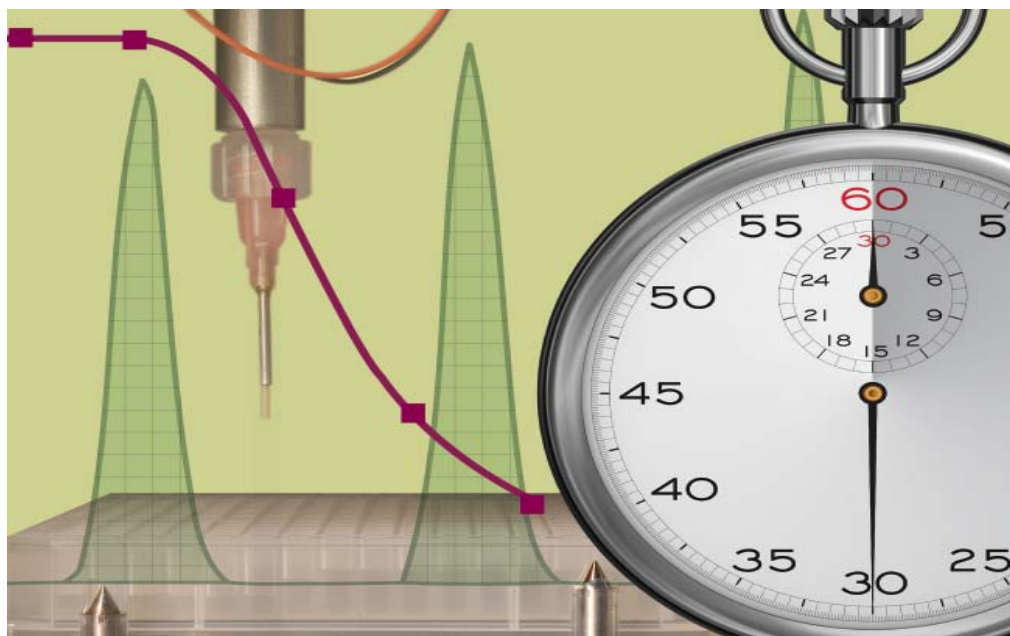


RapidFire 300® - for *in vitro* ADME

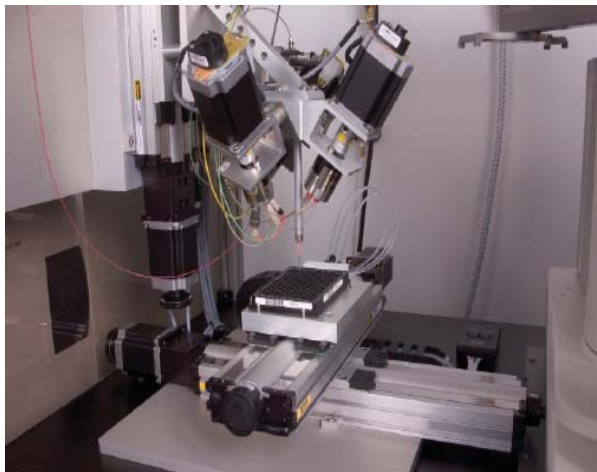


Faster ADME Data – No compromises

Decrease your *in vitro* ADME assay bottleneck by streamlining your drug discovery workflow with the RapidFire® 300 Mass Spectrometry System (RF-MS) optimized for *in vitro* ADME applications. The RapidFire System integrates fast, serial, on-line sample purification with mass spectrometric analysis. With sample cycle times of six seconds per sample, this system can obtain a 7 point IC50 curve in just 42 seconds -- less time than a single sample using standard HPLC methods. The system integrates with any manufacturer's triple quadrupole mass spectrometer and provides output data in plate reader format that is compatible with existing LIMS systems. The RapidFire 300 is capable of analyzing most *in vitro* ADME assays without modification to existing methods or workflows. Multiple MS analysis methods can be run with a single setup and unattended 24 hour operation is possible. Data from a variety of *in vitro* ADME assays shows that results from the RapidFire system are comparable to those from traditional LC-MS systems. The RapidFire 300 system increases the efficiency of *in vitro* ADME drug discovery workflows providing high quality data in a fraction of the time required for conventional HPLC techniques.

In vitro ADME Applications

- CYP Inhibition
- Metabolic Stability
- P-glycoprotein Inhibition (Digoxin)
- Plasma Protein Binding
- Permeability (Caco-2 and PAMPA)
- CYP Induction



Label-free detection -

Use of RapidFire technology requires no labels or surrogate substrates. The system enables detection of native compounds without the need for fluorescence, luminescence or radioactivity. The result is *in vitro* ADME without compromises.

Unattended 24-hour operation -

The RapidFire 300 system incorporates a variety of features that enable unattended, 24-hour operation, permitting researchers to dedicate time to other tasks as well as to run the instrument overnight without the need for operator oversight. Automated cartridge changing hardware can extend unattended operating hours or be loaded with multiple packing materials for method development applications. Integrated optical sensors automatically detect fluid flow and the software flags any aberrant samples.

Mass spec quality data at plate-reader assay speed -

RapidFire technology provides researchers with a significant competitive edge by decreasing the time required for screening. Each sample is processed in just six to eight seconds, yielding much faster throughput than traditional mass spectrometry based methods. A single 384 well plate can be read in under 40 minutes.

Flexibility in screening -

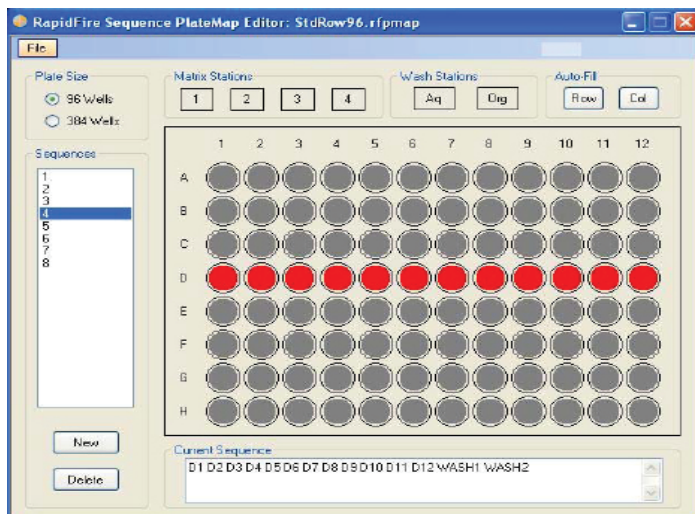
The sequence-based injection of the RapidFire 300 facilitates an expanded plate map functionality such that individual wells of each plate can be analyzed using separate methods. The system can accommodate up to 18 standard 96 or 384 well microtiter plates.

Integrated sample preparation -

The RapidFire system requires no up-front sample preparation. The system samples directly from quenched assay plates without the need for solid-phase extraction or any other sample desalting/pre-processing.

RapidFire Sample Preparation & Injection Cycle

1. Sip (withdraw) sample
2. Inject sample on RF cartridge
3. Wash cartridge (desalt)
4. Elute sample & inject into MS
5. Re-equilibrate cartridge
6. Repeat



RapidFire Software

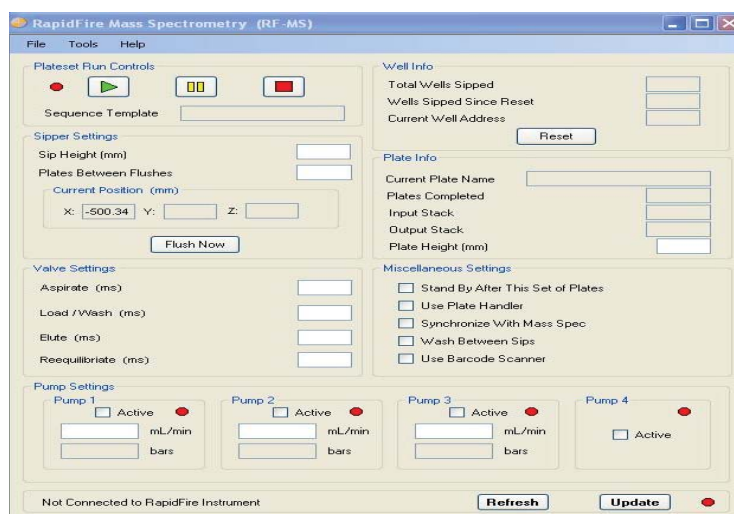
The RapidFire 300's software enables a broader range of capabilities. The user interface is easier to use and more intuitive. This software is also network enabled allowing users to monitor instrument progress at their desktop.

Expanded Plate Maps

The new plate map editor available on the RapidFire 300 enables sequence-based functionality. Injection in any plate configuration allows for custom design of sequences which may be saved.

Data Integration

RapidFire's proprietary data integration software package, Integrator, transforms direct mass spectrometry data output into data files similar to that of plate reader-based systems, compatible with all standard LIMS and databases.



System Details

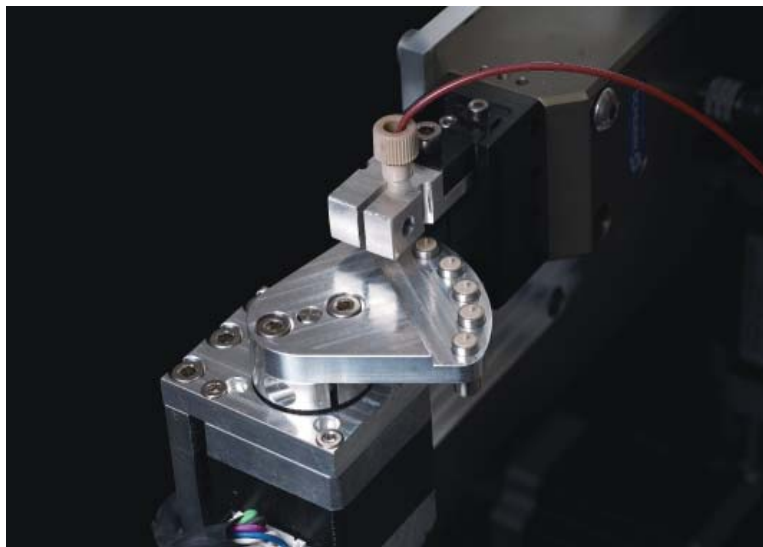
The RapidFire 300 System features an end-to-end PEEK flow path which minimizes carryover effects. Washing of the sample interface, or sipper tube between samples with subsequent aqueous and organic wash solutions further eliminates any sample-to-sample carryover. Sample tracking is accomplished by RapidFire's integrated universal barcode reader – a barcode number is associated with each unique plate file as samples move throughout the system. All procedures are actively monitored during standard operation. The optical sensor and liquid detection functions of the system ensure uninterrupted instrument operation.

- Weight: 700 lbs (320 kgs)
- Footprint: 60 in wide x 72 in high x 30 in deep (152 cm x 183 cm x 76 cm)
- Electrical: Two 115V, 10 AMP circuits or one 115V, 20 AMP circuit
- Warranty: 1-year parts, labor and travel

RapidFire Solid Phase Extraction Cartridges

RapidFire cartridges are available in a variety of chemistries shown in the table below. The assay development cartridge cassette allows for the use of six different packing materials per run enabling analysis by multiple chemistries in a method development mode.

Packing Material	Catalog #
C4	RFCP4A
Cyano	RFCP4B
C18	RFCP4C
C8	RFCP4E
Phenyl	RFCP4F
Client Specified	RFCP4Z



The RapidFire cartridge cassette enables assay development by offering a variety of packing chemistries.

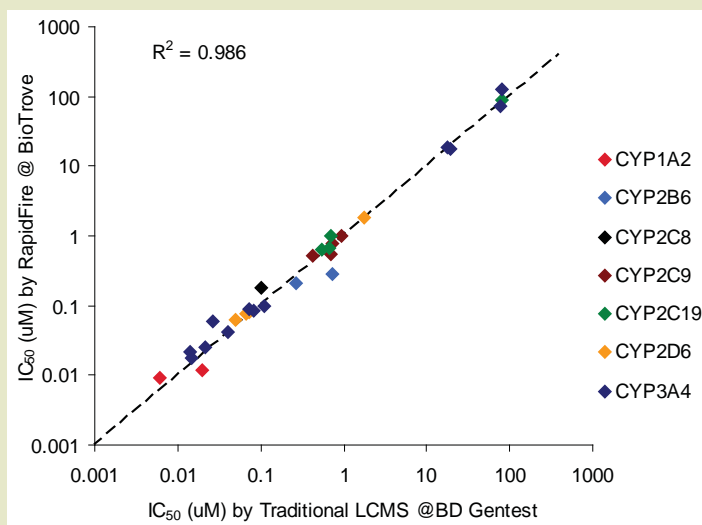
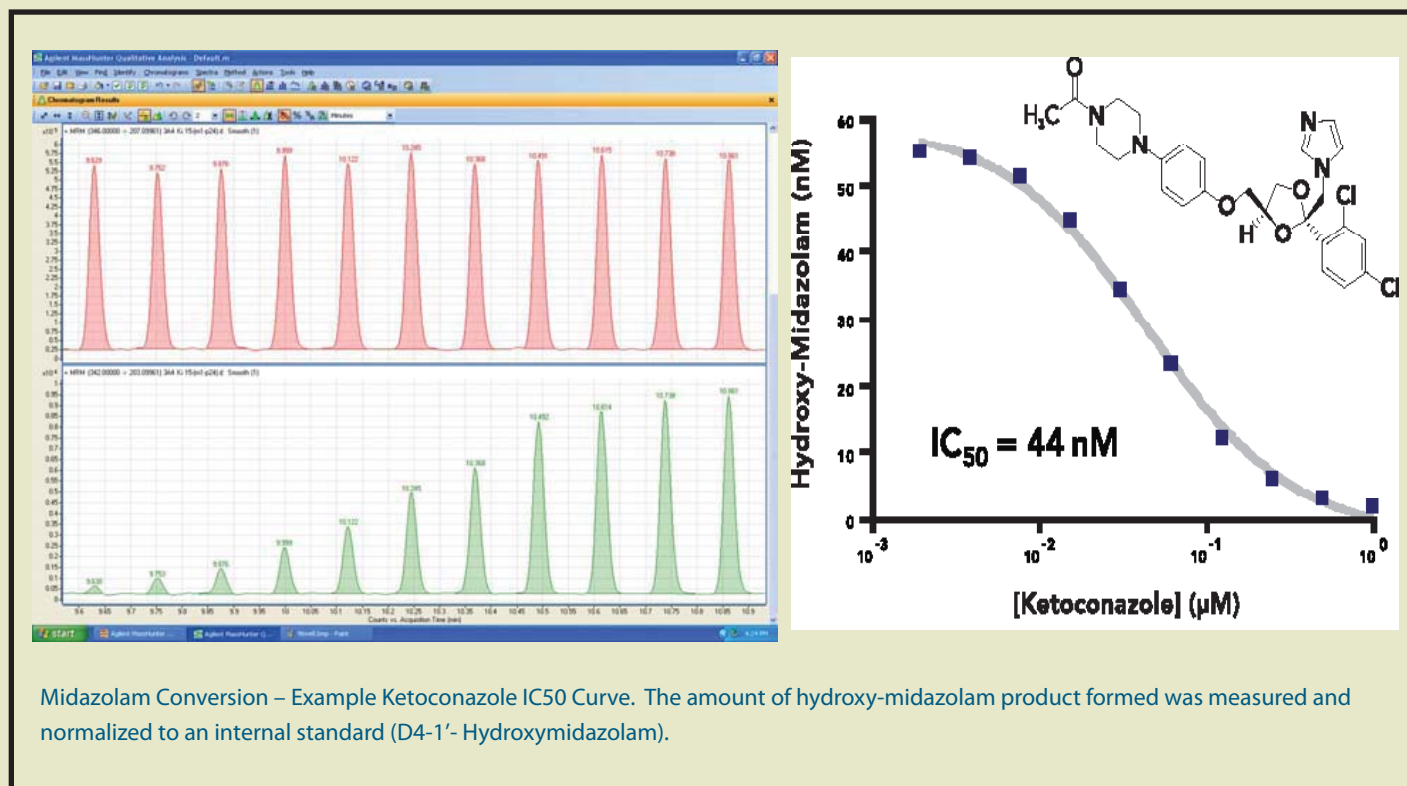


ADME Applications

The RapidFire 300 is capable of analyzing most *in vitro* ADME assays that use a MS endpoint. Assays as varied as CYP inhibition, metabolic stability and permeability have all successfully been processed on the RapidFire system. The RapidFire system is capable of analyzing these assays quickly with no further sample preparation requirements and of delivering results comparable to traditional LC-MS methods. A few examples of ADME assays using RapidFire are described in the following pages.

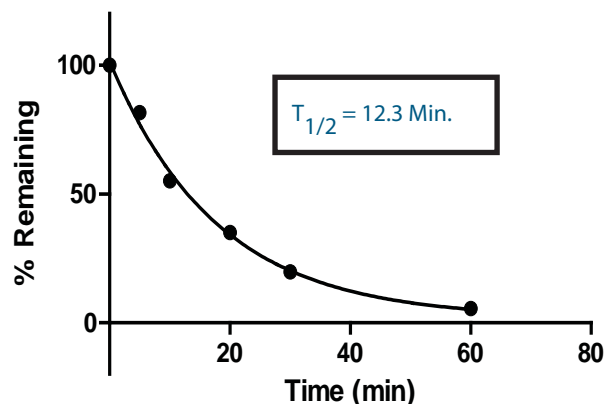
CYP Inhibition

CYP inhibition is one of the most important *in vitro* ADME assays performed in drug discovery. The RapidFire system is compatible with the list of FDA recommended drug probes for the study of cytochrome P450 inhibition assays. The RapidFire 300 system is installed with pre-validated analytical methods for CYP inhibition assays. The figure below shows sample IC₅₀ data for the inhibition of midazolam by ketoconazole in human liver microsomes and a strong correlation between RapidFire technology and traditional LC-MS for CYP inhibition assays.



Available CYP Inhibition Assays

CYP1A2	(tacrine, melatonin)
CYP2B6	(bupropion)
CYP2C8	(amodiaquin, taxol)
CYP2C9	(tolbutamide, diclofenac)
CYP2C19	(S-mephenytoin, omeprazole)
CYP2D6	(dextromethorphan, bufuralol)
CYP2E1	(chlorzoxazone)
CYP3A4/5	(midazolam, nifedipine, erythromycin, testosterone)



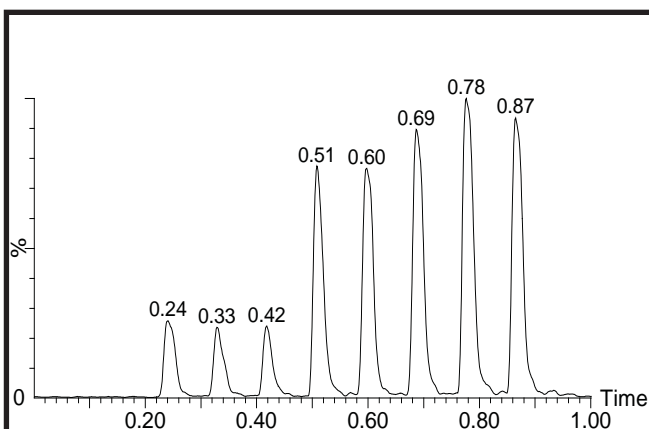
Example of the microsomal stability assay. Diclofenac (1 μ M final concentration) was incubated with human liver microsomes (0.5 mg/ml). The reaction was initiated by the addition of NADPH and stopped by an acetonitrile + IS quench. Half-life was calculated based on first-order reaction kinetics.

Metabolic Stability

The metabolic stability of test compounds in human liver microsomes is a common assay to predict the clearance and rank order new drug candidates. An example (diclofenac) assay using the RapidFire system demonstrates that the half-life for this and many other compounds were in agreement with literature values. The flexibility of the RapidFire 300 system allows the processing of large numbers of compounds for metabolic stability without interruption.

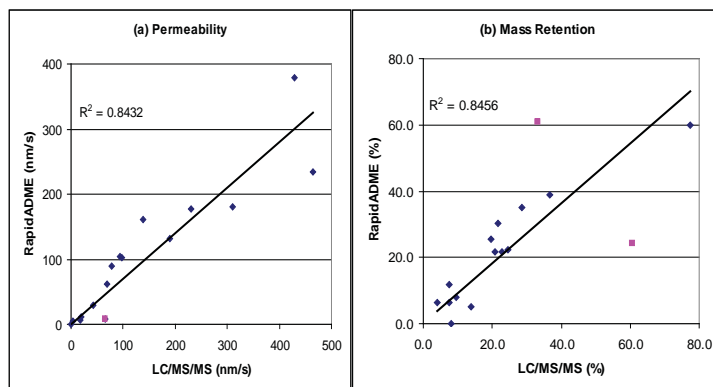
Permeability

The PAMPA assay is an *in vitro* permeability assay used to predict *in vivo* bioavailability and to rank order candidate compounds. Sample PAMPA assay data using the RapidFire system is shown below. Again, the RapidFire system produced data that was comparable to that from traditional LC-MS systems. The flexibility of the RapidFire 300 system allows the analysis of large numbers of permeability assays without interruption.



Example PAMPA data from RapidFire System, samples were injected in the order of receiver (triplicate), donor (triplicate), and initial dosing solution (duplicate).

Data courtesy of D.B. Kassel, Takeda San Diego, Inc. 2008



Comparison of PAMPA results using RapidFire and LC-MS/MS.

Sixteen compounds were tested: (a) Permeability, all but one (■) of these compounds were assigned the same permeability classes in both methods: low (<1.5), medium (1.5-20), or high (>20); (b) mass retention, 2 outliers (■) were not included in the regression analysis.

RapidFire *in vitro* ADME service

In vitro ADME service at BIOCIUS enables hassle-free outsourcing of ADME assays and screening. Quenched CYP Inhibition or metabolic stability assay plates are sent to BioTrove where they are then analyzed by experienced scientists on the RapidFire 300 System. Short turn-around times and data delivered in a plate-reader style format enables easy interpretation as well as seamless integration into existing laboratory workflow. BIOCIUS's experienced scientists have screened millions of wells for our clients in the pharmaceutical and biotechnology industries in support of their drug discovery efforts.



Decrease your *in vitro* ADME bottleneck

Streamline your drug discovery workflow with the high-throughput RapidFire 300 Mass Spectrometry System. RapidFire instrumentation in your laboratory can eliminate in vitro ADME bottlenecks by providing mass spectrometry quality data > 10 times faster than conventional LC-MS methods. Unattended 24 hour operation enables researchers to devote time to other tasks. Alternatively, BIOCIUS's in vitro ADME service enables laboratories to outsource these studies with guaranteed fast turn-around times.



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At BIOCIUS we are committed to the success of
our partners and collaborators.

For information on the RapidFire 300® for in vitro ADME Service,
please contact Sales@BIOCIUS.com
