (AEMS) setup for contactless electrospray ionisation mass spectrometry (ESI-MS).

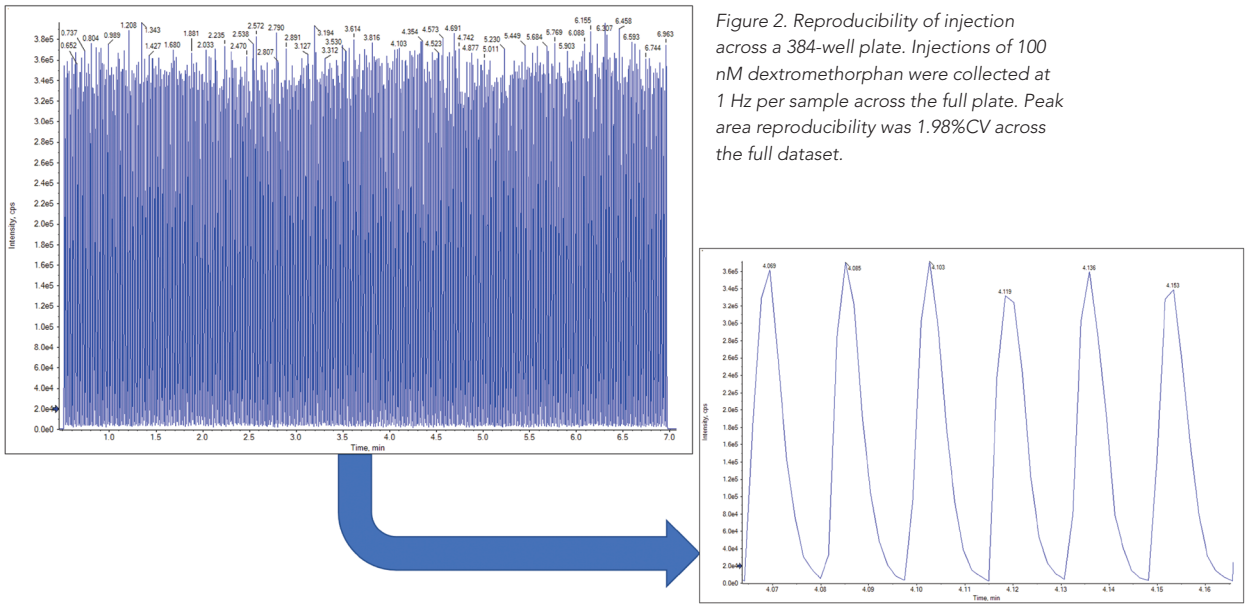

Figure 2. Reproducibility of injection across a 384-well plate. Injections of 100 nM dextromethorphan were collected at 1 Hz per sample across the full plate. Peak area reproducibility was 1.98%CV across the full dataset.

Pushing the Throughput Barrier

Researchers at Boehringer Ingelheim successfully applied an AEMS setup for ESI-MS using an ADE-OPI-MS setup for high-throughput drug metabolism and pharmacokinetics analysis using dextromethorphan and d3-dextrorphan (each 100 nM) as simulants [2]. The interface of an ATS-G4P acoustic droplet emission system with a QTRAP® 6500+ mass spectrometer with a modified OptiFlow® Turbo V Ion Source resulted in creation of the ADE-OPI-MS setup. Various modifications and additional equipment resulted in a system that pushes the speed limit of ESI-MS to 3 Hz (or 3 samples per second) for sampling and detection with baseline separation of each sample.

At a sampling rate of 3 Hz, the contactless sample injection method took significantly less time (7.5-fold faster) than the fastest ESI-based systems, and was even faster than the maximum high-throughput screening throughput of MALDI at 2.5 Hz [2]. Furthermore, using lower sampling rates of 1 or 3 Hz, unprocessed samples containing high concentrations of plasma, cell lysate and other matrices (enzyme assay buffer and crude dog plasma) were analysed without observance of ion suppression, suggesting that physiological conditions of samples can be maintained prior to injection [2].

Acceleration of Drug Discovery

With no LC requirements, AEMS eliminates the need to develop separation methods, troubleshoot LC problems, and wait for LC columns to wash and equilibrate and analytes to elute. Faster turnaround times are possible for the same number of samples and compounds, or more compounds, larger sample sets and bigger cohorts can be analysed in the same time frame as conventional LC-MS. As a result, dead ends and false positives can be found earlier, allowing teams to make critical decisions faster and dramatically accelerate project turnaround times from months to weeks, days or even hours or minutes.

Although there have been significant advances in high-throughput technologies for rapidly screening reaction conditions, collecting analytical data has been an issue. With current MS systems, it can take hours or days to analyse high-density experiments. Combining an ultra-throughput (UT) reader platform with the direct sampling associated with ADE-OPI-MS has been shown by Pfizer scientists to overcome this limitation and support parallel medicinal chemistry and reaction screening efforts [7].

In this study, three sets of experiments were conducted that mimic standard high-throughput experimentation (HTE) and parallel medicinal chemistry (PMC) screening processes:

1. Reaction optimisation for a single transformation (palladium-catalysed C–N coupling of 3-bromopyridine with 4-phenylpiperidine) with variable catalyst and base conditions.
2. Parallel synthesis of a library of drug-like small-molecules involving reaction of common template structures with different monomeric reagents (amidation of two secondary amine templates, each with 96 carboxylic acids for a total of 192 reactions).
3. Similar library synthesis but involving the reaction of a larger template substrate with small-molecule reagents (amide formation between a commercially available DNA fragment possessing an amine reaction point and a set of 96 acids).

The results for each ADE-OPI-MS experiment were compared to those obtained using mainstream high-throughput MS platforms. In all three cases, much less sample (a few nanolitres vs. dozens of nanolitres or microliters) was required, allowing more material to be saved for further analysis. The sampling speed was also an order of magnitude faster, such as ~7.5 minutes for ADE-OPI-MS vs. 5 hours for UHPLC-MS in experiment two.

Importantly, the use of ESI-MS provided accurate quantitative data similar to that obtained using LC-MS and better than that observed with MALDI, without the need for an internal standard, even for reactions involving larger, complex species such as synthetically modified oligonucleotides. In addition, the coverage was greater than that possible with MADLI. Furthermore, use of a high-resolution TOF MS instrument enabled unambiguous identification of both smaller and larger substrates.

While all of the analyses were performed offline after reaction quenching, the researchers suggest that due to the ability to directly sample without any need for sample cleanup, online reaction monitoring should be possible with ADE-OPI-MS, offering the potential to evaluate reaction kinetics in real time.

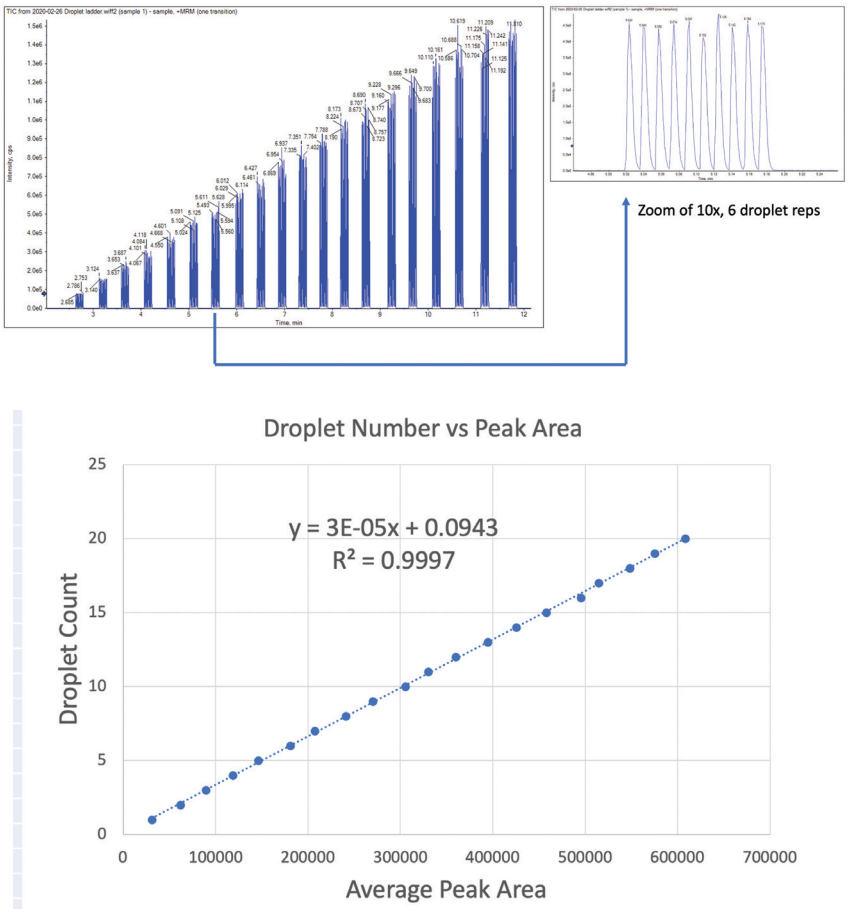

Figure 3. Linearity of injection. Droplet ladder (bottom) showed a reproducible, linear response from 1 through 20 droplets. 10 replicates were collected for each point on the droplet ladder (top).

Reproducible Analytical Performance

Despite the elimination of LC, the rapid delivery of sample via ADE to the OPI provides reproducible performance, as demonstrated with the Echo® MS System (SCIEX, Redwood City, CA). When a 384-well plate containing 100 nM dextromethorphan in 10% v/v methanol/water solutions in every well was analysed, MRM peak areas of 1.98% were achieved across all 384 wells (Figure 2). Additionally, all 384 wells were analysed in just under 7 minutes [4].

Furthermore, when a 'droplet ladder' study of 1 to 20 droplets (10 replicates for each) was generated from one of the dextromethorphan sample wells, the %CV obtained for the 10 replicates across the droplet ladder was <3% with excellent linearity and R^2 of 0.9997 (Figure 3) [3]. The ability to specify the number of droplets is analogous to adjusting an injection volume in standard HPLC work.

Little Sample Preparation Needed

The ability to achieve this type of reproducibility even for complex or 'dirty' samples was observed by the Boehringer Ingelheim researchers. For solutions of fentanyl in protein precipitated plasma, 1:1 plasma in water and untreated plasma, the best performance in establishing concentration curves was obtained with the untreated plasma solution, unlike the results with conventional LC-MS (Figure 4) [4].

This result occurs because in AEMS, when the sample droplet from the ADE enters the OPI, solvent sweeps the droplet to the mass spectrometer. This built-in 'dilute-and-shoot' process minimises matrix suppression effects that would normally result from injecting straight plasma into the MS. The result is higher ionisation efficiency for analytes and excellent sensitivity - all without any sample preparation.

A further study involved the analysis of angiotensin in yeast fermentation broths (Figure 5) [5]. The samples were prepared by lysing the active broth with 4:1 v/v acetonitrile/water and adding angiotensin standard to represent 25, 30 and 50 mg/L angiotensin in broth. The samples were then diluted with 40:60 v/v acetonitrile/water to 1x, 10x and 100x dilutions and subsequently centrifuged for 15 mins at 14000 rpm.

Very high reproducibility with consistent and precise quantification was observed across all dilutions, even for the least dilute sample. Even with such a complex matrix, almost no sample preparation was required.

Overcoming Matrix Suppression Effects

In addition to affording an acceptable level of sensitivity, reproducibility and linearity, the AEMS approach negates the matrix suppression effects commonly observed with LC-MS methods. This behaviour was demonstrated using the Echo® MS System by analysing standards prepared in plasma fortified with PEG400 that were treated as quality controls when the data was processed [6]. PEG400 is a common formulation agent used in pharmaceutical research that can have significant suppressive effects in electrospray mass spectrometry.

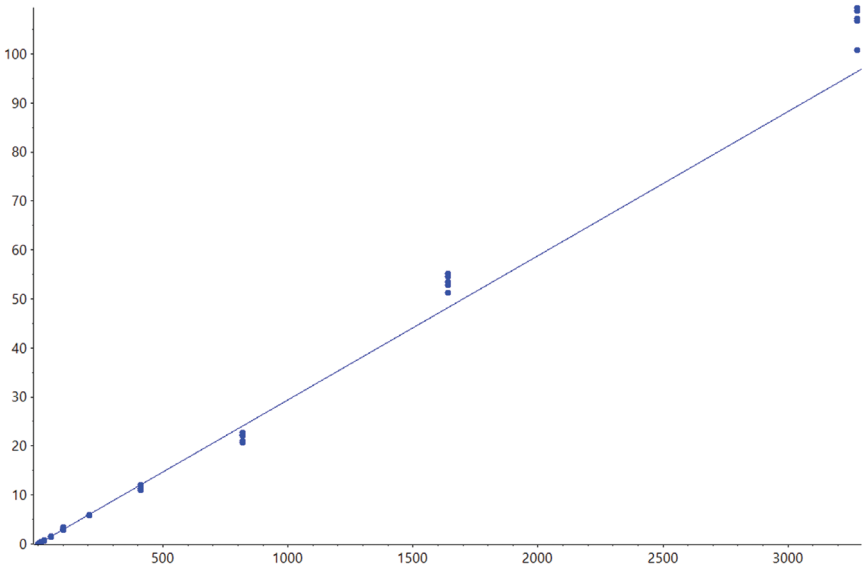

Figure 4. Example calibration curve for fentanyl in untreated plasma. Concentration curve from X to Y ng/mL of fentanyl in untreated plasma was generated and run with n=5 ejections at each concentration level.

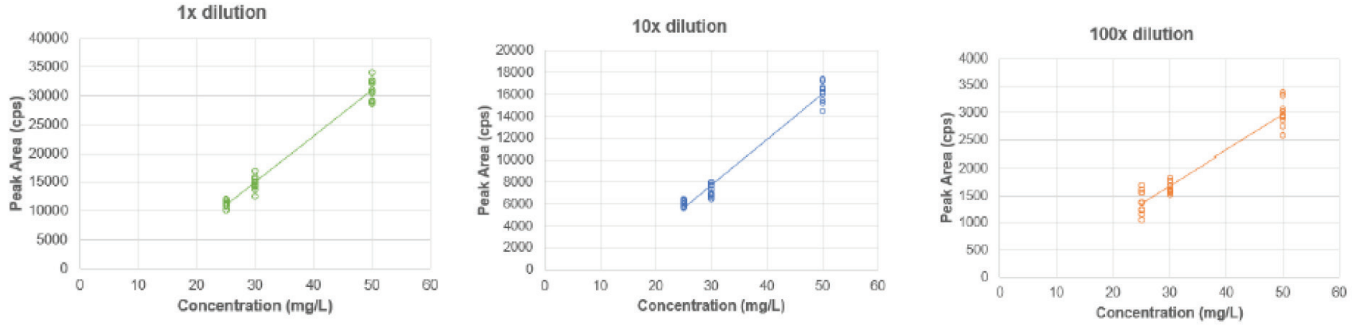

Figure 5. Concentration curve for angiotensin in all three broth dilutions. Good linearity and reproducibility were observed for the sample concentrations out of the minimally prepared samples.

Matrix	Peak Area %cv	Average Calculated Concentration	Average Accuracy
Plasma, no PEG400	*7.7*	*104.8*	*102.4%*
Plasma w/ 0.01% PEG400 by volume	*5.3*	*99.4*	*97.1%*
Plasma w/ 0.10% PEG400 by volume	*3.2*	*76.7*	*74.9%*

Table 1. Matrix effects observed for different PEG400 concentrations. Standards (top row) and QC samples prepared at the 102.4 ng/ml level. Values are of n=5.

The effects of different levels of PEG400 in untreated plasma can be seen in Table 1 at the 102.4 ng/mL level as an example. The deviation from nominal at other concentrations was very similar to the 102.4 level for the two PEG400 levels.

Wide Applicability

In addition to high-throughput parallel medicinal chemistry [2], AEMS has been investigated in numerous applications, including high-throughput pharmacology screening, label-free in situ enzyme kinetics and in vitro and in vivo adsorption, distribution, metabolism, elimination, pharmacokinetic (PK) and biomarker analysis [6]. Whether analysing chemical reactions or performing in vivo and in vitro biological analytical quantification, the AEMS approach compared favourably to conventional techniques.

AEMS has the potential to ultimately drive the adoption of MS across a number of fields where it has previously been deemed impractical. AEMS can also be used in many other fields beyond the pharmaceutical industry that require high sample-readout speed, such as synthetic biology and food and environmental analysis. In all of these applications, scientists get the benefits of simplicity and speed, plus more confidence in their results.

Potential to Change Drug Discovery

The use of ESI-MS permits excellent quantification performance in terms of sensitivity, reproducibility, and linear dynamic range without carry-over, and is applicable to a wide range of analytes. It is evident that the AEMS approach provides significant increases in sample analysis while maintaining, or perhaps improving, the analytical performance of conventional techniques. For routine analysis, the speed and simplicity of using this revolutionary platform offers an attractive solution for high-turnaround bioanalytical laboratories.

AEMS technology also might have the potential to change drug discovery and the development of precision therapies. In the search for safe and efficacious drugs, speed of decision making is critical to the discovery process and to the identification of potential targets that should be advanced for further development. With the potential to impact the entire workflow from start to finish, including sample prep, data acquisition and data processing, AEMS aim to reduce the timescale to produce data used in key decision making.

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