High-Throughput *In Vitro* ADME Analysis with Agilent RapidFire/MS Systems: Permeability Assays

Application Note

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Introduction

During the drug discovery process, permeability assays such as Caco-2 (cell line) and PAMPA (parallel artificial membrane permeation assay) are utilized to mimic the absorption of potential drug candidates by the cells of the human intestine. Depending on a potential drug's permeability characteristics, it will be ranked versus other candidates for its efficacy as an oral therapeutic. Analysis early in the drug discovery process involves large sample sets, making a high-throughput approach desirable. Agilent RapidFire/MS systems combine high-throughput sample processing with triple quadrupole (QQQ) or time-of flight (TOF) mass spectrometry (MS) to streamline ADME assay analysis.

Standard Caco-2 and PAMPA assays were performed on a variety of drug candidates. The Agilent RapidFire High-throughput Mass Spectrometry System, interfaced to either a QQQ or a TOF MS, was used to analyze the assay plates. Using this setup, samples were processed in less than 10 seconds each. The ultra-fast system produced results similar to traditional LC/MS methods in a fraction of the time, demonstrating that RapidFire is both a cost-efficient and a highly productive means of assaying permeability during the drug discovery process.

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Using RapidFire/Q-TOF for PAMPA Assays

PAMPA assays were performed on diverse set of 81 proprietary drug discovery compounds at three different pH conditions and the permeability values from RapidFire/TOF data were correlated to data from LC/MS/MS analysis. RapidFire/TOF analysis utilized a single generic MS source and a solid phase extraction (SPE) method, while LC/MS/MS required multiple reaction monitoring (MRM) optimization for each compound. Sample analysis cycle times for the RapidFire system were more than 10 times faster than LC/MS/MS. The strong correlation (Figure 1)[1] shows that the two analysis methods produce comparable results. In addition to the decrease in cycle time of the Agilent RapidFire system, these results indicate that the MRM method development can be eliminated for this assay, thus providing additional workflow efficiency.

$y=0.9353x - 0.369$
$R^2= 0.8935$

pH 4

$y=0.9066x - 0.4741$
$R^2= 0.7351$

pH 6.8
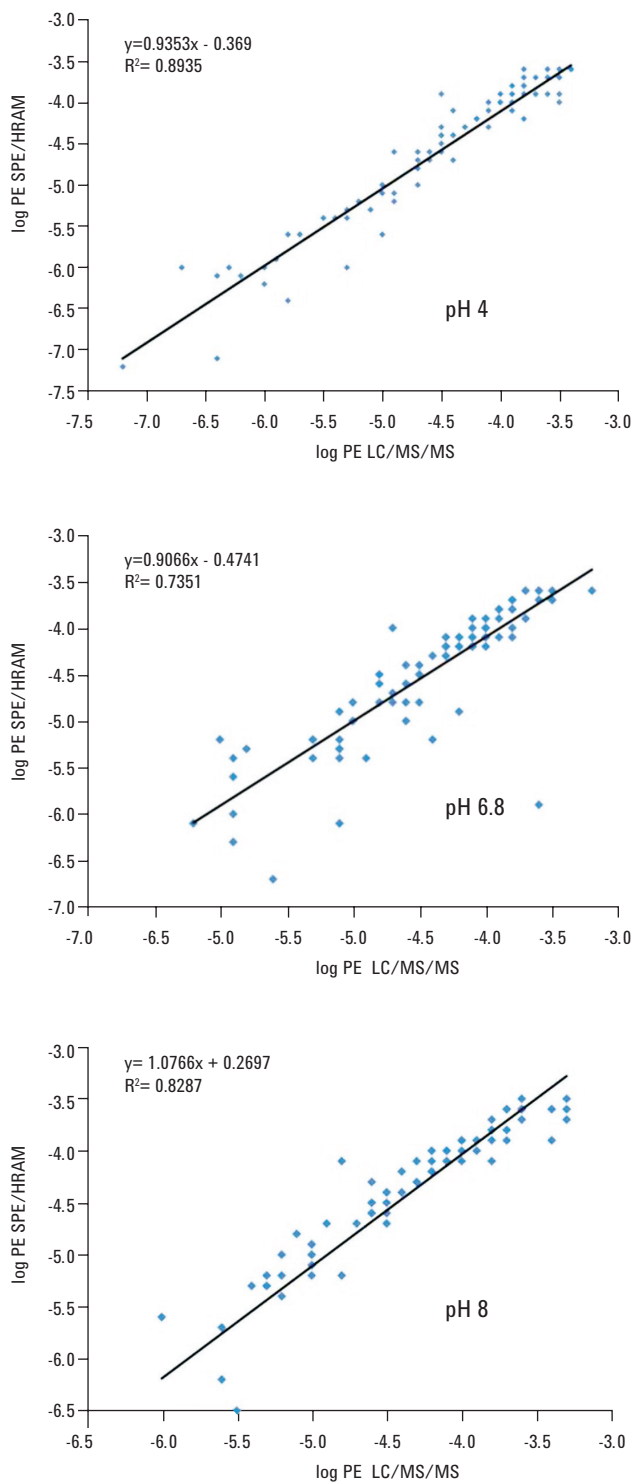
$y= 1.0766x + 0.2697$
$R^2= 0.8287$

pH 8

Figure 1. Correlation of permeability values from PAMPA assays using three different pH conditions for 81 drug discovery candidates. Data from SPE/high-resolution accurate mass (HRAM) (RapidFire/TOF) correlated well with standard LC/MS/MS analysis.

Using RapidFire/MS/MS for Permeability Assays

In a second study, both Caco-2 and PAMPA assays were performed, and the permeability results correlated for RapidFire/MS/MS versus LC/MS/MS. Correlation of permeability coefficients (PAPP) derived from a validated LC/MS/MS system and the RapidFire/MS/MS system. Samples were generated from a Caco-2 assay, which is a surrogate for the *in vivo* permeability of an orally administered compound (Figures 2 & 3)[2]. Sample analysis cycle times for the RapidFire system were 10 times faster than LC/MS/MS. Again, the strong results correlation illustrates that incorporating the RapidFire system into analysis of these assays retains data integrity while greatly increasing laboratory productivity.
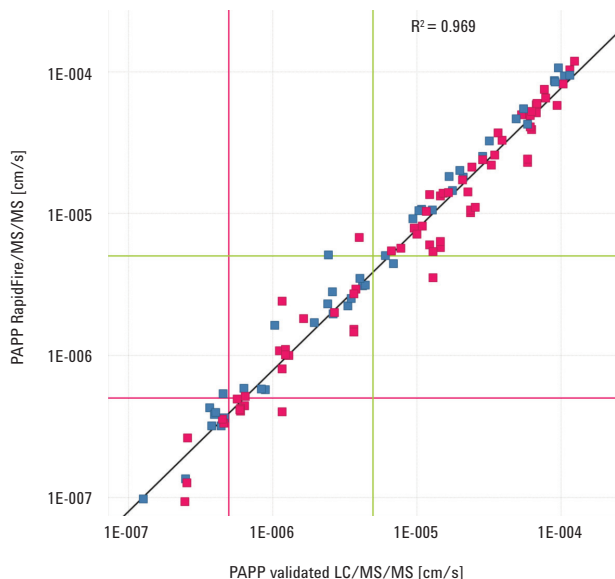

Figure 2. Correlation of permeability coefficients (PAPP) for 110 Caco-2 experiments. Blue squares indicate same day sample measurement and red squares indicate a measurement delay of > one day and one additional freeze-thaw cycle.
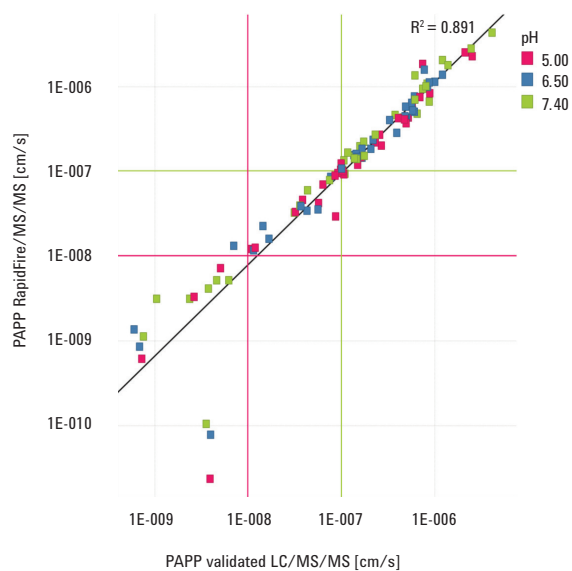

Figure 3. Correlation of permeability coefficients (PAPP) for 35 (PAMPA) experiments. Compounds were investigated at different pH values and display low (<1 × 10^{-8} cm/s), medium (>1 × 10^{-8}, but <1 × 10^{-7} cm/s), or high permeability in the assay.

Conclusions

The Agilent RapidFire High-throughput Mass Spectrometry system, interfaced to either a QQQ or a Q-TOF MS, was used to analyze PAMPA or Caco-2 permeability assay samples. The results from two sets of experiments illustrate the key benefits of the RapidFire System: sample processing speeds of six to ten seconds per sample resulting in a 10-fold increase in throughput compared to conventional methods, and equivalent permeability results compared to conventional LC/MS/MS methods. The RapidFire system significantly increased the efficiency and throughput of conventional laboratory workflow for these assays.

References

1. Hatsis, P. *et al.* Reducing Bottlenecks in ADME Sample Analysis using Solid Phase Extraction with a Quadrupole Time-of-Flight Mass Spectrometer. Podium talk by Novartis/Biocius at the 2010 ASMS meeting.

2. Luippold, A. *et al.* Application of a Rapid and Integrated Analysis System (RIAS) as a High-Throughput Processing Tool for *In Vitro* ADME Samples by Liquid Chromatography/ Tandem Mass Spectrometry. *J Biomol Screen,* **2011**, 16(3):370-377.

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