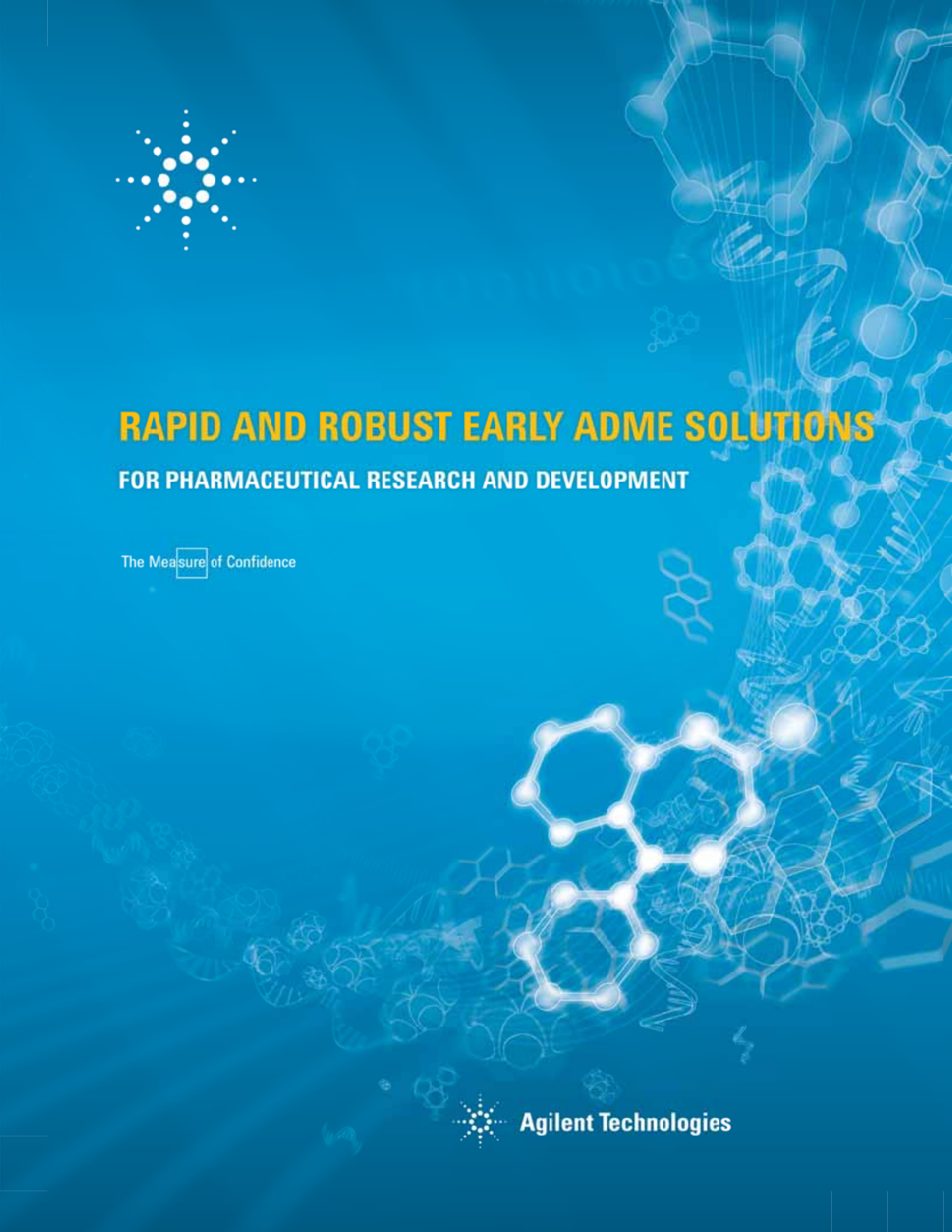

RAPID AND ROBUST EARLY ADME SOLUTIONS

FOR PHARMACEUTICAL RESEARCH AND DEVELOPMENT

The Measure of Confidence

Agilent Technologies

REALIZE EFFECTIVE PHARMACEUTICALS

Risk Mitigation Through Early ADME Studies

The discovery and development of novel therapeutics is highly complex, with companies constantly adjusting the balance between efficacy, safety, speed, and cost. Rising regulatory pressures and increasing efforts to reduce costly late-stage attrition puts a greater emphasis on obtaining robust ADME data as early as possible for drug candidates.[1] By eliminating compounds with toxic liabilities during discovery, instead of during clinical development, more resources can be focused on compounds with a greater likelihood of success.

Additionally, current Metabolites in Safety Testing (MIST) guidelines require a complete toxicological evaluation, identification, and characterization of all unique or significant (>10 % of parent) circulating metabolites observed in humans. This places new requirements and importance on metabolite profiling, identification, and characterization studies during early clinical development.[2,3,4]

While early ADME studies are a promising avenue for identifying and advancing molecules with the best safety profiles, they can be challenging to implement because the large number of compounds that need to be assessed by an ADME group may be prohibitive when multiple projects and therapeutic targets are assessed (Figure 1). Robust, high-throughput assays and automated workflows can be used to overcome the tight timelines required for successful, cost-effective drug development.

With high-performance analytical instruments that work together to seamlessly implement early ADME workflows, Agilent provides the right set of tools for all of your early ADME needs. From high-throughput analysis of metabolites in complex biological matrices to detailed structural analysis and robust automated liquid handling systems, Agilent helps you efficiently obtain the data you need to increase your chances of success.

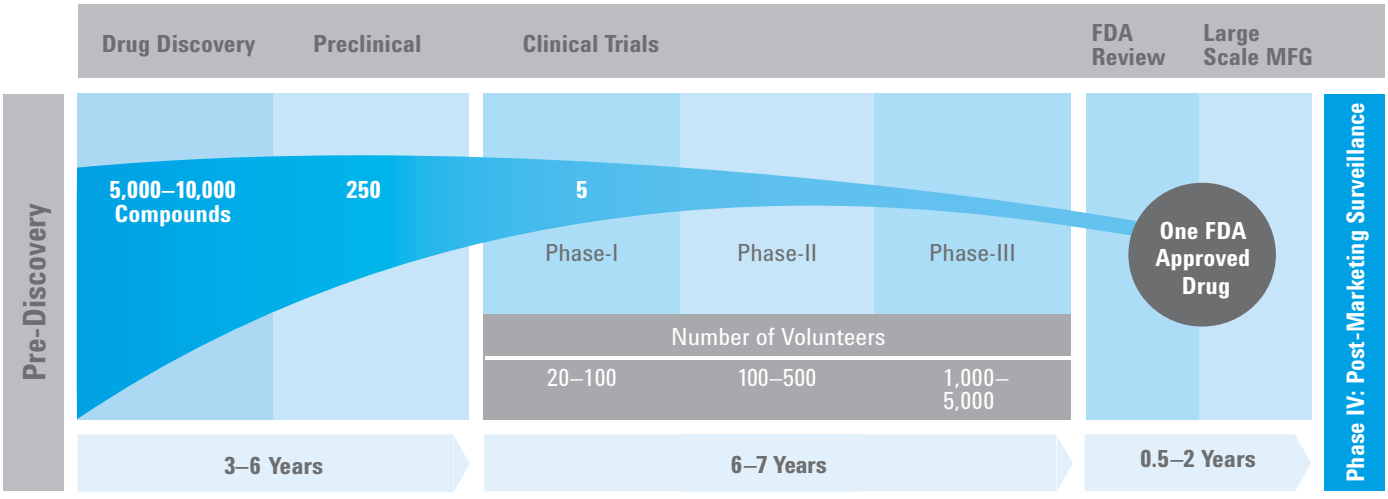

Figure 1. The large number of compounds in the drug discovery phase poses challenges for conducting efficient ADME studies.

Confidently increase your odds of success with Agilent—Solutions for early ADME studies

ADME Applications

Analyses of samples from *in vivo* ADME studies, including bile, urine, feces, and plasma

In vitro ADME assays, including metabolic stability, CYP inhibition/induction, permeability, and PgP inhibition

Drug metabolite characterization, quantitation, and mixture analysis

ADME Workflow

High-throughput ADME assays → Qualitative and quantitative ADME analyses → Metabolite purification → Drug metabolite characterization

Agilent Solutions

Agilent RapidFire/MS System
p. 4

Agilent 6500 Series Q-TOF
p. 6

Agilent 1290 Infinity Series LC
p. 10

Agilent 600 MHz NMR System
p. 8

Agilent Bravo Automated Liquid Handling Platform
p. 12

Agilent 6400 Series Triple Quadrupole
p. 6

Agilent 1260 Infinity Series Analytical-scale Fraction-Collectors
p. 10

Agilent NMR Probe Technologies
p. 8

ACCELERATE ANALYSIS

Unparalleled Throughput with RapidFire/MS

Quickly advancing the safest drug candidates involves making go/no-go decisions on a large number of compounds based on early ADME data. This requires performing ADME studies on very large numbers of compounds in the discovery setting at the throughputs necessary to provide data quickly, without compromising on the quality of the information generated. The Agilent RapidFire/MS platform provides the unmatched throughput at the plate-reader speeds required to do just that. Each sample is processed in 6–10 seconds and a single 384-well plate can be read in less than 40 minutes.

Faster data acquisition

The Agilent RapidFire/MS High-throughput system (Figure 2) removes barriers to discovery by eliminating the liquid chromatography step. It takes samples directly from quenched assay plates, removing the need for any time-consuming, manual sample preprocessing. Therefore, each sample can be analyzed in as little as 6–10 seconds.

By integrating SPE with mass spectrometry, RapidFire technology enables the analysis of hundreds of compounds in just a few hours, thus clearing the time and resource bottlenecks that arise when large numbers of early ADME samples require testing (Figure 3).

Figure 2. The RapidFire 360 System

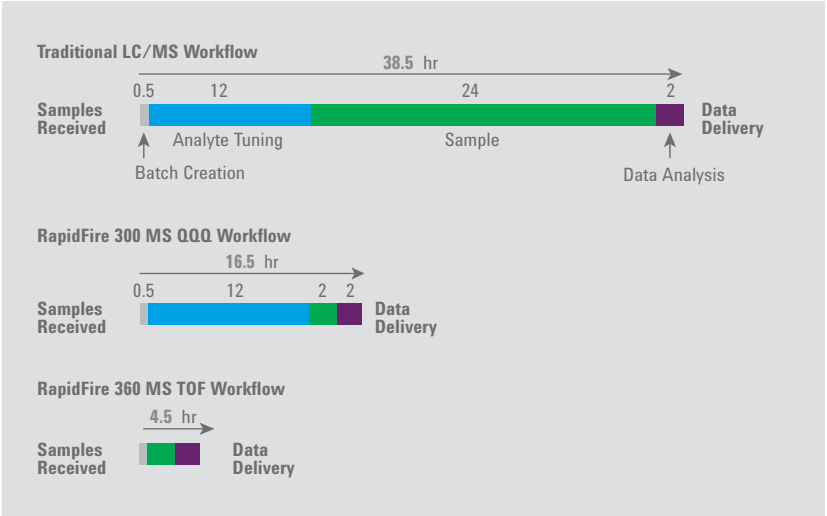

Figure 3. Faster ADME workflows are achieved with the RapidFire/MS system.

Greater versatility

The RapidFire/MS system has the flexibility necessary to meet the specific requirements of different ADME assays. It offers an array of SPE cartridge chemistries that work with a broad variety of compounds, including hydrophobic, hydrophilic, and aromatic small molecules, as well as peptides, proteins, and oligonucleotides.

For highly flexible ADME studies, the RapidFire system is optimized for integration with Agilent Triple Quadrupole MS, TOF/MS, and Q-TOF/MS instruments. With TOF or Q-TOF/MS, quantitative data can be obtained in the same experiment that provides qualitative identification of the major metabolites, further increasing efficiency (see p. 6).

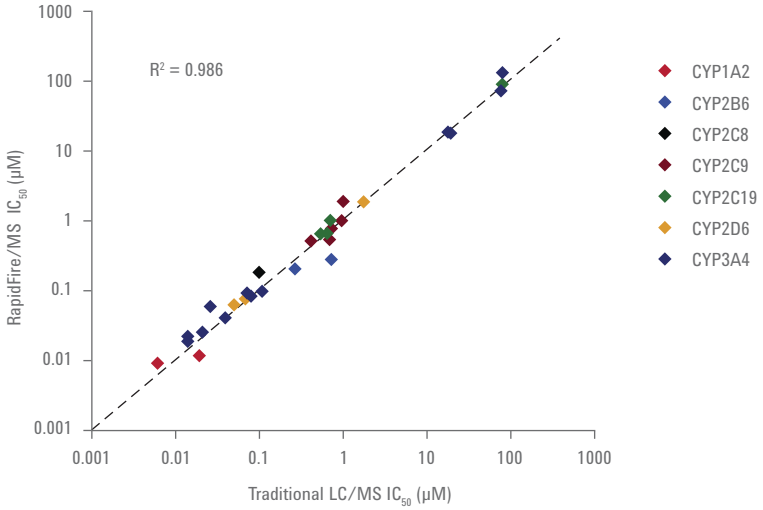

Figure 4. Data from eight different CYP enzyme/substrate pairs using traditional LC/MS and RapidFire/MS technology. The dotted line represents a line of unity.[5] (See Agilent application note 5990-9184EN.)

Uncompromising performance

With the RapidFire system, quality is not sacrificed for speed. Data obtained using the RapidFire/MS system correlates well with LC/MS data obtained in a number of different ADME assays, such as metabolic stability, CYP induction, CYP inhibition, plasma protein binding, P-glycoprotein inhibition, and permeability (Figures 4, 5).

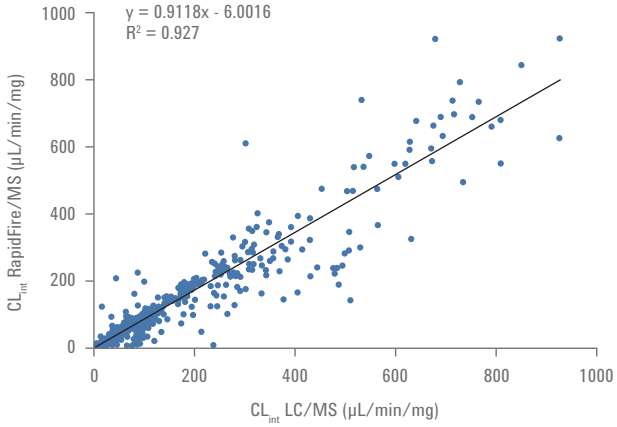

Figure 5. Correlation of microsomal stability results for 597 discovery compounds. The analysis was carried out using LC/MS/MS on a RapidFire 360 coupled to an Agilent 6530 Q-TOF. The R^2 values show very high agreement between the data sets.[6] *Data courtesy of Novartis.*

Agilent Discovery Services

Agilent is proud to offer our customers access to RapidFire *in vitro* ADME analysis through Agilent Discovery Services in order to increase efficiencies while preserving your laboratory's assay method integrity. We use the RapidFire High-throughput Mass Spectrometry system to analyze CYP inhibition/induction, microsomal stability, PAMPA, and plasma protein binding assays. Your results are delivered within 3 days. Data are in a format that is easy to interpret and use with existing laboratory workflows (LIMS). Our in-house scientists have used RapidFire to screen tens of millions of compounds, helping organizations to a accelerate their discovery workflows.

For more information, visit **www.agilent.com/lifesciences/discoveryservices**

Simultaneous Qualitative and Quantitative Measurements

As the pressure to increase productivity continues to challenge the pharmaceutical industry, ADME scientists have been looking for ways to conduct experiments faster and more efficiently. The Agilent 6500 series of Q-TOF LC/MS instruments offers the sensitivity, mass accuracy, and resolution required to enable simultaneous qualitative and quantitative data collection, greatly increasing efficiency (Figure 6).

High sensitivity and mass accuracy for increased productivity

While Agilent's 6400 Series Triple Quadrupole instruments provide mass sensitivity (down to zeptomole levels) and are an excellent choice when quantitation is all you need, Agilent's 6500 Series Q-TOF LC/MS systems are the instruments of choice for combining quantitation with qualitative characterization.

Agilent's 6500 Series Q-TOF LC/MS instruments provide the highest sensitivity of any high-resolution LC/MS instrument. When combined with sub-ppm mass accuracy in both MS and MS/MS, the 6500 Series Q-TOFs deliver high-confidence metabolite identification and structure elucidation for routine analyses of 1 µM substrate concentrations (Figures 7 and 8).

By combining quantitative and qualitative measurements, you no longer need to re-run the same samples on different instruments. Therefore, a complete set of analytical data is obtained in significantly less time, accelerating your ability to make critical go/no-go decisions about candidates using ADME information.

Figure 6. The 6550 Accurate-Mass Q-TOF LC/MS system provides the high sensitivity and mass accuracy needed for simultaneous quantitative and qualitative ADME analyses.

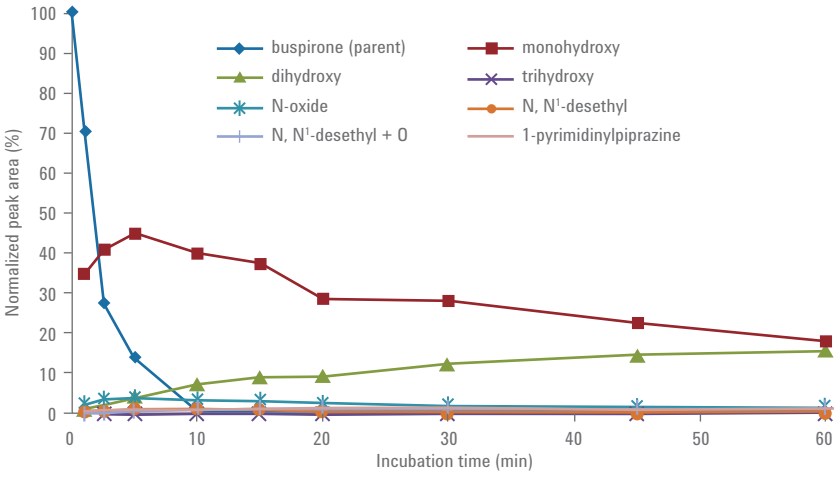

Figure 7. Metabolic stability and metabolite profiling of buspirone in rat liver microsomes analyzed on an Agilent 6550 Q-TOF LC/MS system. The profiles of buspirone and its metabolites demonstrate broad coverage of the high- and low-abundance metabolites during the 60-minute time course. (See Agilent publication 5990-9209EN.)

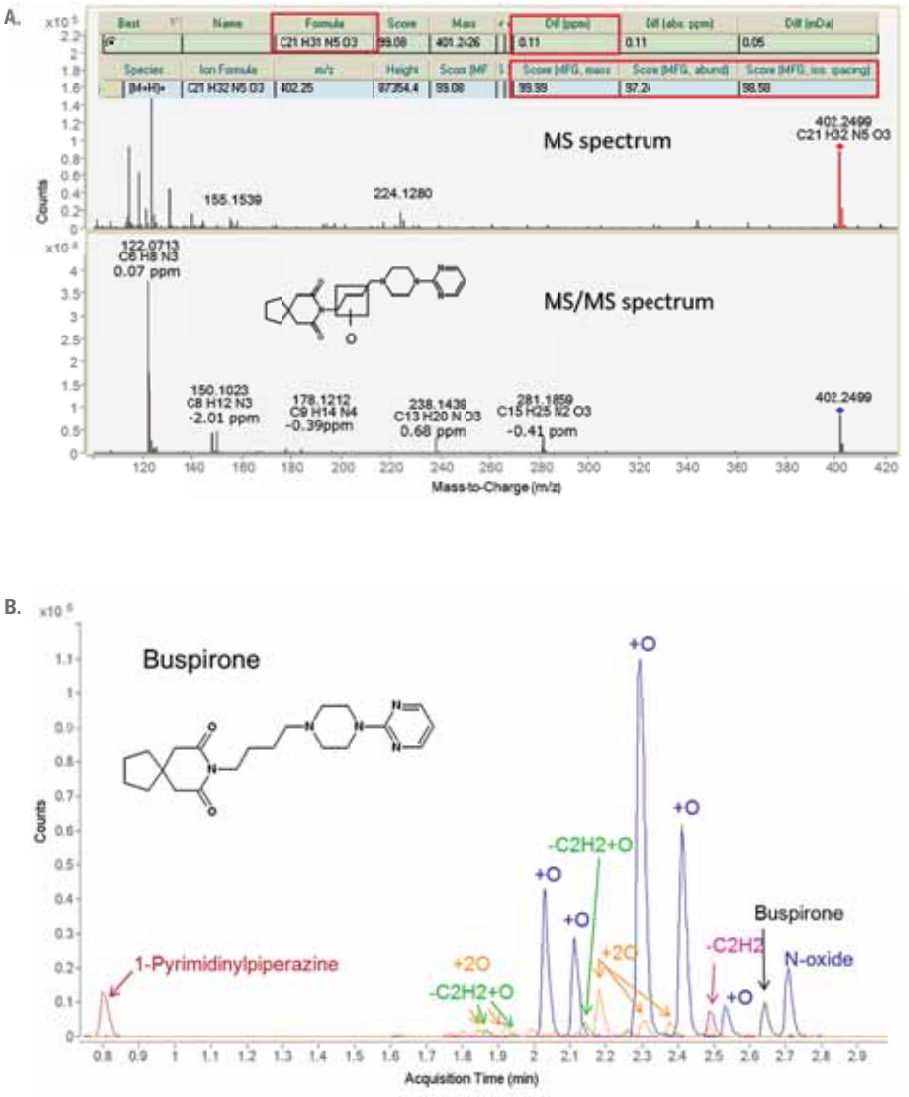

Figure 8. MS and MS/MS spectra of a buspirone monohydroxy metabolite from a 10-minute incubation sample (A). The sub-ppm mass accuracy of the precursor and fragment ions, along with the excellent isotopic fidelity (overall score > 99 %), provided high-confidence metabolite identification and structure elucidation. Excellent separation and broad metabolite coverage was achieved in three and a half minutes (B). (See Agilent publication 5990-9209EN.)

Simplicity in implementation

To achieve more efficient data acquisition, the Agilent 6550 Q-TOF provides a number of features that simplify and streamline experimental setup. With Q-TOF technology, less time is spent developing compound-specific MRM methods. Instead, for most ADME assays, generic MS methods and experimental conditions can be used for analyzing multiple compounds.

Furthermore, Agilent's Q-TOFs feature internal reference mass calibration with every run, and the ability to import work lists from spreadsheets, reducing the amount of time the operator spends optimizing the system and setting up experiments.

Powerful analysis

Identification is simplified with the Agilent MassHunter software package. In addition to data acquisition and instrument control, the software incorporates advanced data mining and processing tools that let you rapidly and accurately extract all available information from the compounds in your samples.

MassHunter software implements powerful algorithms that speed your analysis of ADME data, including:

- Molecular Feature Extractor (MFE) to rapidly find all detectable compounds in your sample
- Molecular Formula Generator (MFG) to derive molecular formulas for unknowns
- Molecular Structure Correlator (MSC) to aid in the identification of unknowns and confirm proposed structures

The compound-centric focus makes it easier for you to use and view your data. You can review one compound at a time or analyze multi-compound batches.

MAXIMIZE UNDERSTANDING

NMR Analysis for Detailed Structure Elucidation

NMR is the gold-standard, unmatched analytical technique for structure verification and identification of partial or complete unknowns. Thus, it is the method of choice for determining structures of metabolites directly from biological matrices or, more routinely, following purification.

Agilent offers a wide range of NMR systems for the in-depth structure analysis of candidate compounds and their metabolites, including high-field NMR systems, advanced probe technologies, automated sample loading, and our powerful VnmrJ 3 software (Figure 9).

For structure verification or full structure determination Agilent NMR systems provide the insight you need to select the most promising compounds for advancement.

- High-field NMR systems designed to fit in compact spaces
- Cryogenic probes for increased signal-to-noise on sample-limited metabolites
- VnmrJ 3 software for push-button experiment setup and data acquisition

From straightforward to sophisticated experiments

The Agilent portfolio of high-field NMR systems provides exceptional magnet stability and homogeneity for an optimal data acquisition environment.

The DirectDrive 2 console architecture incorporates the latest electronic technology to deliver precisely timed RF and gradient events, outstanding dynamic range and sensitivity, and flatter baselines than previously possible. Demanding data acquisition sequences with numerous selective excitation events, such as Hadamard NMR, can be accomplished with push-button ease. By removing inconsistencies and unwanted delays in data acquisition, the Agilent NMR consoles enable you to spend less time optimizing methods and more time analyzing results.

Superior characterization of low-level samples

When your analyte is present in low amounts, as is often the case for drug metabolites, we offer high-field systems and cryogenic NMR probes, such as the 3 mm MicroSample Cold Probe. With designs that deliver stable, homogeneous magnetic fields, superior line shapes, solid RF homogeneity, and excellent salt tolerance, our systems can handle a wide range of compounds, solvents, and buffers (Figure 10).

Figure 9. Structural elucidation of metabolites using NMR; 600 MHz NMR system (left) and 3 mm MicroSample Cold Probe (right). To better accommodate laboratory space constraints, our narrow-bore, actively shielded 600 MHz magnet fits in the same footprint as a traditional 400 MHz magnet.

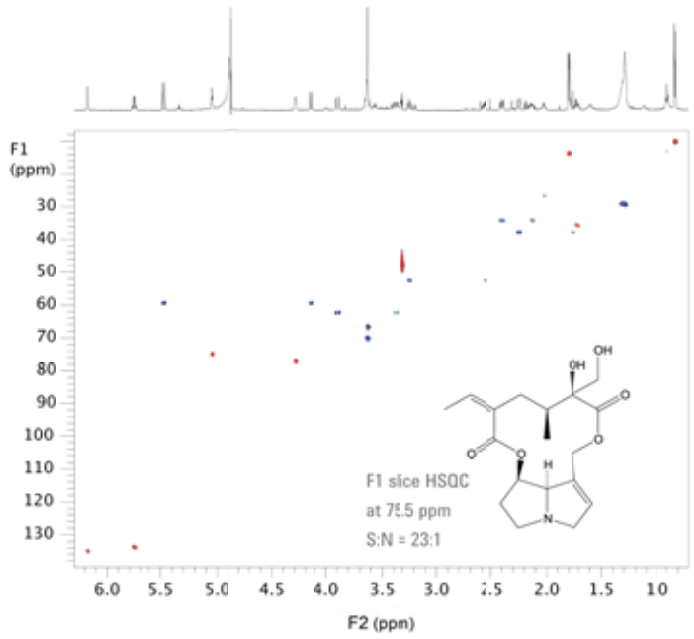

Figure 10. Agilent cold probes deliver data with higher signal-to-noise using limited sample in less time. This HSQC spectrum, collected on a 10 µg sample of retrorsine, represents routine analysis with a 600 MHz 3mm MicroSample Cold Probe.

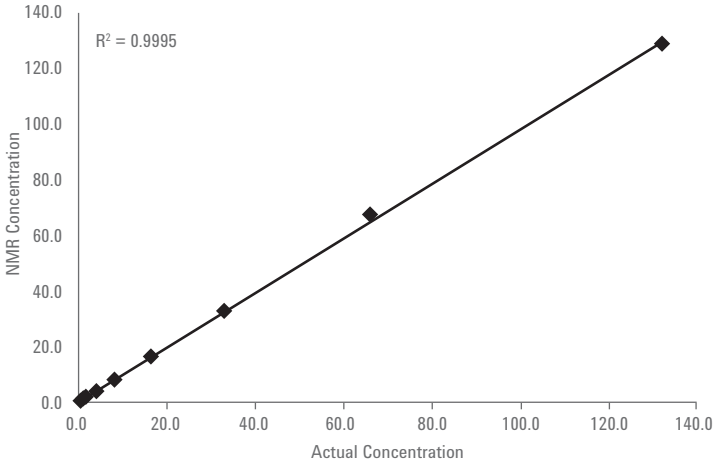

Figure 11. Extremely accurate and precise quantitative NMR, using the new qNMR tools in VnmrJ 3 software and calibration with an external standard. (See Agilent publication 5990-7601EN.)

While used most often for structure analysis, NMR is increasingly considered a critical quantitative tool. With advances in probe technologies, NMR is very relevant for quantitation studies of drug metabolites in biological matrices.[7] Agilent NMR systems, with VnmrJ 3 software tools, provide easy and accurate absolute quantitation using external standards. The superior field homogeneity and stability of our systems enable highly accurate quantitation without the need for internal standards (Figure 11) or error-prone electronic referencing approaches. Periodic calibration with an external standard can deliver accuracy as high as 99.9% and precision of 0.59%, while performing calibration with each study can result in accuracy and precision as high as 100% and 0.35%, respectively.

Sophisticated experiments, easy implementation

The sample-centric design of VnmrJ 3 software makes it easy to set up experiments, collect data, and analyze results. With a large variety of built-in pulse sequences and experimental protocols, all organized for convenient setup and use, sophisticated experiments are easy to implement.

VnmrJ 3 software simplifies metabolite characterization through automated processes such as Adaptive NMR and easily implemented experimental strategies that enable mixture analysis and solvent suppression.

- Set up selective excitation experiments
- Automate variable temperature experiments
- Automate quantitation

SIMPLIFY PURIFICATION

Achieve the Highest Recovery and Purity

High recovery rates and purity levels are key issues for the isolation and purification of valuable drug metabolites. Agilent offers robust preparative LC solutions for the purification of nanogram to gram sample quantities. The Agilent 1260 Infinity Series Analytical-scale Purification System (Figure 12) handles flow rates from 100 µL/min to 10 mL/min at pressures up to 600 bar, making it the system of choice for metabolite purification in the nanogram to low milligram range, on columns with internal diameters between 2.1 and 9.4 mm. Additionally, the modularity of the Agilent 1260 Infinity Purification Systems offers you outstanding flexibility in terms of application and bench space.

Precise and confident sample collection

Whether you need to trigger fraction collection based on the mass, refractive index (RI), Evaporative Light Scattering Detector (ELSD), UV signal, or retention time of your metabolite, or a combination of these attributes, the Agilent 1260 Infinity Series Analytical-scale Fraction Collectors provides high-confidence fraction collection that integrates seamlessly with our industry-leading LC systems that include pumps, columns, splitters, and detectors for reliable and efficient metabolite purification. Our patent-protected fraction delay sensing (FDS) technology enables fast and reliable collection of even very narrow chromatographic peaks with the highest possible recovery and minimal cross-over between fractions, thus preventing loss of precious metabolites (Figure 13).

Various collection strategies are possible, including pooling of fractions from multiple runs into the same well and pre-defining recovery locations to ensure nothing is lost. Sample as well as fraction cooling is also available to keep pre- or post-purification sample or fraction degradation to a minimum. For convenience, fractions can be collected in a variety of formats such as well plates, vials, and test tubes.

Figure 12. Agilent 1260 Infinity Series LC coupled to an Agilent 6100 Series Single Quadrupole Mass Spectrometer.

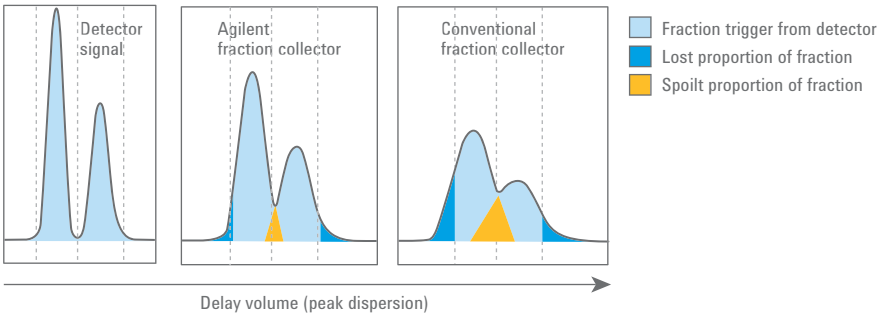

Figure 13. Agilent fraction collectors are designed to have the lowest delay volumes to avoid peak dispersion and carry-over between fractions, ensuring the highest recovery and purity for your fractions, especially at low flow rates. (See Agilent publication 5990-8840EN)

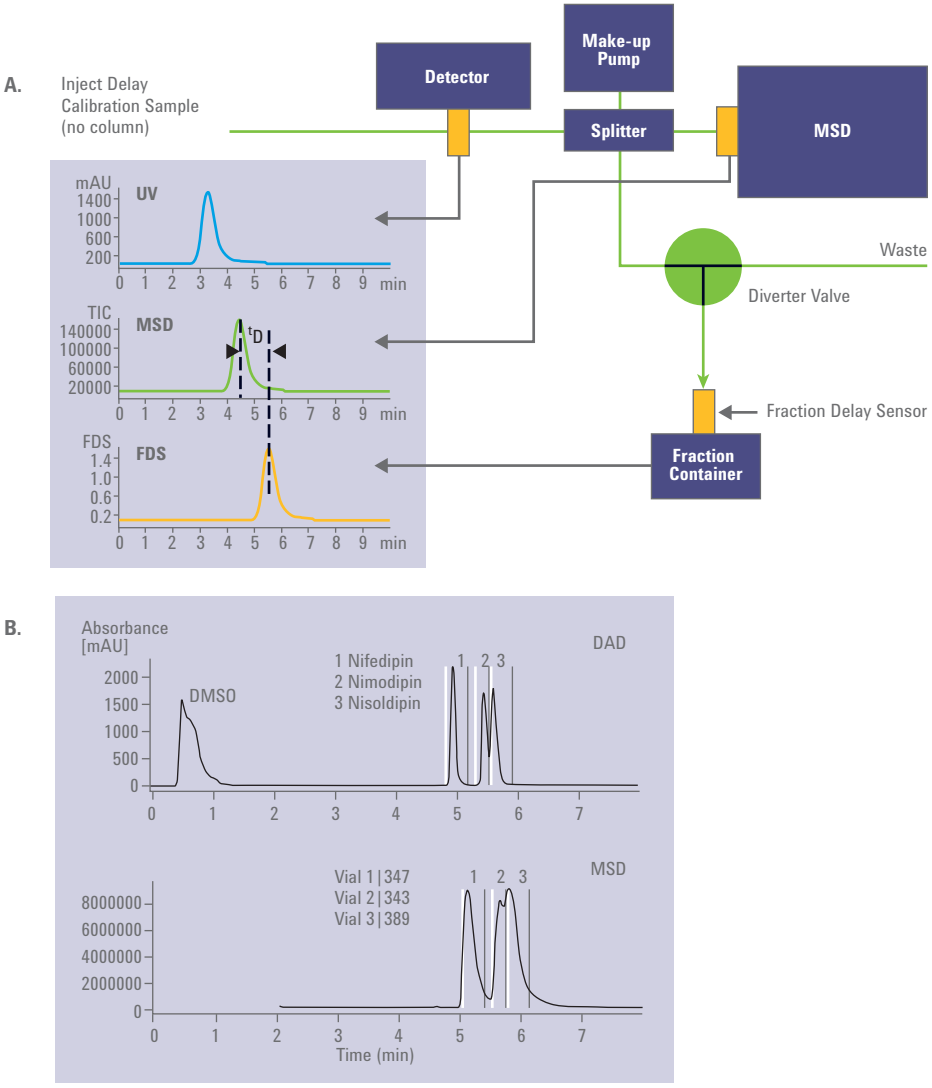

Figure 14. Mass-based system design incorporating FDS for precise collection of fractions without the loss of precious material. (A) Mass-based purification triggers fraction collection based MS as well as UV signal. In cases where MSD peaks are broad, UV-triggering can allow for reduced carry-over and the highest recovery and purity of fractions (B). (See Agilent publication 5988-7113EN.)

The Agilent OpenLAB Chromatography Data System (CDS) ChemStation Edition software includes a graphical fraction preview tool that helps you visualize the optimization of all relevant fraction trigger parameters based on previously acquired data. Alternatively, you can use the Agilent 1200 Infinity Series Instant Pilot to collect fractions manually. The fraction preview tool in OpenLAB CDS also intuitively visualizes changes of fraction trigger values in the chromatogram. Enhanced safety features such as leak, over/under pressure sensing, and forced fume extraction make it possible to walk away from the purification with confidence.

Mass-triggered metabolite purification

When the MS signal is stronger than the UV response or the metabolite of interest lacks a UV chromophore altogether, then a mass trigger may be the ideal solution for purifying the metabolite. The Agilent 1260 Infinity Series LC can be equipped with a mass spectrometer to allow mass-based fraction collection (Figures 12 and 14). A single, intuitive software program is all you need to run complex analytical UHPLC separations and scale up to LC/MS purification.

A combination of UV and MS triggering can be used to minimize cross-over between fractions in the case of broad MS peaks.

INCREASE REPRODUCIBILITY

Automation Speeds Reliable Measurement

Since consistency and reproducibility are critical for ADME assays, it is an ideal application for automation. With a variety of automation solutions for liquid and microplate handling, as well as robotic arms and enclosed automated workstations, Agilent Automation Solutions are customizable, reliable, safe and scalable, and can be integrated with third-party instruments.

- Serial dilutions
- Sample picking
- Labware transfer, storage, and tracking
- Barcode tracking
- Microplate sealing, piercing, storage, centrifugation, and labeling
- Microplate replication
- Assay plate generation
- Customizable enzymatic and cell-based assays

Figure 15. The Agilent Bravo Automated Liquid Handling Platform and Bravo deck layout for a CYP450 assay, with one Orbital Shaking Station (location 8). One stack of five microplates (location 7), three reservoirs (locations 1–3), and three tip boxes (locations 4–6) are placed manually on the deck before the protocol is started. (See Agilent publication 5990-3550EN.)

Automating ADME assays

The Agilent Bravo Automated Liquid Handling Platform is a flexible, highly customizable automated liquid handler that can be easily configured for early ADME assays (Figure 15). The Bravo deck has nine positions which accommodate 96-, 384-, or 1536-well microplates, and can be configured for heating, cooling, shaking, vacuum filtration, and magnetic bead separation. Deck positions accommodate tip boxes, sample microplates, and reservoirs. This versatility facilitates the automation of many different ADME assays, such as Cytochrome P450 (Figure 15), while ensuring consistent and reproducible results.

The Bravo Platform fits on a bench top or in a laminar flow hood, and can be integrated with other instruments or the fully automated Agilent BioCel systems (Figure 16).

Managing microplates

Agilent's microplate handlers, sealers, piercers, centrifuges, and barcode labelers offer unparalleled performance for your ADME studies. Easily integrating with other Agilent instruments such as the Bravo Platform or third-party instruments, our automation tools keep your ADME studies moving.

Customizable integration

For true flexibility in automation and dedicated automation stations, Agilent offers the BenchCel, BenchBot, and BioCel systems, controlled by Agilent VWorks software, the most flexible and efficient scheduling software available. BenchCel Workstations can be adapted for both complex and straightforward workflows, delivering more walkaway time and greater throughput when compared to traditional manual methods. Highly configurable and able to physically integrate both Agilent and over 100 non-Agilent instruments,these robust platforms are the ultimate in high-throughput early ADME.

The Agilent BioCel 900 System for quantifying cytochrome P450 (CYP450) activity illustrates one way to implement robust, high-throughput ADME assays (Figures 16 and 17). The small footprint and open-access design of the BioCel System make it an ideal solution for any medium- to high-throughput ADME application, and not only increase productivity but also reproducibility. All microplates are handled and processed in the same manner, including constant incubation times, ensuring reliable and repeatable results for clearer, easier selection of compounds for advancement.

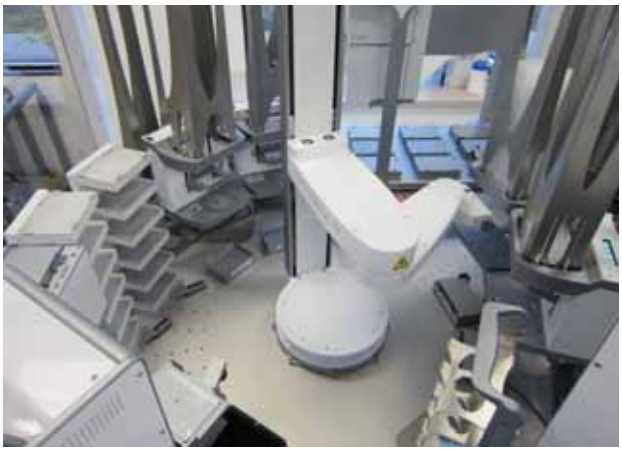

Figure 16. A customized BioCel automation solution, consisting of a Bravo Platform, PlateLoc Sealer, Hotel and LabWare Stackers, and a DDR robot.

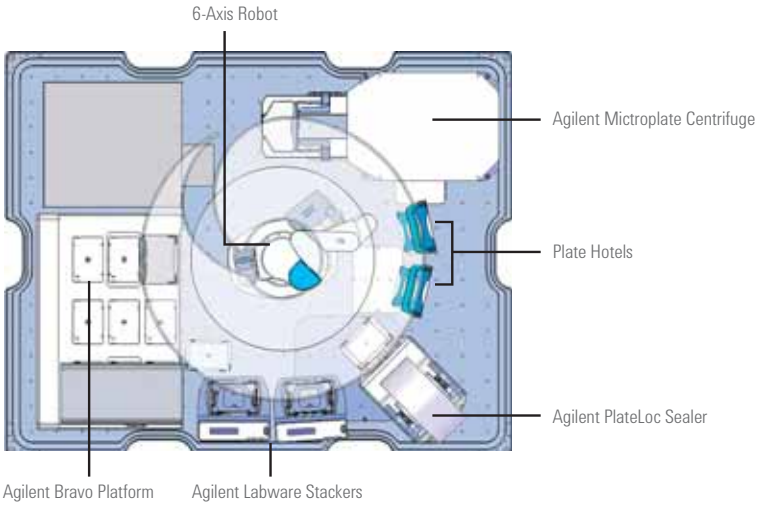

Figure 17. An Agilent BioCel 900 System configured for high-throughput CYP450 assays. (See Agilent publication 5990-3551EN.)

EXPAND CAPABILITIES

Reliable Sample Preparation

Agilent offers the right chemistry cartridge or column for your sample preparation and analysis needs.

Flexible sample preparation solutions

Agilent Bond Elut kits have been providing solutions in solid phase extraction for decades (Figure 18). They feature over 40 bonded silica phases for high-specificity methods and polymeric phases for rapid method development, with the largest choice of formats and sorbents on the market.

All Bond Elut products combine ease of use with flexibility to meet both manual and automated requirements, whether they are in a 96-well format or on fast-flow particles.

Versatile chromatography solutions

Agilent offers a wide variety of columns so you can use different compound chemistries, depending on your LC needs. Our Poroshell 120 columns use 2.7 µm superficially porous particles and a standard 2 µm frit. This design provides the speed, sensitivity, and resolution advantages of smaller particles while avoiding the clogging associated with smaller frits. They also give excellent peak shape for higher sensitivity and more accurate quantitation.

Agilent's ZORBAX Rapid Resolution High Definition (RRHD) columns benefit from a superior packing process, making them ideal for fast or high-resolution separations. Resolution is maximized through the use of 1.8 µm particles. Stability is guaranteed up to 1200 bar, making them ideal for UHPLC. Additionally, Agilent offers a variety of reversed-phase ZORBAX Eclipse columns available with multiple selectivities for method optimization and a variety of particle sizes ranging from 1.8 to 7 µm for use in the purification of metabolites.

Figure 18. Bond Elut SPE provides reliable, robust sample preparation.

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Agilent Technologies understands better than anyone how to evaluate the ADME properties of candidate molecules early in the process in order to mitigate late stage failures. With a long history of converting cutting-edge technologies into robust tools for routine analyses, and a strong focus on workflow-oriented design, Agilent is uniquely positioned to help ADME scientists achieve the efficiencies and quality they need for successful drug discovery. From sample preparation to analysis, purification, and identification, Agilent offers instruments with superior performance for faster, more confident drug discovery decisions.

Agilent Service Guarantee

In addition to our continually evolving products, Agilent offers the industry's only 10-year value guarantee. Agilent guarantees you at least 10 years of instrument use from your date of purchase, or we'll credit you with the residual value of that system toward an upgraded model. It's our way of assuring you of a reliable purchase and protecting your investment.

Related Literature

Agilent RapidFire 360 High-throughput Mass Spectrometry System. *Agilent Publication* 5990-8231EN.

Agilent Discovery Services for Assay Development, HTS, and ADME. *Agilent Publication* 5991-0200EN.

Agilent 6550 iFunnel Q-TOF LC/MS System. *Agilent Publication* 5990-8346EN.

Agilent NMR Chemistry Solutions. *Agilent Publication* 5990-7615EN.

Agilent 1260 Infinity Purification Systems —Infinitely Better Purity and Recovery. *Agilent Publication* 5990-6223EN.

Agilent Bravo Automated Liquid Handling Platform. *Agilent Publication* 5990-8633EN.

ZORBAX Family of Columns Brochure. *Agilent Publication* 5990-8795EN.

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