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Lead Discovery References Using RapidFire

Journal Articles

2011 Bioorganic and Medicinal Chemistry Letters – Pfizer

N-benzylimidazole carboxamides as potent, orally active stearoylCoA desaturase-1 inhibitors.

Atkinson KA, Beretta EE, Brown JA, Castrodad M, Chen Y, Cosgrove JM, Du P, Litchfield J, Makowski M, Martin K, McLellan TJ, Neagu C, Perry DA, Piotrowski DW, Steppan CM, Trilles R. Pfizer Global Research & Development, Groton Laboratories, Pfizer Inc., Groton, CT 06340, USA.
Bioorg Med Chem Lett. 2011 Mar 15;21(6):1621-5. Epub 2011 Jan 31.
Web link: http://www.ncbi.nlm.nih.gov/pubmed/21324691

Abstract: A potent, small molecule inhibitor with a favorable pharmacokinetic profile to allow for sustained SCD inhibition in vivo was identified. Starting from a low MW acyl guanidine (5a), identified with a RapidFire High-Throughput Mass Spectrometry (RF-MS) assay, iterative library design was used to rapidly probe the amide and tail regions of the molecule. Singleton synthesis was used to probe core changes. Biological evaluation of a SCD inhibitor (5b) included in vitro potency at SCD-1 and in vivo modulation of the plasma desaturation index (DI) in rats on a low essential fatty acid (LEFA) diet. In addition to dose-dependent decrease in DI, effects on rodent ocular tissue were noted. Therefore, in rat, these SCD inhibitors only recapitulate a portion of phenotype exhibited by the SCD-1 knockout mouse.

2011 Journal of Biomolecular Screening

Advances in Label-Free Screening Approaches for Studying Sirtuin-Mediated Deacetylation.

Rye PT, Frick LE, Ozbal CC, Lamarr WA.
J Biomol Screen. 2011 Sep 12. [Epub ahead of print].
Web link: http://jbx.sagepub.com/content/early/2011/09/10/1087057111420291.abstract

Abstract: The sirtuin enzymes, a class of NAD+-dependent histone deacetylases, are a focal point of epigenetic research because of their roles in regulating gene expression and cellular differentiation by deacetylating histones and a host of transcription factors, including p53. Here, the authors present two label-free screening methodologies to study sirtuin activity using high-throughput mass spectrometry. The first method involves the detection of native peptides and provides a platform for more detailed mechanistic studies by enabling the concurrent and direct measurement of multiple modification states. The second method obviates the need for substrate-specific assay development by measuring the O-acetyl-ADP-ribose co-product formed by sirtuin-dependent deacetylation. Both methodologies were applied to investigating the deacetylation of multiple-peptide substrates by multiple-sirtuin enzymes. Kinetic data, including binding constants, inhibition, and, in some cases, activation, are demonstrated to correlate well, both between the methodologies and with previous literature precedent. In addition, the ability to monitor sirtuin activity via O-acetyl-ADP-ribose production permits experimentation on whole-protein substrates. The deacetylation of whole-histone proteins by SIRT3, and inhibition thereof, is presented and demonstrates the feasibility of screening sirtuins using more biologically relevant molecules.

2011 Journal of Biomolecular Screening

Advances in Label-Free Screening Approaches for Studying Histone Acetyltransferases.

Rye PT, Frick LE, Ozbal CC, Lamarr WA.
J Biomol Screen. 2011 Sep 9. [Epub ahead of print]
Web link: http://jbx.sagepub.com/content/early/2011/09/08/1087057111418653.abstract

Abstract: Histone acetyltransferases (HATs) catalyze the transfer of an acetyl group from an acetyl-coenzyme A donor molecule to specific lysine residues within proteins. The acetylation state of proteins, particularly histones, is known to modulate their intermolecular binding properties and control various cellular processes, most notably transcriptional activation. In addition, deregulation of HAT activity has been linked to the development of a number of cancers; therefore, compounds that affect these enzymes have strong potential as therapeutic agents. The research presented here demonstrates three label-free HAT screening approaches, all based on the fast and direct measurement of one or more substrate-product pairs by high-throughput mass spectrometry techniques. The first approach involves monitoring all

possible acetylation states of a peptide concurrently to measure HAT activity. The second approach measures acetylation reactions, on both peptides and whole protein substrates, via direct detection of the acetyl-coenzyme A cosubstrate and coenzyme A coproduct. Lastly, the authors demonstrate the ability to monitor directly the acetylation state of whole histone proteins in the same high-throughput manner using time-of-flight mass spectrometry. The generation of compound-mediated inhibition data using each of these techniques establishes mass spectrometry as a versatile, label-free, and biologically relevant screening approach to this challenging target class.

2011 Journal of Biomolecular Screening -- Pfizer/BIOCIUS

High-Throughput Screening Assay for Sphingosine Kinase Inhibitors in Whole Blood Using RapidFire Mass Spectrometry

Maureen K. Highkin, Matthew P. Yates, Olga V. Nemirovskiy, William A. Lamarr, Grace E. Munie, John W. Rains, Jaime L. Masferrer, Marek M. Nagiec
J Biomol Screen February 4, 2011 vol. 16 no. 2 272-277
Web link: http://jbx.sagepub.com/content/16/2/272.abstract
Note: This was also presented as a poster during APA 2010.

Abstract: To facilitate discovery of compounds modulating sphingosine-1-phosphate (S1P) signaling, the authors used high-throughput mass spectrometry technology to measure S1P formation in human whole blood. Since blood contains endogenous sphingosine (SPH) and S1P, mass spectrometry was chosen to detect the conversion of an exogenously added 17-carbon-long variant of sphingosine, C17SPH, into C17S1P. The authors developed procedures to achieve homogeneous mixing of whole blood in 384-well plates and for a method requiring minimal manipulations to extract S1P from blood in 96- and 384-well plates prior to analyses using the RapidFire® mass spectrometry system.

2010 Journal of Biomolecular Screening – Schering Plough/BioTrove/Seton Hall Univ./Merck/Idaho National Laboratory

Screening for antibacterial inhibitors of the UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC) using a high-throughput mass spectrometry assay.

Langsdorf EF, Malikzay A, Lamarr WA, Daubaras D, Kravec C, Zhang R, Hart R, Monsma F, Black T, Ozbal CC, Miesel L, Lunn CA
J Biomol Screen. 2010 Jan ; 15(1): 52-61
Web link: http://jbx.sagepub.com/content/15/1/52.abstract

Abstract: A high-throughput mass spectrometry assay to measure the catalytic activity of UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase, LpxC, is described.. The results show that mass spectrometry-based screening is a valuable high-throughput screening tool for detecting inhibitors of enzymatic targets involving difficult to detect reactions.

The Situation: Find a way to perform a label-free inhibition screen based on the native LpxC substrate in order to develop a better anti-bacterial drug. LpxC (a deacetylase essential for endotoxin biosynthesis in gram-negative bacteria) is a well-known anti-bacterial and anti-inflammatory drug target, but effective drugs based on inhibitors of this enzyme have been difficult to develop. Typically, scientists have used an optical assay based on a LpxC surrogate substrate to screen compound libraries for LpxC inhibitors. The surrogate substrate has a Km >100x that of the native substrate and this large disparity in Km may be the reason for so many past failures to develop an effective inhibitor.

The Solution: Addressing the Challenges: In collaboration with RapidFire scientists, an assay was developed using the RapidFire automated SPE-based system in conjunction with mass spec analysis of the native LpxC substrate. The RapidFire team quickly performed primary screening analysis using this new LpxC assay on the client's compressed compound library (~250,000 wells). Secondary screens were then used to confirm several new inhibitors.

The Results: Three new patent applications and the potential of a new effective drug to treat gram-negative bacterial infections and their associated inflammatory disease.

2009 ASSAY and Drug Development Technologies – Merck/BioTrove

Last Updated August 22, 2012

Label-free high-throughput screening via mass spectrometry: a single cystathionine quantitative method for multiple applications.

Holt TG, Choi BK, Geoghagen NS, Jensen KK, Luo Q, LaMarr WA, Makara GM, Malkowitz L, Ozbal CC, Xiong Y, Dufresne C, Luo MJ
Assay Drug Dev Technol. 2009 Oct ; 7(5): 495-506
Web link: http://www.liebertonline.com/doi/abs/10.1089/adt.2009.0200

Abstract: Label-free mass spectrometric (MS) technologies are particularly useful for enzyme assay design for drug discovery screens. ... Our results show that the HTMS method was useful for screening samples containing serum, for cell-based assays, and for liver explants. The novel extension of the in vitro analytical method, without modification, to secondary assays resulted in a significant and advantageous economy of development time for the drug discovery project.

2009 Combinational Chemistry and High Throughput Screening

A Case Study on Acetyl-Coenzyme A Carboxylase using RapidFire – Mass Spectrometry (RF-MS)

Maxine Jonas, William A. LaMarr and Can Özbal

Abstract**:** In this review various technologies and approaches for the utilization of mass spectrometry in high throughput analyses are discussed. The use of quadrupole-based mass spectrometry in the screening of chemical libraries again stenzymatic targets for the identification of inhibitors and/or activators is highlighted. The RapidFire mass spectrometry system, an integrated on-line solid-phase extraction system interfaced to a triple-quadrupole mass spectrometer is described in detail, and the identification of a series of inhibitors of the acetyl-coenzyme A carboxylase (ACC) assay is described.

2008 Analytica Chimica Acta – Pfizer/BioTrove

Development of a high-throughput screening assay for stearoyl-CoA desaturase using rat liver microsomes, deuterium labeled stearoyl-CoA and mass spectrometry.

Soulard P, McLaughlin M, Stevens J, Connolly B, Coli R, Wang L, Moore J, Kuo MS, LaMarr WA, Ozbal CC, Bhat BG
Anal Chim Acta. 2008 Oct 3; 627(1): 105-11
Web link: http://www.sciencedirect.com/science/article/pii/S0003267008006867

Abstract: Several recent reports suggest that stearoyl-CoA desaturase 1 (SCD1), the rate-limiting enzyme in monounsaturated fatty acid synthesis, plays an important role in regulating lipid homeostasis and lipid oxidation in metabolically active tissues ... High-throughput mass spec screening of over 1.7 million compounds in compressed format demonstrated that the enzyme target is druggable ... The application of mass spectrometry to high-throughput screening permitted the development of a high-quality screening protocol for an otherwise intractable target, SCD1.

2008 Combinatorial Chemistry & High Throughput Screening – Kalypsys (Wako)/BioTrove

Back to basics: label-free technologies for small molecule screening.

Shiau AK, Massari ME, Ozbal CC
Comb Chem High Throughput Screen. 2008 Mar ; 11(3): 231-7
Web link: http://www.benthamdirect.org/pages/content.php?CCHTS/2008/00000011/00000003/0006A.SGM

Abstract: Small molecule high-throughput screening in drug discovery today is dominated by techniques which are dependent upon artificial labels or reporter systems. While effective, these approaches can be affected by certain experimental limitations, such as conformational restrictions imposed by the selected label or compound fluorescence/quenching. Label-free approaches potentially address many of these issues by allowing researchers to investigate more native systems without fluorescence- or luminescence-based readouts. However, due to throughput and expense constraints, label-free methods have been largely relegated to a supporting role as the basis of secondary assays. In this review, we describe recent improvements in impedance-based, optical biosensor-based, automated patch clamp and mass spectrometry technologies that have enhanced their ease of use and throughput and, hence, their utility for primary screening of small- to medium-sized compound libraries. The ultimate maturation of these techniques will enable drug discovery researchers to screen large chemical libraries against minimally manipulated biological systems.

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2007 Journal of Biomolecular Screening – Bayer/BioTrove

High-throughput mass spectrometry screening for inhibitors of phosphatidylserine decarboxylase.

Forbes CD, Toth JG, Ozbal CC, Lamarr WA, Pendleton JA, Rocks S, Gedrich RW, Osterman DG, Landro JA, Lumb KJ
J Biomol Screen. 2007 Aug ; 12(5): 628-34
Web link: http://jbx.sagepub.com/content/12/5/628.abstract

Abstract: A high-throughput mass spectrometry assay to measure the catalytic activity of phosphatidylserine decarboxylase (PISD) is described. PISD converts phosphatidylserine to phosphatidylethanolamine during lipid synthesis. Traditional methods of measuring PISD activity are low throughput and unsuitable for the high-throughput screening of large compound libraries. The high-throughput mass spectrometry assay directly measures phosphatidylserine and phosphatidylethanolamine using the RapidFire trade mark platform at a rate of 1 sample every 7.5 s. The assay is robust, with an average Z' value of 0.79 from a screen of 9920 compounds. Of 60 compounds selected for confirmation, 54 are active in dose-response studies. The application of high-throughput mass spectrometry permitted a high-quality screen to be performed for an otherwise intractable target.

2007 Journal of Biomolecular Screening – Bayer/BioTrove

High-throughput screening by mass spectrometry: comparison with the scintillation proximity assay with a focused-file screen of AKT1/PKB alpha.

Quercia AK, LaMarr WA, Myung J, Ozbal CC, Landro JA, Lumb KJ
J Biomol Screen. 2007 Jun ; 12(4): 473-80
Web link: http://jbx.sagepub.com/content/12/4/473.abstract

Abstract: Mass spectrometry is an emerging format for label-free high-throughput screening. The main limitation of mass spectrometry is throughput, due to the requirement to purify samples prior to ionization. Here the authors compare an automated high-throughput mass spectrometry (HTMS) system (RapidFire) with the scintillation proximity assay (SPA). The cancer therapy target AKT1/PKBalpha was screened against a focused library of kinase inhibitors and IC50 values determined for all compounds that exhibit > 50% inhibition. A selection of additional compounds that exhibited less than or = 50% inhibition in the primary screen was chosen as controls to confirm inactives. The selection of compounds is expected to identify common actives, common inactives, false positives, and false negatives. Agreement is found between HTMS and SPA in terms of primary hit identification and hit confirmation.

2004 ASSAY and Drug Development Technologies – BioTrove

High Throughput Screening via Mass Spectrometry: A Case Study Using Acetylcholinesterase

Can C. .zbal, William A. LaMarr, John R. Linton, Donald F. Green, Arrin Katz,
Thomas B. Morrison, and Colin J.H. Brenan

Abstract: Mass spectrometry-based screening can be applied to a wide range of targets, including those intractable targets that use substrates such as lipids, fatty acids, phospholipids, steroids, prostaglandins, and other compounds not generally amenable to conventional screening techniques.The major limitation to this approach is throughput, making HTS via mass spectrometry impractical.We present a mass spectrometry-based technique and hardware for lead discovery applications. Massspectrometry enables the design of label-free assays using biologically native substrates for a widerange of enzymatic targets. This system can be used for the direct quantification of analytes incomplex reaction mixtures with typical throughputs of 4Ð5 s per sample. A mass spectrometrybasedassay was developed to identify inhibitors of acetylcholinesterase, an enzyme with clinicalimportance in AlzheimerÕs disease. The system was used to screen a small chemical library. Severalpotent inhibitors were identified, and the IC50 values of the inhibitors were determined.

Lead Discovery Posters and Presentations

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2012 SLAS – Agilent Technologies (Poster)

A Label-Free Screening Approach to Epigenetic Drug Discovery: Using Mass Spectrometry to Monitor Protein and Nucleic Acid Modification Events

William A. LaMarr, Peter T. Rye, Lauren E. Frick, Agilent Technologies, Inc. Wakefield, MA 01880

2012 SLAS – Agilent Technologies (Poster)

High-throughput Measurement of DNA Mono- and Oligo-nucleotides by RapidFire/TOF-MS

William A. LaMarr1, Deyu Li2, Vipender Singh2, John M. Essigmann2, and Peter T. Rye1 1) Agilent Technologies, Wakefield, MA. 2) MIT, Departments of Chemistry and Biological Engineering, Cambridge, MA.

2011 SBS – BIOCIUS (poster)

Advances in Label-Free Screening Approaches for Studying Histone Acetyltransferases

Histone acetyltransferases (HATs) catalyze the transfer of an acetyl group from an acetyl-coenzyme A donor molecule to sequence specific lysine residues within proteins The acetylation state of proteins particularly histones is known to modulate their intermolecular binding properties and control various cellular processes, most notably transcriptional activation. Additionally, deregulation of HAT activity has been linked to the development of a number of cancers; therefore, compounds that affect these enzymes have strong potential as therapeutic agents. The research presented here demonstrates three label free HAT screening approaches all based upon the fast and direct measurement of one or more substrate/product pairs by high-throughput mass spectrometry techniques. One approach involves monitoring all possible acetylation states of a peptide concurrently to measure HAT activity. Another approach measures cosubstrate and coenzyme A coproduct. Lastly, we demonstrate the ability to monitor directly the acetylation state of whole histone proteins in the same high-throughput manner using time-of-flight (TOF) mass spectrometry. The generation of compound-mediated inhibition data using each of these techniques establishes mass spectrometry as a versatile label-free and biologically-relevant screening approach to this challenging target class.

2010 ASMS -- Bristol-Myers Squibb (poster)

Development of a High-Throughput MS-Based Bioanalytical Method for Assessment of P-Glycoprotein Inhibition

Evaluation of drug-drug interactions between potential drug candidates and transporters such as P-glycoprotein (P-gp) is an integral part of early liability screening in drug discovery. Inhibition of P-gp can be determined by performing bi-directional Caco-2 permeability studies with digoxin as a substrate probe. Existing methodologies include either assaying H3- digoxin with liquid scintillation counting or assaying non-labeled digoxin with LC-MS detection at a speed of several minutes per sample. Our goal was to develop an analytical method that was “label-free” and provided ample throughput to support this potentially high-volume assay. Mass spectrometric detection was the ideal choice for digoxin analysis because it is “label-free”, sensitive, specific, and reliable. However, it is not possible to achieve a bioanalytical throughput high enough to support a screening assay using traditional LC-MS/MS. To address this, we developed an ultra-fast method for digoxin analysis on the BIOCIUS RapidFire™ (RF)-MS/MS system with a cycle time of ~9 seconds per sample. In order to validate the accuracy of the RFMS/ MS method, a traditional LC-MS/MS method was established for results comparison. Data processing was made feasible through the development of in-house macros that utilize XLfit for standard curve linear regression and calculation of preliminary results. The resulting data suggests that the RF-MS/MS method generates equivalent results to both LC-MS/MS and radiolabel assays in determining inhibitors of P-glycoprotein.

2010 ASMS – BIOCIUS (poster)

Last Updated August 22, 2012

Evaluation of Accurate Mass TOF-MS for Use in High Throughput PAMPA & Plasma Protein Binding Screens

Assays that measure permeability (i.e. PAMPA) and plasma protein binding (PPB) are used to screen drug compounds early in the discovery process to eliminate poor performers and/or to identify compounds that need to be modified. Mass spectrometry has emerged as the preferred analysis method for these assays due to its sensitivity, specificity, and robustness. Traditionally, these assays utilize tandem MS, which is limited by its requirement for compound dependent method development. The use of accurate mass offered by time of flight (TOF) mass spectrometers, which eliminates the need for MRM optimization, was investigated here and has implications for a wide range of other in vitro ADME assays where MS method development is a bottleneck.

A diverse set of commercially available drug compounds with a broad spectrum of physical chemical properties were assayed. Samples were analyzed at a rate of 6-8 seconds per sample using an ultrafast SPE–MS system consisting of a RapidFire RF300 interfaced to an ABSciex API4000 or a RapidFire RF360 interfaced to an Agilent 6530 Q-TOF. MS conditions for each compound were optimized on the QqQ while generic MS conditions were used for all compounds on the Q-TOF. A single generic SPE condition was used for all analyzes.

For each assay, the results using the SPE-TOF were comparable to literature values and correlated very well with results from the SPE-MS/MS system (R2> 0.9). Interestingly, the Q-TOF successfully analyzed compounds that could not be analyzed on the QqQ. The tight correlation in assay values determined on the two different instruments suggests that the use of exact mass MS instrumentation is a valid alternative to MRM method development for the analysis of PAMPA & plasma protein binding assays. Eliminating MRM method development significantly streamlined workflow and improved laboratory efficiency. These generic SPE-TOF methods may also be useful for other in vitro ADME assays that typically utilize QqQ analysis.

2009 ASMS -- GSK (poster)
Label-free High-throughput Whole Protein Kinase Screening Assay

Protein kinases are involved in the regulation of multiple cellular processes by catalyzing phosphorylation of target substrates. Dysregulation of kinase activity has been found in a variety of diseases; therefore, protein kinase inhibitors offer an opportunity to target these diseases. To facilitate the screening and characterization of kinase inhibitors, we developed a mass spec-based high throughput screening method to measure their effect on kinase activity, permitting the use of whole protein substrates which advantageously allows detection of both competitive and non-competitive inhibitors. In addition, this assay does not rely on site-specific antibodies, allowing detection of phosphorylated serine, threonine, or tyrosine residues. Lastly, mass spectrometry facilitates quantitative interrogation of complex kinetics in proteins with multiple phosphorylation sites.

2009 ASMS -- OSI (poster)
Discovery of Novel Inhibitors of Serine Palmitoyltransferase (SPT) by Mass Spectrometry-based High-throughput Screening (HTS)

Obesity has been linked with increased ceramide accumulation and resultant impaired insulin sensitivity. SPT may represent an attractive enzyme to target within the ceramide synthetic pathway in order to treat type 2 diabetes. A mass spectrometry based HTS approach to identify inhibitors of mammalian SPT was used to screen a library of >250,000 small molecules. Screening identified potent SPT inhibitors from multiple chemotypes. A mass spectrometry-based HTS approach was developed to identify inhibitors of SPT, an enzyme that was previously intractable to conventional screening methods. The screen successfully identified potent inhibitors of mammalian SPT activity across several distinct chemotypes.

ASMS 2009 -- Merck (User Symposium Presentation)
"The Application of RapidFire MS in a High-throughput Production Assay Lab"

Last Updated August 22, 2012

This presentation, authored by Merck Research Labs: Deborah Zink, Tom Holt, Jonathon Lum, Marta Arocho, Anne Vergnon, Gerica Galviz, Claude Dufresne, deals with Merck's initially outsourced RapidFire project to develop production assays for drug-drug interaction. They state that they went from zero to five production assays in less than 5 months and, ultimately, brought the instrument in-house. They further describe they successfully tested overnight operation of the instrument and that they expected payback on purchase cost in 5 months.

ASMS 2009 -- Boehringer-Ingelheim (User Symposium Presentation)
"A Novel and Integrated Platform for Fully Automated High Throughput LC-MS/MS Analysis of in vitro ADME Sample"

This presentation, authored by Andreas H. Luippold - Boehringer Ingelheim Pharma GmbH & Co KG , describes how BI reduced cycle times from 80 minutes to 13 minutes for a 96 well plate using RapidFire technology.

2009 SBS -- Genzyme (poster)
Discovery of Small Molecule Inhibitors of GM3 Synthase via High-throughput Mass Spectroscopy

A label-free biochemical HTS assay using the RapidFire system was developed and HTS was performed to find small molecule inhibitors of GM3 synthase. A collection of 250,000 compounds were screened at 10 uM in singlicate. 1816 hits have been identified which resulted in a hit rate of 0.7 %, 1217 hits were confirmed in duplicate at 10 uM, resulting in a confirmation rate of 67%. Currently confirmed hits are under evaluation for potency and selectivity. Confirmed potent and specific hits for GM3 synthase from the biochemical screen will help to guide medicinal chemistry efforts to find a small molecule inhibitor of GM3 synthase. RapidFire system enables measurement of enzymatic activity of proteins which are difficult to measure by other known conventional methods. We have shown that RapidFire system can be used for multiple enzyme assays such as GM3 Synthase and ST6Gal-1.

2009 SBS – BIOCIUS (poster)
High-throughput Mass Spectrometric Detection of Histone 3 Demethylation

Here we illustrate the utility of RapidFire-MS to study epigenetic changes directly, using reactions between LSD1 enzyme and a dimethylated Histone3 peptide (H3K4me2) as an example. The RapidFire platform enables high-throughput mass spectrometric analysis of native molecules from in vitro reactions by performing on-line desalting in seconds, as opposed to HLPC, which requires minutes.

Early Phase Identification of Candidates for Epigenetic Drug Targets

The Situation: A biopharmaceutical company focused on the emerging field of epigenetic-based drug targets sought a faster, more efficient way to advance their screening programs. Traditionally, epigenetic targets were screened using conventional screening methods; however, this company was interested in a native analyte-based technology that could provide more biologically relevant data from assays measuring slight modifications in protein structure.

Addressing the Challenges: Working with BIOCIUS in-house scientists, assays for methylases, acetylases, phosphorylases and other modifying enzymes involved in histone and chromatin modulation were developed and optimized. The RapidFire mass spec platform had the ability to investigate reactions that are not practical using HPLC, fluorescence, or luminescence screening methodologies. The technology avoids some confounding effects (such as auto-fluorescence) and provides fast, high capacity sample analysis throughput that is required in early drug discovery.

Results: With the RapidFire system, the client was able to use biologically relevant assays earlier in the discovery process, ultimately providing rapid and accurate data analysis, reducing R&D time, and enabling faster identification of promising new lead compounds for epigenetic drug development.

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Last Updated August 22, 2012

2009 ELRIG – BIOCIUS (poster)
Consistent, High-throughput Metabolic Stability Screening Across Mass Spectrometry Platforms

The metabolic half-life or stability of a drug candidate has important pharmacokinetic and clinical significance in vivo, because it influences both oral bioavailability and plasma concentration of a compound, which in turn, affect its efficacy. Therefore, a high-throughput metabolic stability assay is a valuable tool in the go/no-go decision-making process in drug discovery. However, conventional LC-MS requires several minutes per sample processing time. Using the RapidFire system, we have developed analytical techniques which lower analysis times to only a few seconds per sample, allowing metabolic stability assays to be performed at throughputs consistent with other drug discovery screening platforms. In the present study we evaluated the compatibility of the RapidFire system with 3 MS platforms (ABI/Sciex, ThermoFisher, and Agilent) by measuring half lives using conventional assay methods. The values measured across all MS platforms tested were within a 2-fold window. This data shows that the RapidFire system produces consistent, high-throughput, metabolic stability data across multiple MS platforms enabling performance of these critical assays in a typical drug discovery environment.

2008 Protein Society – Amgen/BioTrove (poster)
Screening for ATP Citrate Lyase Inhibitors Using Mass Spectrometry

Used RapidFire to identify potential ACL inhibitors, screened more than 43,600 compounds in single point and more than 300 IC50 follow-ups. Showed that the RapidFire platform is ideal for identifying and verifying lead compounds such as Compound A and Compound B. RapidFire technology was also used to look at SAR around these most potent compounds as well as investigate reversibility and probe the mechanisms of inhibition.
Using both RapidFire and FT- Orbitrap® mass spectrometry to characterize PKA treated ACL researchers phospho-residue mapped and assayed under Vmax conditions, though report only marginally lower activity with the unphosphorylated ACL enzyme as opposed to Potapova et.al. who report a 6-fold increase in ACL phosphorylated by PKA. In addition, the high resolution of FT-Orbitrap enabled the direct analysis of ACL activity in cells and demonstrates compensatory increase in cellular AcetylCoA levels upon inhibition.

2008 SBS -- Pfizer (poster)
The Use of High Throughput Mass Spectrometry (HTMS) in Drug Discovery

The 1st generation of RapidFire instrument was delivered to Pfizer Groton HTS Center of Emphasis (CoE) in Sept. 2006. In 2007, the HTS CoE has applied the HTMS system to: Assay development and Subset screening for DGAT; Hit to Lead exploration with Stearoyl CoA Desaturase (SCD); Assay Development for SirT1. In 2008, we have expanded our impact by supporting 7 drug targets at various discovery stages.

2007 SBS -- Merck (poster)
Identification of Inhibitors for the Phosphatidylethanolamine N-methytransferase Enzyme by High-Throughput Mass Spectrometry Detection of Reaction Products

Phosphatidylethanolamine N-methyltransferase (PEMT) is the enzyme that transfers methyl groups from SAM to phosphatidylethanolamine (PE), to generate phosphatidylcholine (PC) and SAH. It has been reported that in PEMT -/- mice there is a decrease in plasma PC and cholesterol found in high density lipoproteins when compared to wild type mice. Thus it might be of interest to see how PEMT inhibitors could modulate the levels of plasma lipoproteins. Two inhibitors were identified, with potency equivalent or higher than SAH, and could represent interesting lead molecules for the development of potent and selective inhibitors of PEMT. Such compounds could be used in a relevant animal model of atherosclerosis to evaluate the role of PEMT in this disease

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Last Updated August 22, 2012

2007 SBS -- Bayer (poster)
High-Throughput Mass Spectrometry Screening for Inhibitors of Phosphatidylserine Decarboxylase

Abstract: A high-throughput mass spectrometry assay to measure the catalytic activity of phosphatidylserine decarboxylase (PISD) is described. PISD converts phosphatidylserine (PS) to phosphatidylethanolamine (PE) during lipid synthesis. Traditional methods of measuring PISD activity are low throughput and unsuitable for the high-throughput screening of large compound libraries. The high throughput mass spectrometry assay measures phosphatidylserine and phosphatidylethanolamine directly using the RapidFire platform at a rate of one sample every 7.5 s. The assay is robust, with an average Z′ value of 0.79 from a screen of 9,920 compounds. Of 60 compounds selected for confirmation, 54 are active in doseresponse studies. The application of high-throughput mass spectrometry permitted a high-quality screen to be performed for an otherwise intractable target

ADME References Using RapidFire-MS

Journal Articles

2012 Future Science Ltd. – Agilent Technologies
SPE-MS analysis of absorption, distribution, metabolism and excretion assays: a tool to increase throughput and streamline workflow
Vaughn P. Miller, Agilent Technologies, 11 Audobon Road, Wakefield MA 01880

2011 Analytical Chemistry – J&J/Agilent
Evaluation of a high-throughput online solid phase extraction-tandem mass spectrometry system for in vivo bioanalytical studies. Jian W[1], Romm MV[2], Edom RW[1], Miller VP[2], LaMarr WA[2], Weng N[1]. [1]Johnson & Johnson Pharmaceutical Research & Development, Raritan, New Jersey 08869, United States; [2]Agilent Technologies, Inc. 11 Audubon Rd., Wakefield, MA 01880, USA. Anal. Chem. 83, 8259-8266 (2011).
Web Link:
http://pubs.acs.org/doi/abs/10.1021/ac202017c?mi=scbaih&af=R&pageSize=20&searchText=cyclodextrin

- Evalution of RapidFire/MS for in vivo (PK) bioanalysis: RF-MS/MS correlates with LC-MS/MS

2011 Journal of Biomolecular Screening – Boehringer Ingelheim
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. Andreas H. Luippold[a],Thomas Arnhold[a], Wolfgang Jörg[a] , B Krüger , and Roderich D. Süssmuth[b a] Department of Drug Discovery Support, Boehringer Ingelheim Pharma GmbH & Co KG, Birkendorfer Str. 65, 88397 Biberach an der Riss, Germany; [b] Institut für Chemie, Technische Universität Berlin, Strasse des 17. Juni 124, 10623 Berlin, Germany. Journal of Biomolecular Screening, 16(3):370-7 (2011).
Web Link: http://jbx.sagepub.com/content/16/3/370.abstract

- RF-MS/MS correlates with LC-MS/MS
    - o CYP inhibition
    - o Caco-2
    - o PAMPA

2011 Rapid Communications in Mass Spectrometry – BMS
Ultrafast mass spectrometry based bioanalytical method for digoxin supporting an in vitro Pglycoprotein(P-gp) inhibition screen. Andrew D. Wagner[1], Janet M. Kolb[1], Can C. Özbal[2], John J. Herbst[1], Timothy V. Olah[1], Harold N. Weller[1], Tatyana A. Zvyaga[1] and Wilson Z. Shou[1]* [1]Applied Biotechnology, Bristol-Myers Squibb, 5 Research Parkway, Wallingford, CT 06492, USA; [2]BIOCIUS Life Sciences, Inc. 10P Gill St., Woburn, MA 01801, USA. Rapid Communications in Mass Spectrometry, 25, 1231-1240 (2011).
Web Link:
http://onlinelibrary.wiley.com/doi/10.1002/rcm.4984/abstract;jsessionid=C35156AFB1BA0CE1C759126FB74A49DF.d01t01?systemMessage=Wiley+Online+Library+will+be+dis

rupted+14+May+from+10-12+BST+for+monthly+maintenance

- P-gp digoxin inhibition assay in Caco-2 cells: RF-MS/MS correlates with LC-MS/MS

2010 International Journal of Mass Spectrometry – Boehringer Ingelheim

An integrated platform for fully automated high-throughput LC–MS/MS analysis of in vitro metabolic stability assay samples. Andreas H. Luippold[a],Thomas Arnhold[a], Wolfgang Jörg[a] and Roderich D. Süssmuth[b a] Department of Drug Discovery Support, Boehringer Ingelheim Pharma GmbH & Co KG, Birkendorfer Str. 65, 88397 Biberach an der Riss, Germany; [b] Institut für Chemie, Technische Universität Berlin, Strasse des 17. Juni 124, 10623 Berlin, Germany. International Journal of Mass Spectrometry, 296:1-9 (2010).
Web Link: ftp://ftp.ni.com/pub/branches/germany/artikel/2010/11_nov/ni_10_IJMS.pdf

- Metabolic stability with HLM: RF-MS/MS correlates with LC-MS/MS

Abstract**:** The evaluation of ADME properties contributes importantly to drug candidate selection and therefore is crucial for the drug discovery process. Providing a sufficiently high-throughput capability in laboratories dedicated to early pharmacokinetic studies will thereby shorten the entire drug discovery process. In this paper an integrated and fully automated LC MS/MS-based platform is described, which enables the assessment of *in vitro* metabolic stability, a key ADME parameter. An ultra-rapid injection system was coupled to a triple quadrupole mass spectrometer and hard- and software were customized to perform sample identification, cleanup, MS compound optimization and sample measurement without manual interaction. Conventional chromatography was initially evaluated but was later replaced by solid phase extraction as the only purification step. Ultra-fast robotics, combined with a generic step gradient enables analysis times of 8 sec/sample. Reproducibility and quality of data has been found fully comparable to data generated by validated LC-MS/MS methods. The system handles data for mass spectrometric compound optimization and MRM (Multiple Reaction Monitoring) analytics in a fully automated way and exhibits great potential for a generalized use in ADME replacing currently applied conventional LC-MS/MS approaches.

2010 Journal of Biomolecular Screening – Takeda/BIOCIUS

Development of a High Throughput On-Line Solid Phase Extraction/Tandem Mass Spectrometry Method for Cytochrome P450 Inhibition Screening. Journal of Biomolecular Screening, 15 (4), 447-452 (2010). Kheng B. Lim[1], Can C. Ozbal[2], Daniel B. Kassel[1] ([1]Takeda San Diego, Inc., [2]BIOCIUS Life Sciences).
Web Link: http://jbx.sagepub.com/content/15/4/447.abstract

- CYP inhibition: RF-MS/MS correlates with LC-MS/MS

2010 Rapid Communications in Mass Spectrometry – Novartis

High-Throughput Analysis of In-Vitro Cytochrome P450 Inhibition Samples Using Mass Spectrometry Coupled with an Integrated Liquid Chromatography/Autosampler System.
Ann Brown*, Shari Bickford, Panos Hatsis, Jakal Amin, Leslie Bell, and Shawn Harriman. Metabolism and Pharmacokinetics, Novartis Institutes for Biomedical Research, 250 Massachusetts Avenue Cambridge, MA 02139 USA. Rapid Commun Mass Spectrom. 2010 Apr 30;24(8):1207-10.
Web Link: http://onlinelibrary.wiley.com/doi/10.1002/rcm.4461/full

- CYP inhibition: RF-MS/MS correlates with LC-MS/MS

ADME Posters and Presentations

2012 ISSX/MDO – Agilent Technologies

Evaluation of Ultrafast Online SPE/MS for the Efficient Screening of Cytochrome P540 Inhibition Using Cassette Analysis

Nikunj Parikh1, Michelle V. Romm1, Vaughn P. Miller1, Moritz Wagner2 and William A. LaMarr1 1. Agilent Technologies Inc., Wakefield, MA 2. Agilent Technologies, Waldbronn, Germany

Last Updated August 22, 2012

Abstract: The Cytochrome P450 (CYP) enzymes are involved in many drug metabolism transformations and account for a significant number of bioactivation and metabolic reactions. The presence of some drugs can interfere with the metabolic activity of CYP enzymes causing drug-drug interactions. Analysis of potential drug-drug interactions caused by inhibition of the enzymatic activities of CYP enzymes plays an important role in the drug discovery process. The ever increasing need to evaluate a large number of samples and the need to eliminate weaker candidates in the initial phase of the drug discovery process, has created a bottleneck at LC/MS/MS analysis. We evaluated the use of an ultra-fast online SPE/MS system (the Agilent RapidFire High-throughput Mass Spectrometry system) coupled to an Agilent QqQ and Agilent QTOF for the analysis of pooled samples across several CYP450 isoforms in a single cassette injection mode to provide an even faster and more cost-effective screen.

2010 AAPS – BIOCIUS (poster)
Streamlining ADME Workflow Using SPE-TOF Analysis

The throughput of in vitro ADME analysis is gaining an almost equal footing with data quality as a determinant of laboratory workflow. These assays have traditionally utilized tandem MS, which is limited by its requisite MRM method development that requires several minutes per sample of processing time.

The use of accurate mass offered by high resolution (time of flight: TOF) mass spectrometers, which eliminates the need for MRM optimization, in conjunction with a high throughput solid phase extraction (SPE) system was investigated to assess its ability to enable a faster and more efficient assay analysis workflow.

We compared the assay results for a panel of in vitro ADME assays (CYP inhibition, metabolic stability, PAMPA and plasma protein binding) using ultrafast SPE-MS/MS and SPE-TOF systems for analysis. A chemically diverse set of 50 compounds was used for each assay and all analyses utilized the same generic SPE conditions. All SPE-TOF analyses were performed using the same generic MS conditions with a RapidFire 360 interfaced to an Agilent 6530 Q-TOF run in ESI-TOF mode. The experimental results obtained for all four of the ADME assays by each analysis system were comparable (R2 >0.9).

Using a generic SPE condition, the ultrafast SPE-MS systems consistently produced sample analysis cycle times of 7 seconds/sample with no changes to standard laboratory workflow and no significant sample carryover. Using a generic MS method, the TOF analysis gave comparable results to conventional triple quadruple analysis. Assay results with the SPE-TOF system were comparable to the SPE-MS/MS system for a variety of ADME assays indicating that MRM method development could be eliminated for these assays providing a significant increase in efficiency of laboratory workflow. The SPE-TOF system enabled faster and more efficient assay data analysis across a panel of ADME assays without compromising the quality of results.

2010 AAPS -- Novartis (poster)
Ultra-fast Solubility Sample Analysis Using SPE-MS/MS

We investigated the utility of solid phase extraction (SPE) coupled with mass spectrometry for improving sample throughput of the solubility assay. The current method for solubility sample analysis employs HPLC with ultraviolet absorbance detection (UV). We investigated a SPE-TOF system consisting of an ultra-fast SPE system (RapidFire®) linked to an Agilent 6530 Q-TOF mass spectrometer. The two analytical methods were compared using a set of compounds from Novartis' drug discovery programs that covered a wide range of chemical space and properties. Solubility was measured in triplicate in pH 6.8 buffer and in FASSIF buffer. A four point calibration curve was prepared to quantify the solubility of each sample. The SPE-TOF method had a cycle time of eight seconds compared to two minutes for the HPLC-UV method. This represents a 15-fold improvement in cycle time, which can be used to increase the capacity of this assay. The SPE-TOF method was able to maintain data quality as evidenced by linear standard curves (r2 ≈ 0.99) and back-calculated standards within 30%. Moreover, a correlation plot of the solubility determined using SPE-TOF vs. HPLC/UV was linear with a slope of approximately one and an r2 > 0.8.

2010 APA – J&J/BIOCIUS (poster)
Evaluation of RapidFire Ultra-fast Online SPE-MS/MS System for Analysis of in vivo Bioanalytical Samples. Wenying Jian[1], Michelle V. Romm[2], Richard W. Edom[1], Vaughn P. Miller[2], William A. LaMarr[2], Naidong Weng[1]; [1]Johnson & Johnson Pharmaceutical Research & Development, Raritan, NJ [2]BIOCIUS Life Sciences, Wakefield, MA.

- Human & animal plasma bioanalysis: RF-MS/MS correlates with LC-MS/MS

2010 ISSX – BIOCIUS (poster)

The End of MRM: Ultrafast SPE-TOF Analysis Streamlines Workflow and Increases Throughput of ADME Assays. Michelle V. Romm, Nikunj Parikh, Vaughn P. Miller, William A. LaMarr, Can C. Ozbal.

- RF-TOF correlates with RF-QqQ
  - o CYP inhibition
  - o Microsomal stability
  - o PAMPA
  - o Plasma protein binding

2010 DDI Conference – BD Gentest/BIOCIUS (poster)

Evaluation of SPE-MS/MS (RapidFire®) for Relative Quantitation of Hydroxy-testosterone Metabolites in Cytochrome P450 3A4 Inhibition Assays In Vitro. David M. Stresser, Eric T. Gangl, Andrew K. Mason, Andrew P. Blanchard, Elke S. Perloff, Charles L Crespi and Vaughn P. Miller1. BD Biosciences Discovery Labware, Woburn, MA 01801, USA 1BIOCIUS Life Sciences, Wilmington, MA 01880.

- RF-MS/MS produces robust, quantitative outcomes for the 6B-hydroxytestosterone assay.

2010 DDI Conference – BD Gentest/BIOCIUS (poster)

Validation of an Automated Cytochrome P450 Inhibition Assay Using SPE/MS/MS (RapidFire®) Analysis. Elke S. Perloff, Andrew K. Mason, Michael S. Shanler, Sudarshan Kapadnis, Vaughn P. Miller, William A. LaMarr, and David M. Stresser. BD Biosciences (ESP, AKM, MSS, SK, & DMS), BIOCIUS Life Sciences (VPM & WAL).

- CYP inhibition: RF-MS/MS correlation to LC-MS/MS

2010 ASMS – Novartis/BIOCIUS (podium talk)

Reducing Bottlenecks in ADME Sample Analysis using Solid Phase Extraction with a Quadrupole Time-of-Flight Mass Spectrometer. Panos Hatsis1, Michelle Romm2, Vaughn Miller2, Jakal Amin1, William LaMarr2, Can Ozbal2 and Shawn Harriman1 *1Novartis Institutes for Biomedical Research 2BIOCIUS Lifesciences.*

- RF-TOF correlates with LC-MS/MS
  - o CYP inhibition
  - o Microsomal stability
  - o PAMPA

2010 ASMS – BMS/BIOCIUS (poster)

Development of a High-Throughput MS-Based Bioanalytical Method for Assessment of PGlycoprotein Inhibition. Andrew Wagner[1], Janet Kolb[1], John Herbst[1], Charlie Conway[1], Tatyana Zvyaga[1], Harold Weller[1], Wilson Shou[1], Can "Jon" Ozbal[2]; [1]Bristol-Myers Squibb Company; Applied Biotechnology [2]BIOCIUS Life Sciences, Inc.

- High-throughput P-glycoprotein inhibition assay
  - o RF-MS/MS correlates with LC-MS/MS

2010 ASMS – Novartis (poster)

Comparison of LC/MS/MS and a Fully Integrated Autosampler/Solid Phase Extraction System for the Analysis of Permeability Samples*;* Adam C Amaral; Christopher Caldwell; Panos Hatsis; Jakal Amin; Shawn Harriman; *Novartis, Cambridge, MA.*

- RF-MS/MS correlates with LC-MS/MS
  - o PAMPA

2010 ASMS – BIOCIUS (poster)

Evaluation of Accurate Mass TOF-MS for Use in High Throughput PAMPA & Plasma Protein

Binding Screens. Michelle V. Romm, Nikunj Parikh, Vaughn P. Miller, William A. LaMarr, Can C. Ozbal.

- RF-TOF correlates with RF-QqQ
  - o PAMPA
  - o Plasma protein binding

2010 SBS – BIOCIUS (poster)

A High-Throughput In Vitro ADME Assay Panel: Incorporation of SPE-MS/MS Into Standard Laboratory Workflow. Michelle V. Romm, Nikunj Parikh, Vaughn P. Miller, William A. LaMarr, Can C. Ozbal.

- RF-TOF correlates with RF-QqQ
  - o CYP inhibition
  - o Microsomal stability
  - o PAMPA
  - o Plasma protein binding

2009 ISSX – BioTrove (poster)

Evaluation of accurate mass TOF-MS for use in high throughput metabolic stability screening. Michelle V. Romm, Nikunj Parikh, Vaughn P. Miller, William A. LaMarr, and Can C. Özbal.

- Metabolic Stability: TOF correlates with QqQ

2009 ISSX – AstraZeneca/BioTrove (poster)

Comparison of High-Throughput Fluorescence, Traditional LC/MS/MS and BioTrove RapidFire uHTMS Analysis to Assess Cytochrome P450 Inhibition for Use in Drug Discovery. Reema Harish[1], Garrett Ainslie[1], Michael Rooney[1], Patrick Brassil[1], Simon Roberts[1], Vaughn Miller[2] and Caroline Rivard[1]*.
[1]Drug Metabolism and Pharmacokinetics, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451.
[2]BioTrove Inc, 12 Gill St, Ste 4000, Woburn, MA 01801.

- CYP inhibition: RF-MS correlation to LC-MS/MS

2009 ISSX – BD Gentest/BioTrove (poster)

Evaluation of High Throughput Screening Methods for Time-Dependent Inhibition of CYP3A4 Utilizing RapidFire LC/MS/MS Technology. Vaughn P. Miller[1], Can C. Ozbal[1], Elke S. Perloff[2], Shangara S. Dehal[2], Andrew K. Mason[2], Andrew P. Blanchard[2], Charles L. Crespi[2], David M. Stresser[2], and William A. LaMarr[1]
[1] BioTrove, Inc., 10P Gill St. Woburn, MA 01801, USA; [2] BD Biosciences, 6 Henshaw Street, Woburn, MA 01801, USA.

- CYP inhibition: RF-MS/MS correlation to LC-MS/MS

2009 ISSX – BD Gentest/BioTrove (poster)

Comparison of RapidFire® Ultra High Throughput LC/MS/MS with Traditional LC/MS/MS for Cytochrome P450 Inhibition Testing. Elke S. Perloff[1], Shangara S. Dehal[1], Andrew K. Mason[1], Andrew P. Blanchard[1], William A. LaMarr[2], Can C. Ozbal[2], Vaughn P. Miller[2], Charles L. Crespi[1], and David M. Stresser[1]
[1]BD Biosciences Discovery Labware, BD Gentest℠ Contract Research Services, Woburn, MA 01801, USA
[2]BioTrove, Inc. 10P Gill St. Woburn, MA 01801, USA.

- CYP inhibition: RF-MS/MS correlation to LC-MS/MS

2009 ELRIG – BioTrove (poster)

Consistent High-throughput Metabolic Stability Screening Across Mass Spectrometry Platforms. Michelle V. Romm, Nikunj Parikh, Vaughn Miller, William A. LaMarr, Selena Larkin, Can C. Ozbal.

- Metabolic stability data correlates with RF using 3 different MS QqQ vendor platforms

Agilent Technologies

Last Updated August 22, 2012

2009 ASMS – Merck (BioTrove Users Group presentation)

The application of RapidFire MS in a high-throughput production assay lab. Merck Research Labs: Deborah Zink, Tom Holt, Jonathon Lum, Marta Arocho, Anne Vergnon, Gerica Galviz, Claude Dufresne. BioTrove: William LaMarr, Can Ozbal, Maxine Jonas, Arrin Katz.

- CYP inhibition: RF/MS/MS correlation to LC-MS/MS

2009 ASMS – BMS (poster)

Application of the BioTrove RapidFire™ Ultra-fast Online SPE-MS/MS System for Compoundspecific Analysis of in vitro ADME Samples. Anthony Paiva; Andrew Wagner; Xianmei Cai; Ying Li; Shu Li; Janet Kolb; Aaron Walker; John Herbst; Charlie Conway; Harold Weller; Wilson Shou; Bristol-Myers Squibb, Wallingford, CT.

- RF-MS/MS correlation to LC-MS/MS
    - o Caco-2 permeability

2009 ASMS – BioTrove (poster)

Evaluation of accurate mass TOF-MS for use in high throughput CYP450 inhibition screening. William A. LaMarr, Michelle V. Romm, Nikunj Parikh, Lauren Frick, Can C. Ozbal.

- CYP Inhibition using RF/TOF

2009 ASMS – Boehringer Ingelheim (podium talk)

A Novel and Integrated Platform for Fully Automated High-Throughput LC-MS/MS Analysis of In Vitro ADME Samples. Andreas H. Luippold, Thomas Arnold, Wolfgang Jorg, Klaus Klinder & Kurt Schumacher, Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany.

- RF-MS correlation to LC-MS/MS
    - o CYP Inhibition
    - o Metabolic Stability
    - o PAMPA
    - o Caco-2

2009 SBS – BioTrove (poster)

Consistent High-throughput CYP Inhibition Data Across Mass Spectrometry Platforms. William A. LaMarr, Michelle V. Romm, Nikunj Parikh, Vaughn Miller, Can C. Ozbal.

- CYP Inhibition data correlates with RF using 3 different MS QqQ vendor platforms

2008 ASMS - Takeda (poster)

An Integrated Approach for an Ultra-High Throughput On-Line SPE-MS/MS System and its Applications to ADME Assays. Rongda Xu, Marianne T. Quintos, Melinda Manuel, Joshua E. Cramlett, Kheng B. Lim, and Daniel B. Kassel; Takeda San Diego, Inc., San Diego, CA 92121.

- RF-MS/MS correlation to LC-MS/MS
    - o Metabolic Stability
    - o Plasma Protein Binding
    - o PAMPA

2008 SBS – Exelixis (poster)

CYP450 Inhibition Profiling Using High Throughput RapidFire Mass Spectrometry Assays. Sean Wu, Lory Tan, Jing Wang, Stefan Engst, Wentao Zhang* and Kirk McMillan Exelixis, Inc., South San Francisco, CA 94083.

- CYP inhibition: RF/MS/MS correlation to LC-MS/MS

2007 AAPS - AstraZeneca (poster)

Comparison of BioTrove RapidFire uHTMS and Traditional LC/MS/MS Analysis to Assess Cytochrome P450 Inhibition Utilizing Clinically Relevant Probe Substrates. Ethan Hoffmann[1], Caroline Rivard[1], David Ayres[1], Can Ozbal[2], Steve Good[1] and Patrick Brassil[1] AstraZeneca R&D Boston, 35 Gatehouse Drive,

Waltham, MA 02451 U.S.A [2]BioTrove Inc, 12 Gill St, Ste 4000, Woburn, MA 01801.

- CYP inhibition: RF-MS/MS correlate with LC-MS/MS

2007 ASMS – Takeda (podium talk)

Development of an Ultra High Throughput MS/MS CYP Inhibition Assay.

Daniel B. Kassel[1]; Kheng Lim[1]; Can C Ozabal[2]; William A LaMarr[2]
[1]Takeda San Diego, Inc, San Diego, CA; [2]BioTrove, Inc., Woburn, MA.

- CYP inhibition: RF-MS/MS correlation to LC-MS/MS

Clinical/Forensic Toxicology References Using RapidFire-MS

2012 AACC – Agilent Technologies (Poster)

Ultrafast Analysis of Drugs of Abuse using SPE/MS/MS

Nikunj R. Parikh[1], Kari Schlicht[1], Michelle V. Romm[1], Vaughn P. Miller[1], and William A. LaMarr[1], 1Agilent Technologies Inc., Wakefield, MA

2012 AACC – Agilent Technologies (Poster)

High-Throughput Analysis of Immunosuppressant Drugs in Whole Blood Using Ultra-fast SPE/MS/MS

Kari E. Schlicht[1], Pierre Wallemacq[2] , Vaughn P. Miller[1], and William LaMarr[1] 1. Agilent Technologies Inc, Wakefield, MA 2. Cliniques universitaires St-Luc, UCL, Brussels, Belgium

2012 ASMS – Agilent Technologies (Poster)

High-Throughput Analysis of Drugs of Abuse in Urine Using Ultra-fast SPE/MS/MS for Forensic Toxicology

Michelle Romm; Kari Schlicht; Matthew Woodcock; Vaughn Miller; Can "Jon" Ozbal; William A. Lamarr

2012 ASMS – Agilent Technologies (Poster)

Automated High-Throughptu Analysis of Proteins in Blood-based Matrices Combining Immunoaffinity Purification and Ultra-fast SPE/MS/MS (AssayMAP)

Michelle V. Romm1, Zachary Van Den Heuvel2, Nikunj Parikh1, Vaughn P. Miller1 and William A. LaMarr1 1.

Abstract: Measuring biomarkers in plasma is challenging due to the complexity of the matrix. Currently, immunoaffinity purification followed by LC/MS/MS is often employed to isolate and quantify proteins or peptides. However, both steps are limited by inefficiency; immunoaffinity purification is labor intensive, while LC/MS/MS analysis requires minutes per sample. Automation of the immunoaffinity purification step, allowing for simultaneous preparation of 96 samples, followed by online SPE/MS/MS analysis at a rate of seconds per sample could greatly improve the throughput of this workflow. We investigated the feasibility of using such an automated high-throughput workflow to analyze both proteins and peptides from blood-based matrices.

2012 ISSX/MDO – Agilent Technologies (Poster)

High-Throughput Analysis of 1'-Hydroxymidazolam in Plasma Using Ultra-fast SPE/MS/MS

Last Updated August 22, 2012

Michelle V. Romm1, Nikunj Parikh1, Vaughn P. Miller1, Moritz Wagner2, and William A. LaMarr1 1. Agilent Technologies, Wakefield, MA 2. Agilent Technologies, Waldbronn, Germany

Abstract: There is an increasing demand for greater throughputs and efficiencies of mass spectrometry-based bioanalysis by DMPK researchers. We evaluated the ability of an ultra-fast SPE/MS/MS system to analyze small molecule analytes in a plasma based matrix with much faster sample cycle times and similar analytical results compared to LC/MS/MS methods. Critical bioanalytical parameters were systematically investigated using the small molecule analyte 1'-hydroxymidazolam spiked into plasma. 1'-Hydroxymidazolam is the primary CYP3A4 metabolite of the benzodiazepine midazolam and is often used for clinical drug-drug interaction studies. Comparable accuracy, precision, and linearity were achieved at rates 10-30 fold faster than traditional LC/MS/MS methods. Hydroxymidazolam calibration curves and quality control standards were prepared in a wide dynamic range by spiking blank matrix with 1'-hydroxymidazolam. The samples were then precipitated with acetonitrile containing an isotopically labeled internal standard, followed by dilution. Sample analysis was performed at a rate of <10 seconds per sample using an Agilent RapidFire ultra-fast autosampler/online SPE system (reverse phase) coupled to an Agilent 6460 QQQ mass spectrometer. SPE methods were optimized for the analyte and the SPE/MS/MS results were compared to LC/MS/MS analysis. Data analysis was performed using RapidFire Integrator software. Hydroxymidazolam had excellent linearity within the measured range of 10-1000 ng/ml with an R^2 value greater than 0.995. Intra- and interday accuracies and precision for all concentrations were within 10%. A robustness test comprising of 1500 sequential injections of the same hydroxymidazolam concentration in protein precipitated plasma revealed a CV of less than 3% with no changes to peak signal or SPE cartridge pressure. Ionization suppression was present, but it could be effectively managed by using the stable isotope labeled internal standard (IS). Carryover was found to be < 1% of the low standard. A very strong correlation of SPE/MS/MS results from the same samples analyzed by traditional LC/MS/MS was seen (correlation coefficient = 0.999). The ultra-fast SPE/MS/MS system had comparable accuracy, precision and linearity results to LC/MS/MS for 1'hydroxymidazolam in plasma. This methodology is capable of throughputs >400 samples per hour. The SPE/MS/MS system may be useful for the efficient bioanalysis of 1'-hydroxymidazolam clinical drug-drug interaction studies and for analysis of similar small molecules in plasma from animal and human research studies.

2012 MSACL – Agilent Technologies (Poster)

Rapid Analysis of Drug Analytes in Uring for Forensic Toxicology Using Ultra-fast Online SPE/MS/MS

Michelle V. Romm1, Kari Schlicht1, Matthew Woodcock2, Vaughn P. Miller1 and William A. LaMarr1 1. Agilent Technologies Inc., Wakefield, MA 2. Dominion Diagnostics, LLC, North Kingstown, RI

Abstract: Forensic drug testing has traditionally utilized GC/MS as the analytical method of detection. In the present study, we evaluated the ability of an ultra-fast SPE/MS/MS system to analyze metabolites of some of the drugs of abuse including benzoylecgonine (BE), the major metabolite of cocaine, and 11-nor-9-delta-9-tetrahydrocannabinol (THCCOOH), the major metabolite of marijuana, in urine with much faster sample cycle times (under 15 seconds per sample) and similar analytical results compared to GC/MS or LC/MS assays.

2012 MSACL – Agilent Technologies (Poster)

Ultra-fast analysis of Small Molecule Analytes in Urine and Blood-based Matrices using an SPE/MS/MS System

Michelle V. Romm, Nikunj Parikh, Kari Schlicht, Vaughn P. Miller and William A. LaMarr; Agilent Technologies Inc., Wakefield, MA

Abstract: Mass spectrometry-based analysis in clinical research and forensic toxicology has emerged as a viable analytical method due to it's sensitivity, specificity, and robustness. We evaluated the ability of an ultra-fast SPE/MS/MS system to analyze small molecule analytes in human urine or blood-based matrices with much faster sample cycle times and similar analytical results compared to LC/MS/MS assays. Hydroxymidazolam was investigated in plasma and the major metabolite of cocaine, benzoylecgonine, was investigated in urine for forensic toxicology.

2012 SOFT – Agilent Technologies (Poster)

Ultra-Fast Online SPE/MS/MS Confirmation Analysis of THCCOOH in Urine for Forensic Toxicology

Michelle V. Romm1, Lawrence J. Andrade2, Matthew Woodcock2, Vaughn P. Miller1 and William A. LaMarr1 1. Agilent Technologies Inc., Wakefield, MA 2. Dominion Diagnostics, LLC, North Kingston, RI

Abstract: Forensic drug testing has traditionally utilized GC/MS and more recently LC/MS as the analytical method of detection. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. We evaluated the ability of an ultra-fast SPE/MS/MS system to accurately measure 11-nor-9-delta-9-tetrahydrocannabinol (THCCOOH, the major metabolite of marijuana) and metabolites of synthetic cannabinoids in urine with much faster sample cycle times (under 15 seconds per sample) and similar analytical results compared to LC/MS/MS assays.

2012 SOFT – Agilent Technologies (Poster)

Screening for Drugs of Abuse in Forensic Toxicology Using an Ultra-Fast Online SPE/MS/MS System

Nikunj R. Parikh1, Kari Schlicht1, Michelle V. Romm1, Vaughn P. Miller1, and William A. LaMarr1, 1Agilent Technologies Inc., Wakefield, MA.

Abstract: Forensic drug screening is extensively used by Law Enforcement officials; employers and pathologists today. Traditionally the screening for these drugs involves analysis by immunoassays followed by a confirmatory test by GC/MS detection and more recently by LC/MS assays. The steady increase in sample size has created a bottleneck in screening for these drugs across different classes and longer turnaround times for confirmatory tests. While immunoassays are convenient and well established; they are not as sensitive or specific as LC/MS which leads to many false negatives and positives. In the present study; we evaluated the ability of an ultra-fast SPE/MS/MS system to screen across different classes of drugs of abuse in urine while approaching the sensitivity and accuracy of LC/MS and maintain the speed and efficiency of a screen (sample cycle times <15 seconds per sample).

2012 US HUPO – Agilent Technologies (Poster)

Development of Automated SISCAPA Assays for High-Throughput Quantitation of Protein Biomarkers

Christine Miller3, Leigh Anderson1, Matt Pope2, Morteza Razavi2, Terry Pearson2

Abstract: SRM-MS allows specific, internally-standardized measurement of protein biomarkers and can achieve sub-nanogram/mL detection levels when specific anti-peptide antibodies are used to enrich target peptides from the plasma digests (SISCAPA). We have developed an automated protocol for implementing this immunoaffinity enrichment of biomarker peptides. The effectiveness of the protocol was evaluated using a multiplex SISCAPA panel based on 11 rabbit monoclonal antibodies to specific peptides from 10 proteins spanning a wide plasma concentration range. Parameters for each of the 11 target peptides and cognate labeled standards have been optimized, permitting use of retention-time scheduled MRM data collection and rapid (3 min) analysis times. Results will be presented demonstrating the performance of the workflow for high-throughput quantitationof protein biomarkers.

2011 AACC – Agilent Technologies (Poster)

High-Throughput Analysis of Levetiracetam in Serum Using Ultra-fast SPE/MS/MS

Michelle V. Romm, Eric W. Korman, Vaughn P. Miller, Christine L. Snozek, Frank W. Crow, Loralie J. Langman and William A. LaMarr

Abstract: Mass spectrometry-based assays have emerged as a viable analytical method due to their sensitivity, specificity, and robustness. We evaluated the ability of an ultra-fast SPE/MS system (Agilent RapidFire High-throughput Mass Spectrometry System) to analyze levetiracetam in human serum with much faster sample cycle times and similar analytical results compared to HPLC or LC/MS/MS assays.

2011 AACC – Agilent Technologies (Poster)

Ultra-fast, Simultaneous Analysis of a Panel of Benzodiazepines in Human Urine using an SPE-TOF System

Vaughn P. Miller1, Michelle V. Romm 1, Nikunj Parikh1, and William A. LaMarr1 1. Agilent Technologies Inc., Wakefield, MA

Abstract: Benzodiazepines are widely prescribed drugs for the treatment of anxiety, sleep disorders and other illnesses. Because addiction and abuse can occur with these drugs, efficient screening methods are critical to clinical, forensic and toxicology laboratories. We evaluated the ability of an ultra-fast SPE-MS system (RapidFire) to analyze a panel of benzodiazepines in human urine with much faster sample cycle times and similar analytical results compared to traditional LCMS systems.

2011 AACC – Agilent Technologies (Poster)

High-Throughput Analysis of Tacrolimus in Whole Blood Using Ultra-Fast SPE/MS/MS

Kari E. Schlicht 1, Eric W. Korman2, Vaughn P. Miller1, Christine L. Snozek2, Frank W. Crow2, Loralie J. Langman2 and William A. LaMarr1 1. Agilent Technologies Inc., Wakefield, MA 2. Mayo Clinic, Rochester, MN

Abstract: In many clinical research laboratories, liquid chromatography-mass spectrometry (LC/MS) methods of analysis of immunosuppressant drugs have proven superior because of their increased sensitivity and selectivity. We have evaluated the ability of an ultrafast SPE/MS/MS system (Agilent RapidFire High-throughput Mass Spectrometry System) to analyze the immunosuppressant drug tacrolimus in whole blood. The results demonstrate the superior speed of SPE/MS/MS to complement the superior sensitivity and selectivity of MS with significantly faster sample cycle times than LC/MS while yielding similar analytical results.

2011 ASMS – Agilent Technologies (Poster)

Ultra-fat Analysis of Benzodiazebines in Human Urine using Dilute and Shoot Methodology and SPE/MS/MS

Lauren E. Frick, Michelle V. Romm, Vaughn P. Miller, and William A. LaMarr

Abstract: Liquid-liquid extraction and protein precipitation represent time-consuming and cumbersome steps in clinical sample preparation, the elimination of which would greatly speed the rate with which labs can analyze clinical samples. Dilute and shoot has been proposed as a preparation-free alternative, though its utility can be limited by matrix effects resulting from the lack of pre-analysis sample cleanup. Here, we apply the method to the detection of a panel of benzodiazepines in human urine using an ultra-fast online SPE cleanup prior to MS/MS analysis. With a cycle time of ~9 seconds per sample, the system offers a considerable speed advantage over chromatography-based dilute and shoot methodologies while maintaining data quality.

2011 ASMS – Agilent Technologies (Poster)

Ultrafast SPE Integrated with TOF-MS Increases the Throughput of Metabolic Stability Assays and Enables Analysis of Metabolites

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Bioanalysis without chromatography: the Evaluation of RapidFire Ultra-fast online SPE-MS/MS System for Quantitation of Drugs in Biological Matrixes

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Abstract: The RapidFire Mass Spectrometry platform is a fully automated system that integrates on-line SPE sample preparation and mass spectrometry analysis, and is capable of conducting analysis at a speed of 5-10 s per sample. Its existing applications mostly involve in vitro high-throughput ADME or therapeutic target screening studies. In the current work, the feasibility of utilizing RapidFire for analysis of in vivo bioanalytical samples was evaluated for the first time. Key potential analytical liabilities of RapidFire, such as matrix suppression (caused by matrix components and dosing vehicles) and carryover, were systematically investigated using structurally diverse commercial compounds. More importantly, quantitation data obtained from the RapidFire system was compared with those from conventional LC-MS/MS analysis to evaluate its performance for bioanalysis.

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