



Fully Integrated High Throughput System for ADME Screens Using Cassette Analysis

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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/MS is the current workhorse for HT ADME screens because of its 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 routinely utilized especially for multi reaction monitoring (MRM) assays with the mixture of analytes bearing the same polarities using triple quadrupole mass spectrometer. When the analytes need to be detected at different polarities, they were usually analyzed by separate injections as it used to take 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 the cassetted methods and review the cassetted data in batch, need to be developed.

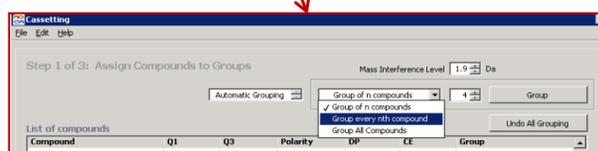
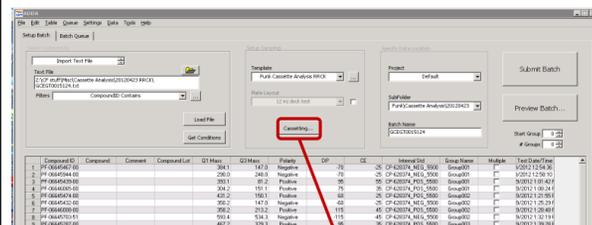
Through collaboration between Pfizer, AB Sciex, Sound Analytics, and Apricot Designs, an integrated system (both ADDA autosampler and controlling software package) has been developed that enables a streamlined workflow for batch sample analysis with mixed polarity cassetting capability, which includes: 1. cassette grouping 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.

EXPERIMENTAL

- All data were generated using an LC/MS/MS system constituted by two PU-980 LC pumps (Jasco, Tokyo, Japan), an Apricot Designed Dual Arm (ADDA) autosampler (Apricot Designs, Covina, CA), and a 5500 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 Tech, 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/MeOH; analytes were then eluted out with 1.5 ml/min 10/45/45 2mM Phosphate buffer/ACN/MeOH.
- Samples from assay incubates were first analyzed using conventional discrete analysis (one-analyte-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.

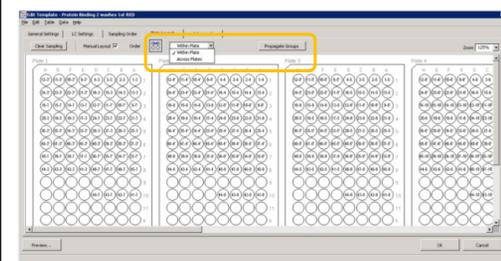
RESULTS & DISCUSSION

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 files 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 were developed to address all the problems identified back then. Each item were addressed and shown below.

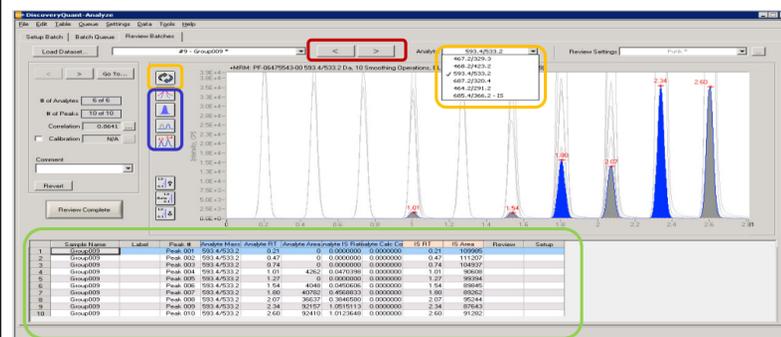


2. Automatic Analyst MRM method generation

- 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.

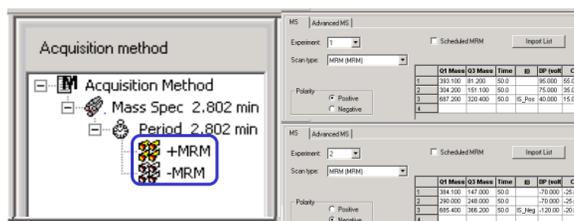


4. Cassetted data review by Discovery Quan-Analyze



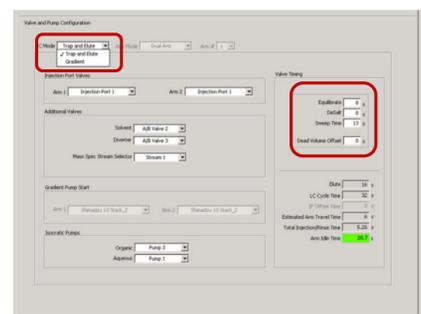
1. Cassetting multi-analytes using ADDA

- ADDA control software allows both automatic and manual grouping.
- Two ways for automatic grouping: **Every n compounds** & **Every nth compounds**. The later is very useful for cassetting samples across multiple plates.
- Alerts when MRMs are too close to each other.



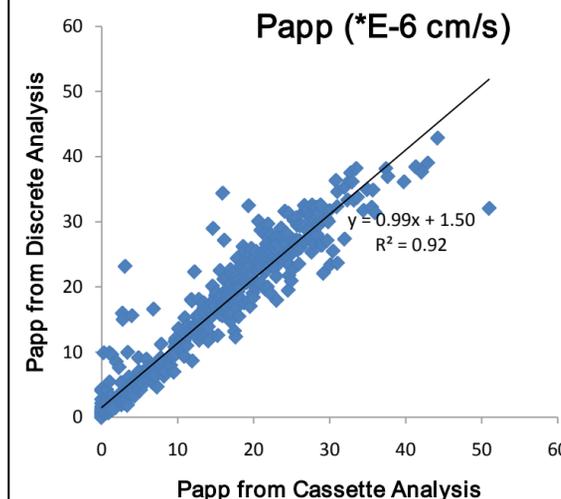
3. Data acquisition using ADDA autosampler

- Big deck for HT screens (12 plates).
- Versatile injection sequencing building & propagate function.
- Full control of gradient settings.



- 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.

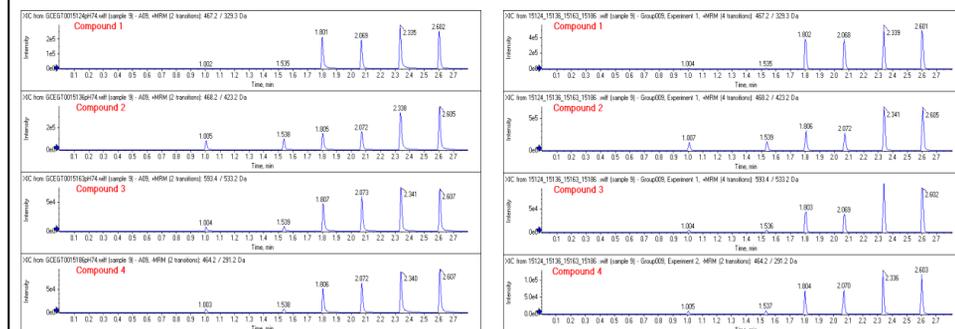
APPLICATION of Cassette Analysis for HT-ADME Screens



MDCK-RRCK Passive Permeability Screen

- Assessment of passive permeability
- 500 tested compounds screened. Every four compounds were dosed together for incubation.
- Good correlation of Papp value between cassette and discrete analysis.

Data comparison between Discrete and Cassette Analysis



- Traditional one-analyte-a-time (four separate sets of injections) **16 sec/injection/analyte**
- Cassetted analysis of four analytes, (single set of injections) **4 sec/injection/analyte**

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 discrete analysis methods. Cassetting of four compounds were demonstrated here but higher degree of cassetting is readily available.

REFERENCES

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