

High Throughput Metabolic Stability Screening by MALDI Triple Quadrupole Analysis

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The FlashQuant™ workstation (MALDI/QqQ) is based on the API 4000™ or 4000 Q TRAP® mass spectrometer and combines the speed of MALDI ionization with the quantification power of a triple quadrupole mass spectrometer for the analysis of small molecules. Analysis by MALDI/QqQ can reach 1 sec/sample readily with sensitivity, accuracy, and precision required for *in vitro* ADME quantification (1), in sharp contrast with current LC/MS/MS methodologies, where the LC separation speed limits the sample introduction rate. In this application note, a workflow suitable for high throughput metabolic stability screening purposes is demonstrated using commercially available liquid handlers and the new FlashQuant workstation.

Sample Preparation and Spotting

Standard rat liver microsomal incubation assays were performed in triplicate with a substrate concentration of 4 μM. Reaction was stopped by acetonitrile precipitation at t = 0, 5, 15, and 30 minutes and supernatant aliquots were transferred into a 96-well plate. Samples were desalted using automated solid phase extraction (SPE) on a Tomtec QUADRA 3 SPE workstation (Tomtec, Hamden, CT). The supernatant was first diluted in water (1:5) and then loaded on a preconditioned Oasis μElution HLB plate (Waters, Milford, MA). The sorbent was washed with 200 μL of 5% methanol solution and sample eluted with 50 μL of 40/60 acetonitrile/2-propanol, followed by a one-to-one dilution with water. The MALDI matrix solution (α-cyano-4-hydroxycinnamic acid), which also contained prazosin as an internal standard, was added to the diluted eluent in a one-to-one ratio before spotting on the target plate using a PerkinElmer JANUS system (PerkinElmer, Waltham, MA). Since carryover is not a problem for the FlashQuant workstation, the samples were analyzed in time order, starting with the high concentration samples (t = 0) first.

Mass Spectrometry Experiment

The FlashQuant workstation was programmed to ablate sample spots in a straight line raster at a speed of 1.5 mm/s, with the laser set at 1000 Hz pulse rate and 20% power. The signal was filtered by the triple quadrupole stage measuring two ion pairs simultaneously in multiple reaction monitoring mode. Signal produced by a laser passage over a spot was recorded and integrated by the FlashQuant™ Software. Results were reported as concentrations relative to t = 0. In this experiment, one sample was ablated every 6 sec.

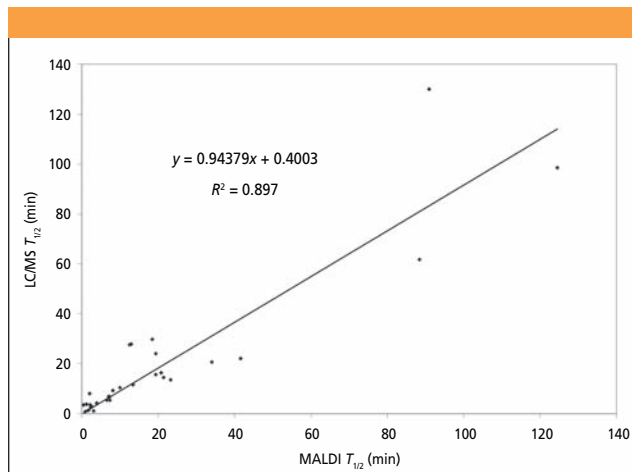


Figure 1: Rat microsome half-lives for 30 reference compounds: MALDI vs. LC/MS.

Results and Discussion

Incubation samples for 30 reference compounds were analyzed by MALDI/QqQ (FlashQuant workstation) and by a generic LC/MS protocol. The metabolic stability profiles produced by the two techniques were highly comparable, with good correlation between half-life values obtained in LC/MS/MS and MALDI/QqQ for these 30 compounds (Figure 1).

Conclusion

The data results show that *in vitro* metabolic stability profiles can be easily produced very quickly and accurately with the FlashQuant workstation. The results obtained using the MALDI/QqQ demonstrated good correlation to the corresponding LC/MS/MS data, proving that the FlashQuant workstation generates results on par with the industry-standard approach, LC/MS/MS, but with much greater speed.

Reference

- (1) J. Gobey, M. Cole, J. Janiszewski, T. Covey, T. Chau, P. Kovarik, *J. Corr. Anal. Chem.* **77**, 5643–5654 (2005).

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