Sensitive, High-Throughput *In Vitro* ADME P-Glycoprotein Inhibition With Agilent RapidFire/MS Systems

Application Note

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Introduction

Interactions between drug candidates and transporters are routinely evaluated during drug discovery. P-glycoprotein (P-gp) is a transporter which is commonly assessed at an early phase of the process to determine if the drug of interest is a substrate or an inhibitor of P-gp, so that poor drug candidates can be eliminated. Since analysis early in the drug discovery process involves large sample sets, a high-throughput approach is desirable. Agilent RapidFire/MS systems enable high-throughput sample processing to streamline ADME assay analysis.

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Using RapidFire High-Throughput Mass Spectrometry to Analyze P-Glycoprotein Inhibition

The RapidFire High-throughput Mass Spectrometry System was employed to assess the effect of the drug candidates on bidirectional transport of the substrate, digoxin, in a Caco-2 cell-based system which expresses the P-gp transporter (Figure 1).

RapidFire analysis of this assay achieved sample cycle times of six to ten seconds per injection, enabling an ultra-fast, bioanalytical method to asses P-gp inhibition.

Solid phase extraction (SPE) based RapidFire does not require the sample preparation of chromatography-based systems, which makes the system a straightforward high-throughput solution. Combined with mass spectrometry, the system delivers the sensitivity required to analyze P-gp inhibition assays and demonstrates excellent correlation to LC/MS/MS and radioactive methods (Figure 2). Side-by-side testing of cyclosporin A P-gp inhibition IC_{50} in all three assay methods yielded similar results as shown in Figure 3. Furthermore, the RapidFire/MS/MS total workflow was more than 10 times faster than the LC/MS/MS method (Table 1), but demonstrated similar sensitivity, selectivity, reproducibility, linearity, and robustness.

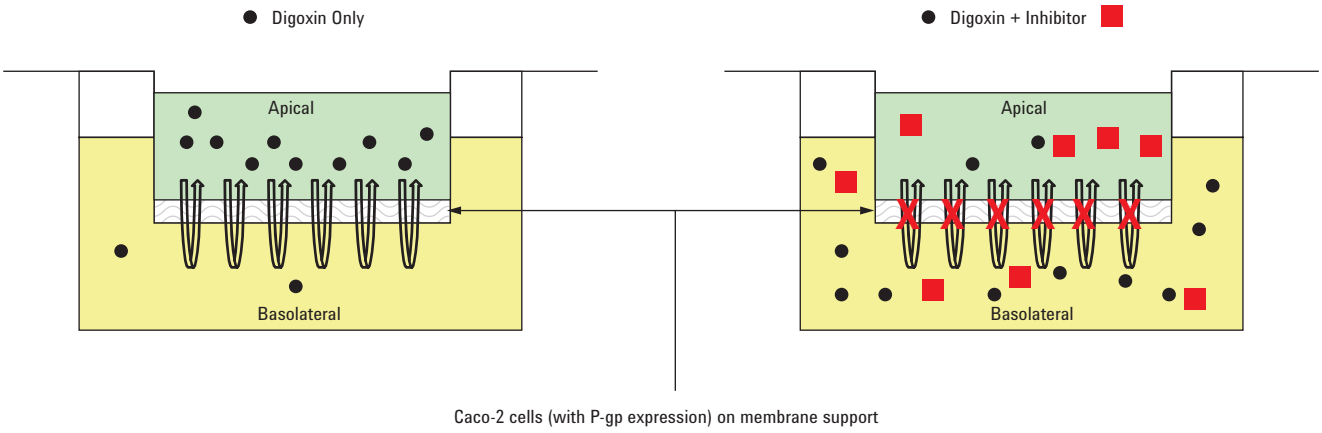
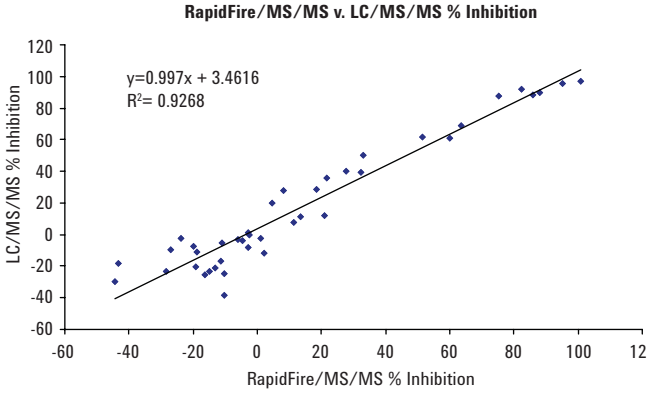
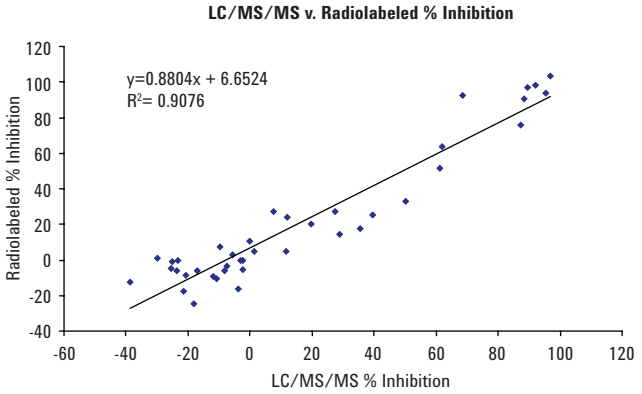


Figure 1. Schematic of the P-gp inhibition assay using a Caco-2 cell model. The basolateral to apical (B to A) direction of the bi-directional assay is shown here.

Table 1. Throughput comparison between the RapidFire/MS/MS and LC/MS/MS analyses of P-gp inhibition samples.

Time (h)	RapidFire/MS/MS	LC/MS/MS
Sample Preparation	0.75	0.75
Sample Analysis	1.50	24.0
Data Review	0.25	0.50
Total	**2.50**	**25.3**





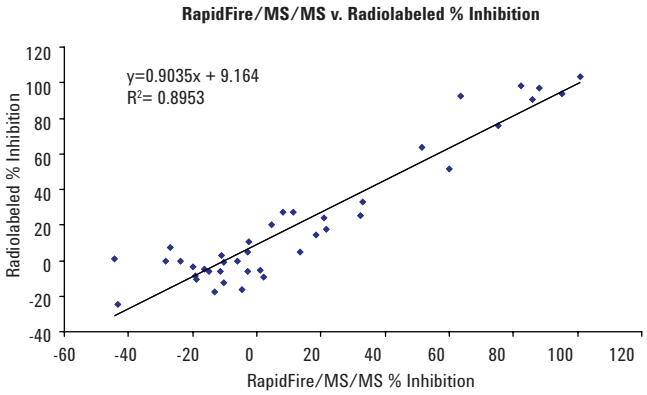
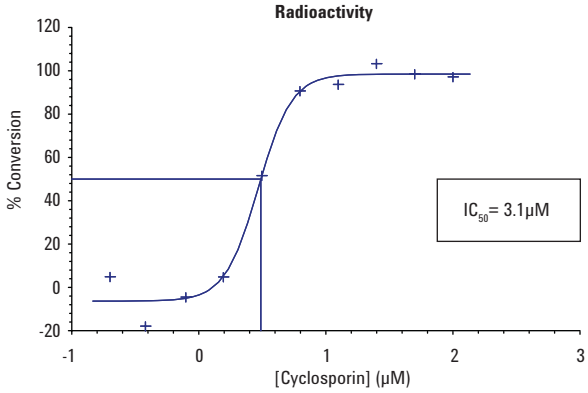
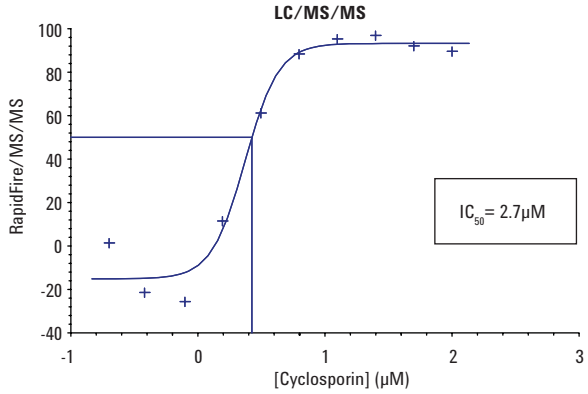


Figure 2. Correlation plots comparing % P-gp inhibition of test compounds in RapidFire/MS/MS, LC/MS/MS, and radiolabel assays.





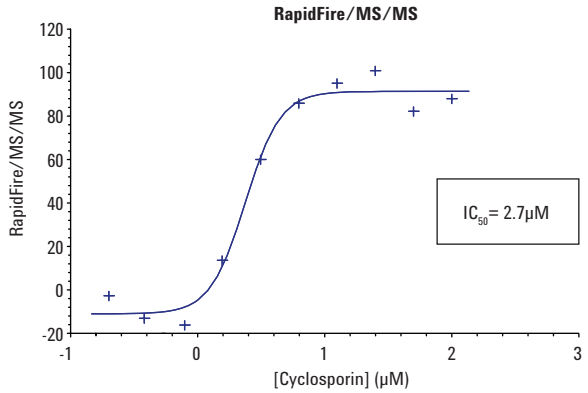


Figure 3. Side-by-side testing of cyclosporin A P-gp inhibition IC_{50} in all three assay methods.

Conclusions

The RapidFire High-throughput Mass Spectrometry System demon-strated a number of key benefits for the assessment of early drug candidates in P-Glycoprotein inhibition assays: rapid sample processing speeds, increased throughput and laboratory efficiency, and equivalent inhibition results as compared to conventional LC/MS methods. As a result, incorporation of RapidFire/MS systems into the *in vitro* ADME phase of the drug discovery process enables efficiency and productivity advances unrivaled by other technologies.

References

1. Wagner, A. *et al*. Ultrafast mass spectrometry based bioanalytical method for digoxin supporting an *in vitro* P-glycoprotein(P-gp) inhibition screen. *Rapid Commun Mass Spectrom*, **2011**, 25:1231-1240.

www.agilent.com/lifesciences/rapidfire

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