Full integration of high throughput system for ADME screens using cassette analysis

**OVERVIEW**

In vitro screens to assess ADME properties of potential drug candidates have been widely adopted in early drug discovery to reduce the attrition rate throughout downstream development. High throughput is mandatory considering the vast number of new chemical entities that need to be screened. LC/MS systems are the current workhorse for HT ADME screens because of their great analytical performance (good detection sensitivity, fast duty cycle, and wide dynamic range), reliability, and robustness. In order to improve the throughput, a lot of efforts have been focused on getting faster LC separation. With the modern UPLC system, or fast LC separation under trap & elute mode with short columns, analysis time has been decreased significantly from minutes to seconds level. The sample analysis throughput can be further improved by multiplexing analytes, or in the cassette analysis mode. This had been largely utilized especially for multi-reaction monitoring (MRM) assays with the mixture of analytes bearing the same polarities using triple quadruple mass spectrometer. When the analytes need to be detected at different polarities, they were usually analyzed by separate injections as it took too long (>1sec) for old generation of mass spectrometers to switch the detection polarities. Such limitation was alleviated as the state-of-the-art instruments like AB Sciex 5500 and newer models allow fast (100ms) polarity switching within each scanning cycle and can produce enough data points across regular LC peaks or even ultra sharp UPLC peaks. Our lab had demonstrated that similar quantitation performance can be achieved using mixed polarity cassette analysis as compared to discrete analysis, in term of detection sensitivity, accuracy, dynamic range, and reproducibility. Back then (2010) it was realized that to fully take advantage of the cassette analysis capability, new software packages, especially those can automatically generate cassette methods and review the cassetted data in batch, need to be developed. Through collaboration between Pfizer, AB Sciex, Sound Analytics, and Apricot Designs, an integrated system (both ADDA autosampler and controlling software package) has been developed that enables a streamlined workflow for batch sample analysis with mixed polarity cassette capability, which includes: 1) cassette groupings options for multi-analytes; 2) analytical method generation and batch submission; 3) sample analysis through integrated sample delivery system; and 4) high throughput data review processes with visualization features. Significant improvement of both sample analysis throughput (4X) and savings (75%) in instrument time and consumables were achieved without compromising data quality for the ADME screens tested.

**RESULTS & DISCUSSION**

The feasibility of cassette analysis with mixed polarities had been fully demonstrated on ABSciex 5500 system (2010). Back then, cassetted Analyst acquisition methods were generated by manually combining multi MRM transitions for in vitro screen samples in 96 well plates. Injection sequences were built manually on a CTC Pal autosampler. Data review of cassetted runs were also slow as every transition had to be individually reviewed. Obviously further development of special sample and data handling software packages was needed for HT ADME screens to fully utilize the cassette analysis technology. Over the past a couple of years, a fully integrated system was developed to address all the problems identified back then. Each item was addressed and shown below.

1. Cassette analysis with fast polarity switch enables higher sample analysis throughput, monitored, though every four compounds were dosed together for incubation. For compounds (most of time have mixed polarities) were monitored simultaneously analysis (one-analyte-a-time)—within each injection only one compound was cassetted methods and review the cassetted data in batch, need to be developed.

2. Automatic Analyst MRM method generation
   - ADDA sort and regroup all MRMs by polarities with only one polarity switch, as it has proved not feasible to switch multi times.
   - For each polarity, predefined internal standard(s) can be automatically added.
   - ADDA submits the whole batch with acquisition method generated individually for each sample.

3. Data acquisition using ADDA autosampler
   - Big deck for HT screens (12 plates).
   - Versatile injection sequencing & program function.
   - Full control of gradient settings.

4. Cassetted data review by Discovery Quan-Analyze
   - Arrows navigates through group of injections.
   - For each group of injections, MRMs (with both polarities) can be toggled.
   - Various peak integration options.
   - Intuitive data output format and setting templates.

**APPLICATION of Cassette Analysis for HT-ADME Screens**

**RESULTS**

**CONCLUSIONS**

- Cassette analysis with fast polarity switch enables higher sample analysis throughput, provided faster data turnaround time, and generated significant savings (75% or more) in term of instrument time and solvent/consumables consumption.
- ADDA platform integrated versatile sample delivery schemes and powerful software control functionalities such as automatic cassetting method generation and batch building.
- Bottleneck of high throughput data analysis were overcome by new DQ-Analyze software which enables fast data review in batch with powerful visualization tools.
- Real ADME screen results using cassette analysis showed good correlation with conventional analysis methods. Cassettes of four compounds were demonstrated here but higher degree of cassetting is readily available.

**REFERENCES**

(2) Zhang, H., Zalewski V., Schneider R., Janiszewski J., etc. Improving the Throughput of in vitro ADME Screens Using Cassette Analysis and Polarity Switching, AAPS 2010
(3) Di L., Whitney-Puckett, C., Lunderg, J., etc. Development of a New Permeability Assay Using Low-Efflux MDCK Cells

**EXPERIMENTAL**

- All data were generated using an LC/MS/MS system constituted by two PL-680 LC pumps (Lacso, Tokyo, Japan), an Apricot Designed Dual Arm (ADDA) autosampler (Apricot Designs, Covina, CA), and a 5000 triple quad MS (ABSciex, ON, Canada). Data were reviewed through DiscoveryQuan-Analyze software (Sound Analytics, Easy Lyme, CT & ABSciex, ON, Canada).
- All MRM conditions (Q1/Q3/DP/CE) for compounds screened were optimized by DiscoveryQuan-Optimize software (AB Sciex) on a separate mass spectrometer, and stored in a centralized database.
- For sample analysis, 15ul of sample was injected into a short trap column (5mmx1mm, optimized Techn, Oregon City, OR) and analyzed in a trap & elute mode: first trap column was washing with 1.5 ml/min 95/2.5/2.5 2mM Phosphate buffer/ACN/Meth.; analysts were then eluted out with 1.5 ml/min 1045/45 2mM Phosphate buffer/ACN/Meth.
- Samples from assay incubates were first analyzed using conventional discrete analysis (one-analyte-at-a-time)—within each injection only one compound was monitored, though every four compounds were dosed together for incubation.
- For cassette analysis, samples were injected into API 5500 instrument and four test compounds (most of time have mixed polarities) were monitored simultaneously during the same injection.

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