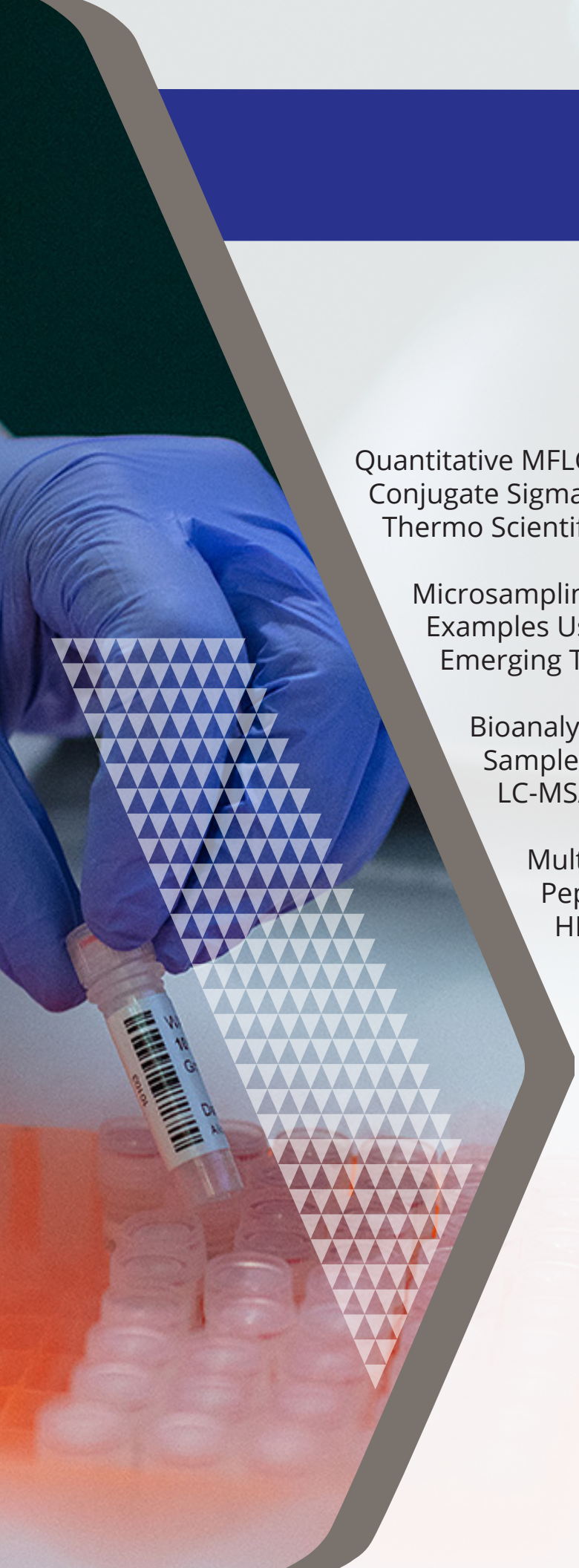
Alturas
Analytics

The LC-MS Experts



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TECH NOTE

OVERVIEW

Purpose
Develop immunocapture extraction and MFLC-MS/MS methods for the determination of SigmaMAb ADC Mimic from rat plasma.

Methods
Rat plasma was spiked with SigmaMAb ADC Mimic and enriched using Thermo Scientific™ MSIA™ D.A.R.T.'S™ Streptavidin microcolumns. The MSIA™ eluate was denatured, reduced, digested and analyzed by MFLC-MS/MS.

Results
The developed method resulted in Accuracy/Precision better than 15% with a dynamic range of 10.0-10,000 ng/mL.

Quantitative MFLC-MS/MS Analysis of the Antibody Drug Conjugate SigmaMAb Extracted from Rat Plasma Using Thermo Scientific™ MSIA™ Microcolumns

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[1]Alturas Analytics, Inc., Moscow, ID, United States
[2]Thermo Fisher Scientific, West Palm Beach, FL, United States

INTRODUCTION

Antibody drug conjugates (ADCs) are potent and specific biopharmaceuticals. These large molecules are typically analyzed using ELISA methods, which may not have the selectivity to accurately quantify the ADC alone. HPLC-MS/MS analysis of the digested ADC is an alternative approach that provides better selectivity. In order to increase the selectivity/sensitivity of the method, analysis was conducted using microflow HPLC (MFLC) and immunocapture sample preparation. Typical immunocapture techniques require the use of labor intensive magnetic beads. Thermo Scientific™ MSIA™ sample preparation is simple and can be fully automated, greatly increasing the sample throughput. The resulting MSIA™ data results in lower limits of quantitation due to the selectivity and extraction efficiency improvements when compared to other non-immunocapture methods such as pellet digestion.

METHODS

MSIA™ SAMPLE PREPARATION NOVUS i

- Sample volume: 25µL
- Add 80 µL SILu™ MAb Infliximab internal standard
- Rinse MSIA™ tips with PBS
- Link streptavidin with CaptureSelect™ Biotin anti-IgG-Fc
- Capture ADC on MSIA™ tips
- Rinse tips with PBS buffer (removes all interferences)
- Elute with 2% formic acid (removes ADC from CaptureSelect™)

DIGESTION PROCEDURE

- To MSIA™ eluate add 14 µL 2M Tris buffer
- Add 5 µL 0.1M TCEP
- Denature by heating at 80˚C for 15 minutes
- Add 8 µL 21.3 mM calcium chloride
- Add 10 µL 0.5M ammonium bicarbonate
- Add 10 µL 80 mg/mL trypsin (incubate 50˚C 1 hour)
- Add 5 µL 40% formic acid

MFLC-MS/MS

- Waters Acquity UPLC® M-Class Systems
- Gradient using acetonitrile and water with 0.1% formic acid
- Flow rate: 50 µL/min
- Column: Phenomenex Kinetex® Biphenyl (50 X 1.0 mm 3 µm)
- Column temperature: 50˚C
- Sciex QTRAP® 6500+ operating in MRM mode
- ESI Positive ion mode

Figure 1: SigmaMAb Antibody Drug Conjugate Mimic (Recombinant Monoclonal IgG1 Human Antibody Linked to dansyl-fluorophores)

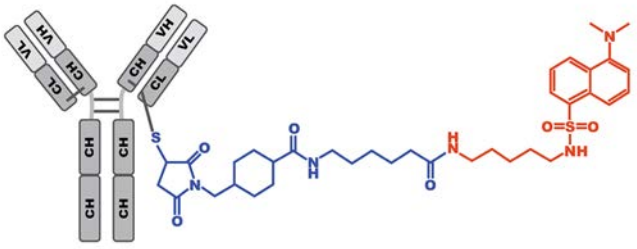

Image courtesy MilliporeSigma.

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Figure 2: Linear Calibration Curve Generated from the Analysis of 16 Standard Curve Samples (8 Concentrations in Duplicate) Extracted from Rat Plasma; r=0.9959, 10.0-10,000 ng/mL

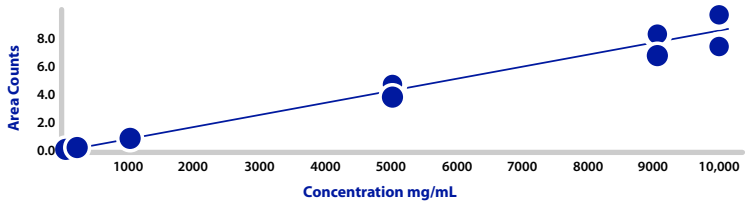


Figure 3: SigmaMAb ADC Mimic (Digested to peptide) 100 ng/mL Extracted from Rat Plasma using Pellet Digestion Chromatogram

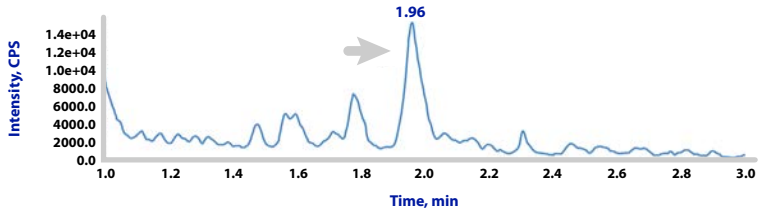


Figure 4: SigmaMAb ADC Mimic (Digested to peptide) 10.0 ng/mL Extracted from Rat Plasma using MSIA Procedure Chromatogram

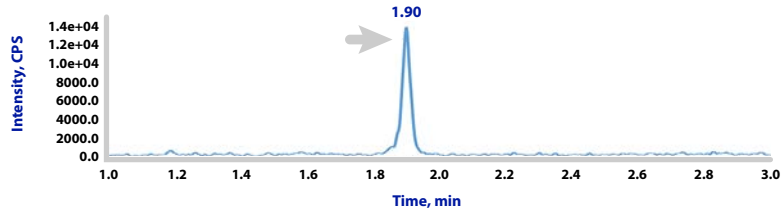
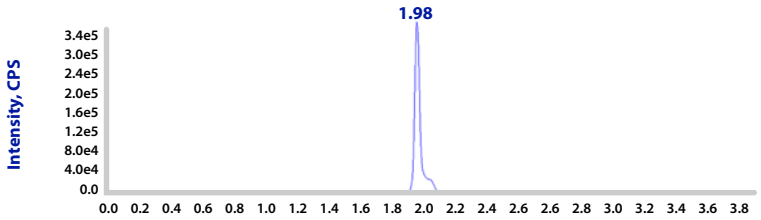


Figure 5: Internal Standard (Digested Infliximab Stable-Isotope Labeled Monoclonal Antibody) Chromatogram



Protein	Peptide Sequence	Molecular Weight	Charge State	Q1	Q3
SigmaMAb ADC Mimic	ALPAPIEK	838	2	420	327
Infliximab Stable-Isotope	*YASESMSGIPSR	1294	2	648	845

*arginine $^{13}C_6$, $^{15}N_2$ labeled

CONCLUSIONS

- Developed MSIA methods for the extraction and analysis of SigmaMAb ADC Mimic from rat plasma
- Method developed is accurate/precise with a LLOQ of 10.0 ng/mL using only 25 µL of plasma
- MSIA method is more selective and sensitive compared to pellet digestion
- MFLC method resulted in a >40% increase in the peptides instrument response when compared to conventional HPLC

Alturas Analytics is a GLP compliant bioanalytical CRO specializing in MS/MS solutions supporting early discovery through late phase clinical trials.

In addition to providing PK support services to pharmaceutical companies worldwide, Alturas maintains an intensive research effort of applying new technologies leading to scientific advancement.

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TECH NOTE

OVERVIEW

Purpose

Non-invasive matrices such as tears, sweat, saliva, and milk offer an enticing alternative to traditional sampling from blood, serum, and plasma. Collection of these matrices is simpler and more affordable than venipuncture. It is also much less unpleasant for patients and study subjects allowing for more frequent sampling, higher compliance, and removal of fear of needles as a barrier to clinical trial recruitment. However, bioanalysis of these matrices is not without challenges. Sample volumes can be very low, the more complex matrices such as milk may require extensive sample preparation, and the use of the matrix must be biologically relevant. Using the example of a method we developed at Alturas Analytics Inc. to measure Tobramycin from human tears, we will discuss practical applications of microsampling of non-invasive matrices for bioanalysis, and improvements offered by emerging technologies.

Objectives

- Understand the benefits and challenges of using non-invasive matrices in bioanalysis
- Explore currently available microsampling technologies and advances in analytical techniques
- Discuss strategies for collecting, extracting, and analyzing non-invasive matrices

Microsampling of Non-Invasive Matrices: Practical Examples Using Tears and a Perspective of Past and Emerging Technologies

Presented by Jason Watts, Ph.D.
Alturas Analytics, Inc., Moscow, ID, United States

INTRODUCTION

Microsampling

» Typically uses ≤50 µL
» Simple collection and storage
» Possibility for at-home sampling
» Site-Centric → Patient-Centric

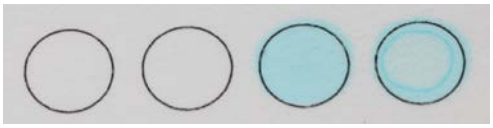
Non-invasive Matrices

» Tears, sweat, saliva, milk
» High correlation with non-protein bound plasma concentrations for many drugs

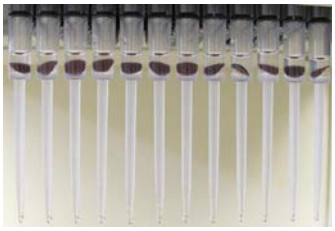
Practical Considerations

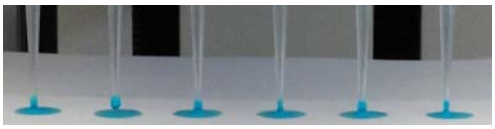
» Can sufficient sensitivity be achieved?
» Analyte stability
» Does the matrix concentration correlate with plasma concentrations?
» What is gained over traditional sampling?

Adapting blood microsampling techniques for non-invasive matrices



» Improved precision and accuracy using color-indicating dyes



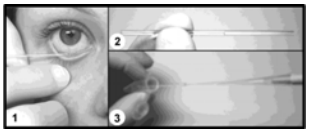


» Improving extraction workflow with common Bioanalytical tools

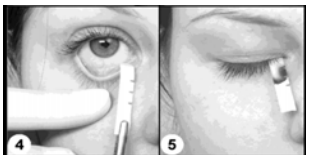
MATRICES

Tears

» Constantly produced at 1.2 µL/min
» Unstimulated volume ~ 7 - 10 µL
» 0.6 - 0.8% protein (0.4% albumin)
» pH 6.5 - 7.6
» Differences between stimulated and unstimulated collection

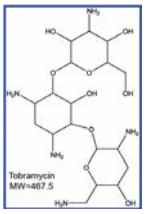


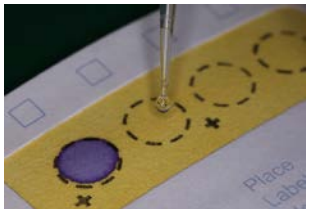
Capillary tube collection
From Posa et al., 2012



Schirmer strip collection
From Posa et al., 2012

Tobramycin from Human Tears





QC Level (µg/mL)	Assay Accuracy and Precision (% ± %CV)	Matrix Factor
12	90.0 ± 4.5	NA
3.0	96.2 ± 0.07	NA
1.5	101 ± 5.7	0.97

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Sweat

» Collected on sweat wipes or patches

» Volume variability: normalized to sodium and potassium levels

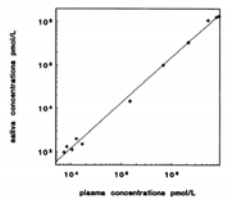
» pH 4 - 6.8 when resting
- High sweat/plasma ratio for basic drugs

» Commonly used for monitoring drugs of abuse

Saliva

» Production and composition
- 0.5 mL/min
- pH ~ 6 - 7
- Excretion of drugs dependent on permeability and protein binding

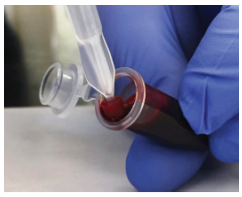
» Production and composition
- Unstimulated
- Stimulated



Correlation between plasma and saliva dexamethasone concentrations
Thijssen et al., 1996

Using blood microsampling device for saliva sampling

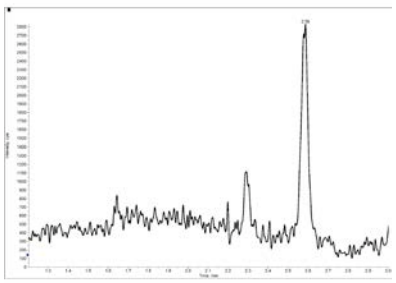
» Mitra® (Neoteryx) microsampling device
- Precise sample collection
- Ideal for pediatric or at home sampling
- 10, 20 or 30 µL sample volume



SIMPLE EXTRACTION OF DEXAMETHASONE FROM SALIVA USING MITRA® MICROSAMPLING DEVICE

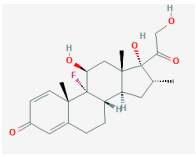
Method

» Collect sample on 20 µL Mitra® tip
» Dry for at least 2 hours at ambient temperature
» Place dried tip in 96 well plate with curve and QCs
» Add 25 µL 100 ng/mL IS (Dex-D4 in ACN:H20 1:1)
» Add 300 µL ACN. Vortex and incubate 30 min
» Transfer extract to fresh plate. Evaporate to dryness
» Reconstitute in 100 µL of ACN:H20 with 0.1% formic acid



0.4 ng/mL Dexamethasone extracted from Saliva

Dexamethasone extracted from saliva



Dexamethasone 392.5 g/mol
Structure from https://pubchem.ncbi.nlm.nih.gov/compound/Dexamethasone

Concentration (ng/mL)	Assay Accuracy and Precision (% ± % CV)	% Recovery
0.8	98.3 ± 0.94%	94
50	103.5 ± 0.68%	117
400	100.1 ± 7.0%	121

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OVERVIEW

Purpose
Demonstrate an accurate, precise, and selective method to analyze Adalimumab in human plasma samples using MSIA™ capture and microflow LC-MS/MS.

Methods
Streptavidin MSIA™ (Mass Spectrometric Immunoassay) D.A.R.T.'S™ were treated with biotinylated human TNF-α in order to capture Adalimumab in human plasma samples. The Adalimumab was eluted from the MSIA™ D.A.R.T.'S™, denatured, reduced, and digested. A signature peptide was measured by microflow LC-MS/MS.

Results
- 1000-20,000 ng/mL dynamic range
- A/P 90% ± 5%
- Blanks <15% of LLOQ response
- 2x faster than magnetic bead immunocapture method

Bioanalysis of Adalimumab in Human Plasma Samples Using MSIA™ Capture and Microflow LC-MS/MS

Sharon DeChenne, Chad Christianson, and Jennifer Zimmer
Alturas Analytics, Inc., Moscow, ID, United States

INTRODUCTION

Humira® (Adalimumab) is the top selling prescription drug in the world. It is FDA approved for the treatment of ten autoimmune diseases including rheumatoid arthritis, Crohn's disease, and ulcerative colitis. Given the number of biosimilars pending Humira® patent expiration in 2023, there is a need for robust and accurate methods for Adalimumab analysis. LC-MS/MS analysis offers a solution to traditional ligand binding assay selectivity challenges. MSIA™ is a precise and accurate immunocapture method that can be fully automated for fast sample preparation.

MATERIALS

- SigmaMAb Adalimumab - MilliporeSigma
- Internal Standard: SILu™MAb Adalimumab Stable-Isotope Labeled Monoclonal Antibody [13C6, 15N4] - Arginine and [13C6, 15N2] - Lysine - MilliporeSigma
- Thermo Scientific™ Finnpipette™
- Biotinylated Human TNF-α Protein - ACROBiosystems
- 300 µL Mass Spectrometric Immunoassay (MSIA™) Streptavidin D.A.R.T.'S™ - Thermo Scientific™
- Water, PBS Buffer, TCEP and Sequencing Grade Modified Trypsin - Promega
- Waters ACQUITY UPLC® M-Class Binary LC System
- Sciex QTRAP® 6500+ operating in MRM mode

METHODS

EXTRACTION

- Plate Preparation
  - Aliquot plasma and internal standard
  - Aliquot rinses (PBS buffer and water)
  - Aliquot Biotinylated TNF-α
  - Aliquot Elution Solvent
- Capture Biotinylated TNF-α with MSIA™ D.A.R.T.'S™, rinse
- Capture Adalimumab with TNF-α-treated MSIA™ D.A.R.T.'S™, rinse
- Elute Adalimumab
- Adjust pH 2M Tris buffer
- Denature/Reduce with Heat (80°C) & 0.1 M TCEP 15 minutes
- Add Ammonium Bicarbonate/Calcium Chloride
- Digest with 10 µL of trypsin (0.8 mg/mL)
- Incubate at 50°C for 1 hour
- Stop digestion with 40% formic acid solution

MICROFLOW LC-MS/MS

- Determined sensitive and selective signature peptide using Skyline predictions
- Quantitation peptide: EVQLVESGGGLVQPGR (2X Charge)
- Adalimumab: 812.9 → 1056.5
- Internal Standard: 818.1 → 1066.6
- Waters ACQUITY UPLC® M-Class Binary LC Systems
- Gradient using acetonitrile and water with 0.1% formic acid
- Flow rate: 10 µL/min
- Column: HALO® Biphenyl (50 X 0.3 mm, 3 µm)
- Column temperature: 50°C
- Sciex 6500+ operating in MRM mode
- ESI
- Positive ion mode
- Optiflow™ Turbo V Source (1-50 µL/min probe)

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Figure 1: 1,000 and 5,000 ng/mL Adalimumab Extracted from Human Plasma

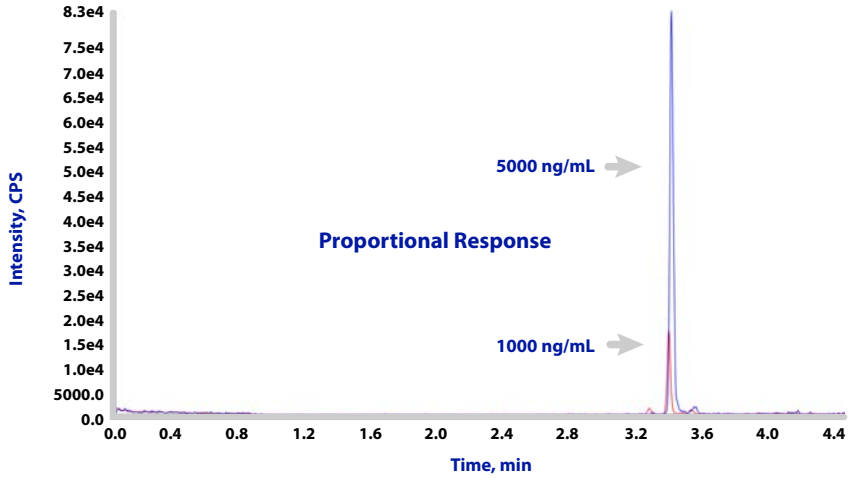
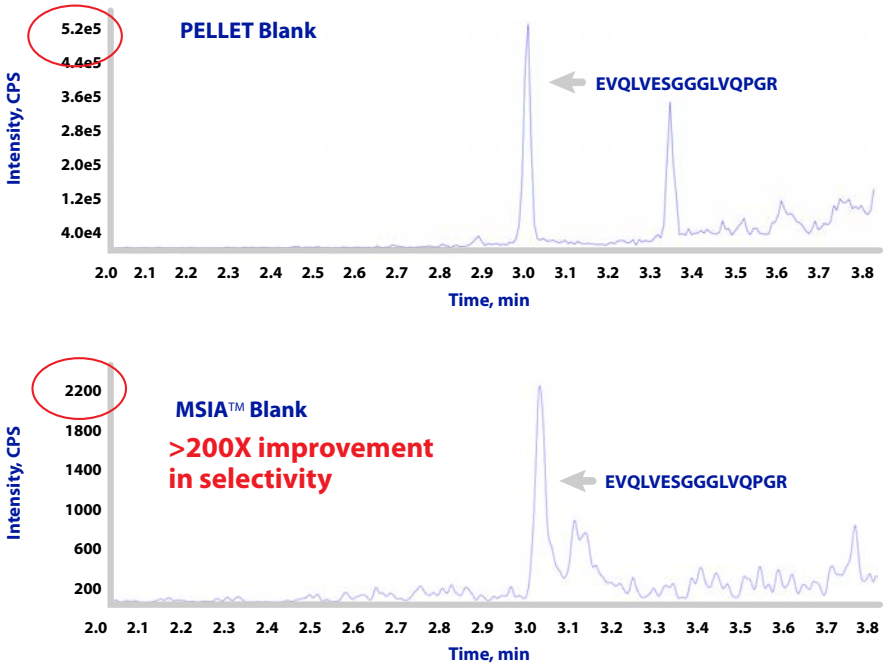


Figure 2: Pellet Extraction vs MSIA™ (blank plasma).



CONCLUSIONS

» An accurate, precise, and selective method was developed to analyze Adalimumab using MSIA™ and Microflow LC-MS/MS. The method is faster than using streptavidin treated beads. This method could also be validated to support clinical studies.

» **Next Step:** Microsampling using MITRA® (Neoteryx) microsampling device, which would be ideal for pediatric or at home sampling.

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TECH NOTE

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OVERVIEW

- Cystine-Dense Peptides (CDP) have vast applications for diagnostic and therapeutic applications.
- CDP structural stability is an important factor for biological function and delivery to target (e.g., oral delivery, etc.).
- Developed a general workflow on CDP screening for translational medicine development using High Pressure Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS).
- CDP compounds that are expressed using adapted Daedalus lentivirus transduction system in HEK293 cells were evaluated.
- Robust multiplex CDP screening method is developed based on chromatographic peak elution, plasma stability and sensitivity.
- The optimized and accurate HPLC-MS/MS method developed for screening can be easily utilized in preclinical therapeutic discovery studies when the selected compounds move further down into the translational medicine development pipeline.
- HPLC-MS/MS CDP quantitation performed on intact peptides (not denatured or digested) with an LLOQ of 10.0 ng/mL for each CDP and accuracies within 20%.

Multiplex Screening for Cystine-Dense Peptides (CDP) Therapeutics Proteins by HPLC-MS/MS

Mi-Youn Brusniak[1] Chad Christianson[2] Jim Olson[1] Emily Witthuhn[2] Shane Needham[2]
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[2]Alturas Analytics, Inc., 1324 Alturas Dr., Moscow, ID 83843, USA

INTRODUCTION

Cystine-Dense Peptides (CDP) can be found as naturally expressed, small proteins from all kingdoms. CDPs have a highly disulphide cross-linked structure and some may exhibit drug-like properties.[1,2] As an example, the protein CTXL_LEIQU from the Egyptian deathstalker scorpion has a known biological function such as selective interaction with MMP2 to inhibit its enzyme activity. More than 680,000 putative CDPs have been bioinformatically identified and ~700 structures were available for us to further classify CDPs based on connectivity.[3] A high-throughput platform expression screening method (up to 20 µg in 1 mL scale plate culture) and large-scale production (up to 10mg/L in 2 L cell culture) has been developed for further translational medicine development such as in-vivo biodistribution. While CDP classes that are found in nature provide great potential, determining each CDP mechanism of action in human biology and mapping out exact pharmacophores to the therapeutic binding partner is time consuming. Thus, we hypothesized that

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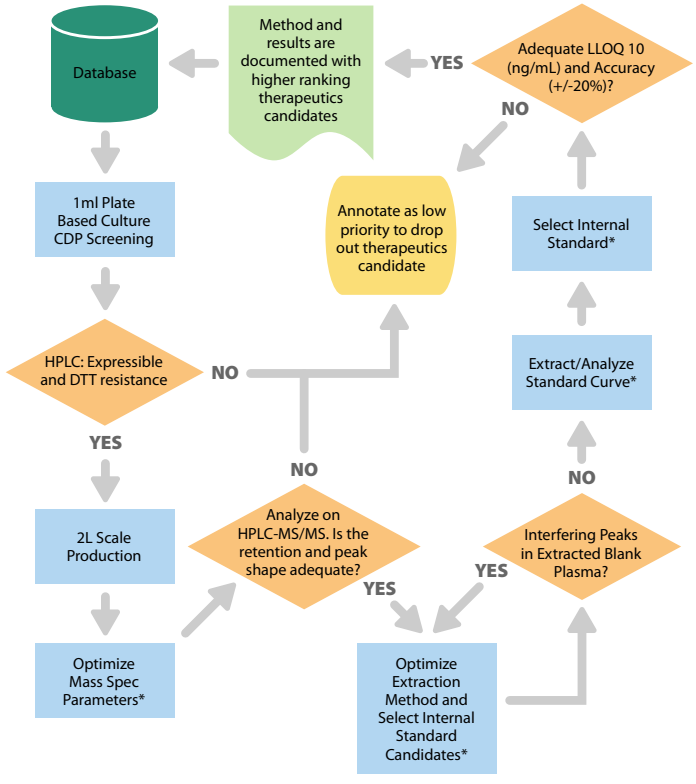
Figure 1: CDP Therapeutic Candidate Priority Schema by Assays. The process annotated with "*" are addressed by HPLC-MS/MS assay.



This work was funded in full by philanthropic support from Project Violet (https://www.fredhutch.org/en/labs/clinical/projects/project-violet.html). FHCRC MDT core provided CDP compounds for this work.

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intact CDP stability (e.g., keeping disulfide cross-linked structural integrity) in plasma or therapeutic target tissues will increase its natural bioavailability and we can further prioritize them in the therapeutic development pipeline. HPLC-MS/MS analysis is well suited for targeted protein quantification[4] and is the industry standard due to the selectivity and sensitivity of the instrumentation.[5,6] Here, we present CDP based therapeutics development schema in prioritizing the therapeutic development candidates including high throughput HPLC-MS/MS bioanalysis of several CDPs in order to determine the stability and Lower Limit of Quantification (LLOQ) which can then be used in future preclinical discovery PK studies. The criteria for candidate selection can be found in **Figure 1**.

METHODS

SPE EXTRACTION

- Add 100µL plasma to 96-well plate
- Add 25 µL of internal standard (IVTD-F*-SVIK)
- Add 100 µL water 0.1% formic acid
- Condition MAX SPE plate with Methanol then water
- Add entire sample volume to SPE plate
- Wash with 500 µL water then 100 mL water 10% methanol
- Elute with 250 µL 2% TCA in methanol (2 times)
- Evaporate eluent and reconstitute with 100 µL water 0.1% formic acid

LC-MS/MS

- Shimadzu Binary LC Systems
- Gradient using acetonitrile and water with formic acid
- Flow rate: 700 µL/minute
- Column: Pursuit 5 Diphenyl (100 X 2.1 mm, 5 µm)
- Column temperature: 50° C
- Sciex QTRAP® 6500+ operating in MRM mode
- ESI
- Positive ion mode

CONCLUSIONS AND DISCUSSION

A schema has been developed to screen CDP compounds for therapeutics translational medicine development based on chromatographic peak elution, plasma stability and sensitivity. We presented the best overall recovery method from plasma to establish the LLOQ by SPE and HPLC-MS/MS (10 ng/mL). The developed method can be used in further therapeutics and development pipelines (e.g. PK). Though we presented a “universal” method for CDP screening the developed method was only successful for 6 out of the 7 CPDs tested. The most polar compound among the candidates was rejected during the selection stage due to poor retention on the LC column. Future investigation is needed to cluster CDPs into groups based on informatics biophysical predicted values (e.g. polarity) and to develop multiple extraction and HPLC-MS/MS methods to apply to varying therapeutically desirable properties.

Table 1: CDP Compound Sequence, Source Organism and Fully Disulfide Bonded Monoisotopic Mass

ID	Target #30	Target #9	Target #12	Target #91	Target #62	Target #19
Sequence	GSEGDCPISEAIKCVEKCK EKVEVCEPGVCKCSG	GSVRIPVSCKHSGQCLKPC DAGMRFGKCMNGKCDCTPK	GSQKILSNRCNNSSECPHC IRIFGTRAAKCINRKCYCYP	GSQFCGTNGKPCVNGQCCG ALRCVVTYHYADGVCLKMNP	GSQIDTNVKCSGSSKCVKIC IDRYNTRGAKCINGRCTCYP	GSGHACYRNCWREG NDEETCKERC
Source Organism	Lychas mucronatus (Chinese swimming scorpion)	Androctonus australis (Sahara scorpion)	Buthus occitanus israelis (Common yellow scorpion)	Macrothele gigas (Japanese funnel web spider)	Tityus discrepans (Venezuelan scorpion)	Hererometrus fulvipes (Indian black scorpion)
Mass	3539.04	4165.93	4543.24	4089.68	4337.94	2799.96
Q1 Ion	885.5	834.1	909.3	818.7	868.4	560.7
Product Ion	1131.2	1152.9	1067.5	954.2	1017.1	664.2
RT (min)	4.22	2.73	3.49	3.83	2.57	2.83
Recovery (%)	90	34	63	43	50	80
r	0.997	0.995	0.998	0.997	0.997	0.995

Figure 2: Q1 Charge Envelope Target #12

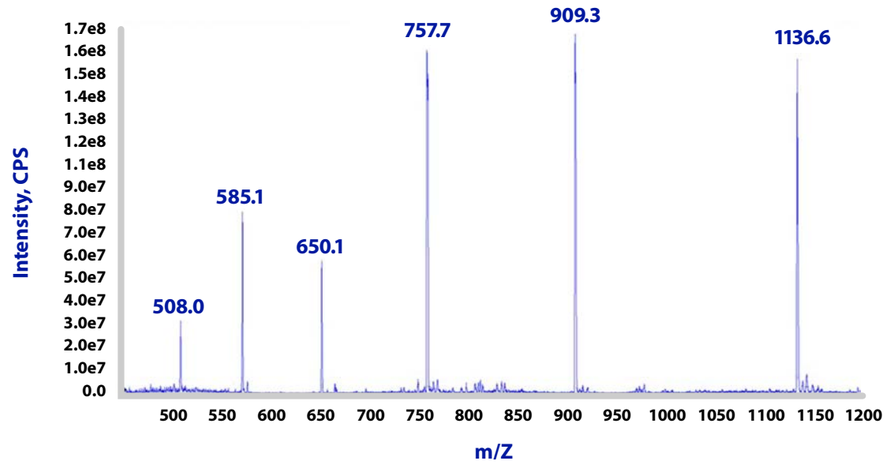
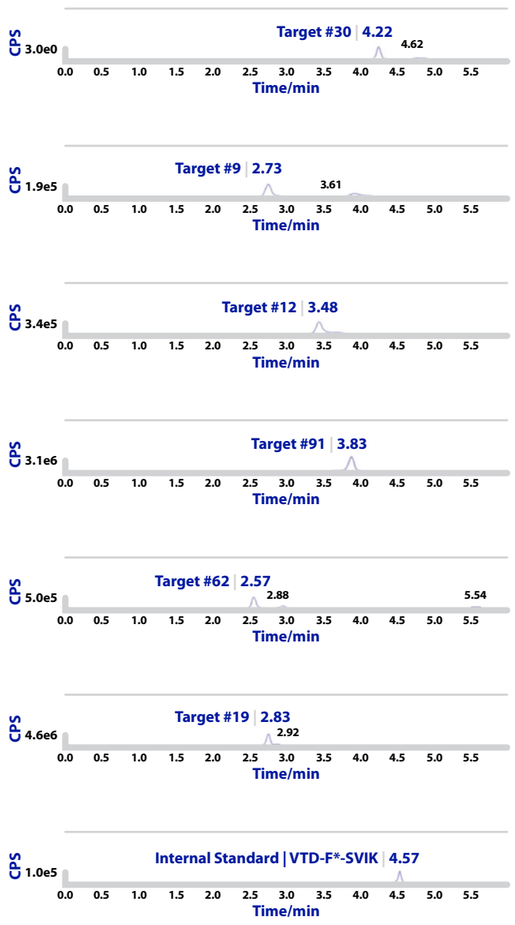


Figure 3: Representative Chromatogram 1000 ng/mL CDPs extracted from Human Plasma



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Alturas Analytics
The LC-MS Experts

SYSTEM ARCHITECTURE

Holmes is a client-server database application. The backend data storage requirement is Microsoft SQL Server. The user interface frontend is Microsoft Access or Microsoft Access Runtime. Programming languages are Microsoft VBA, Microsoft Jet SQL and Microsoft T-SQL.

Using an Electronic System to Record Reagent Tracking and Solution Preparation

Rachel Walker, David Schumacher, Staci Loughney, and Bo Cheng

INTRODUCTION

Holmes (originally CIMS - Chemical Inventory Management System), is a custom developed application by Alturas that has evolved from a small database for tracking of a few basic reagents to a system that currently functions as an electronic laboratory notebook (ELN), illustrated in the timeline below. An off-the-shelf ELN can be cost prohibitive and difficult to implement; the incremental process has resulted in numerous benefits.

Traceability is critical to QA auditors when reviewing laboratory activities that involve the preparation of reagents and sample processing. Through the use of the Holmes database, QA can audit all materials used in each batch of samples. Holmes can also monitor pipette verification through an add-on module.

TIMELINE OF HOLMES DEVELOPMENT (2008-PRESENT)

V1.0 (2008) Initial product. Records and tracks chemical stocks, reagents, mixes and mobile phases only.

V2.0 (2009) More functionalities requested to evolve into a 21 CFR Part 11 compliant electronic notebook; became v3.0.

V3.0 (2009) Keeps records for standards; records preparation of standard solutions. Mix preparation genealogy is recorded and traceable. E-signature and audit trail for all data creation/modification to meet 21 CFR Part 11 standards.

V3.1 (2011) "Client" entity is introduced and standards are assigned client ownership.

V3.2 (2012) Weights are read directly from balances through communication ports.

V3.3 (2013) Creation of serial dilution of working solutions (Cascade Dilution). Added second person e-signature as witness for stock solution preparation.

V4.0 (2017) "Study" and "Batch" entities are introduced to form a complete hierarchy of Client > Study > Batch > Batch Item. Archiving feature is introduced.

V4.1 (2017) Reporting feature added to facilitate report writing/review. Added study-wide search and report feature for neat drug items used in ancestry of items in batch runs in the study, grouped by manufacturer/supplier, lot number and purity. "Stability Profile" introduced.

V4.1.1 (2018) Added study-wide search and reporting feature for standard stock and working solution items in ancestry of mix preparation for all items.

V4.2 (2018) User authentication changed to Windows Active Directory-integrated. Added study-wide search and report for all matrix items used in ancestry of mix preparations. New type of neat drug items added.

V4.3 (2019) Added in-application pipette verification; pipette verification status confirmed when selected for mix preparation.

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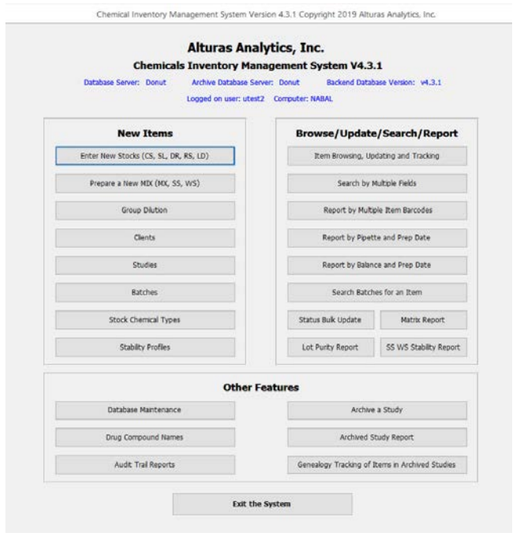


Figure 1: Holmes Menu and Features

The main menu allows the user to navigate within the application. Holmes is a 21 CFR Part 11 compliant environment, including the features below. Additional features are described in Figures 2-4.

Item creation: All reference standards and reagents are assigned a unique Holmes bar code upon receipt, allowing review and tracking of original component items.

Mix creation: Calibration standards, QCs and other solutions used in the analysis of bioanalytical samples are assigned unique bar codes when created. Holmes interfaces directly with balances to capture weights, eliminating transcription errors. Concentrations are automatically calculated and expiration dates are assigned based on validated stability.

Reporting: Reports by client and study generate summary tables listing matrices and standard solutions included in batch records.

REVIEW BATCH RECORDS

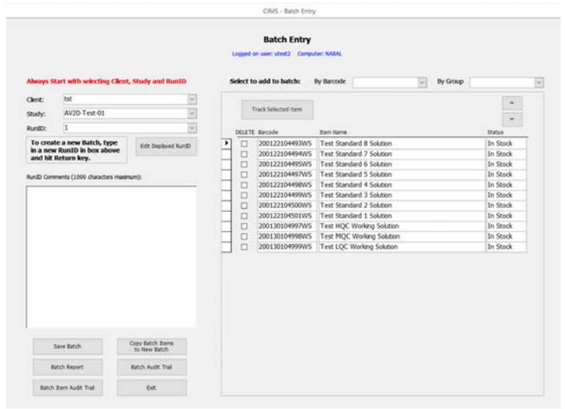


Figure 2: Batch Entry Menu

The Batch Entry menu allows scientists to add items used in each batch to a unique batch record traceable by client, study number and batch number. Batch records include solutions and materials used on each day of analysis. Auditors use this menu to confirm that all items used during the extraction and analysis of a batch were recorded and match the test method. The Batch Audit Trail records the date and time a batch record was created, and the Batch Item Audit Trail lists item addition and removal events.

VERIFY PIPETTES

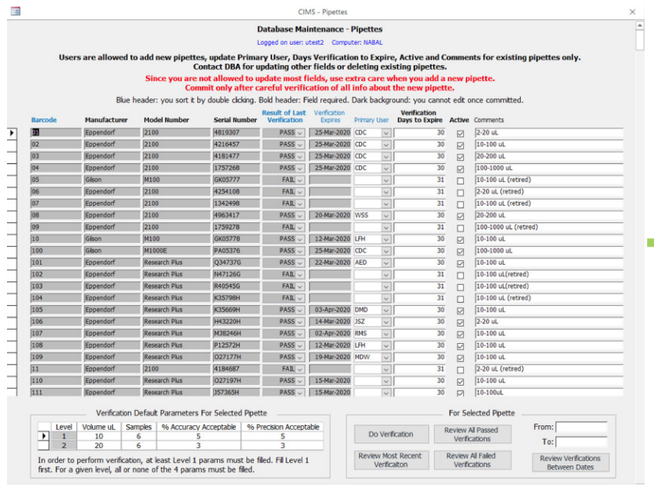
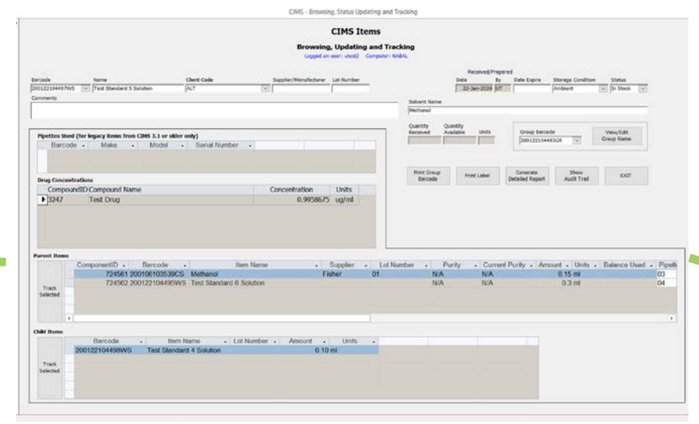


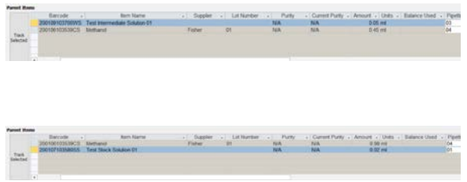
Figure 3: Pipette Tracking and Verification

All pipettes are tracked and gravimetrically verified on a monthly schedule. An analytical balance captures consecutive weighings and transmits the data to Holmes, where is it evaluated against the acceptance criteria designated by SOP.

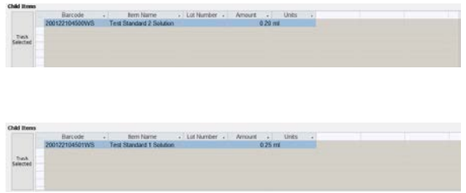
TRACK TO SOURCE RECORDS



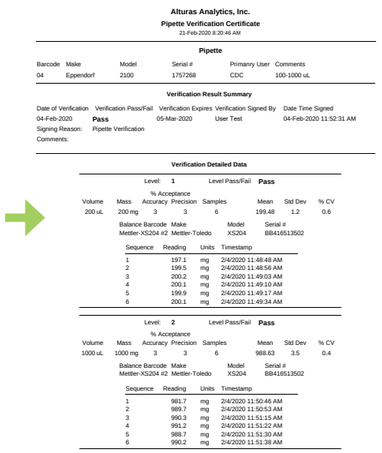
PARENT



CHILD



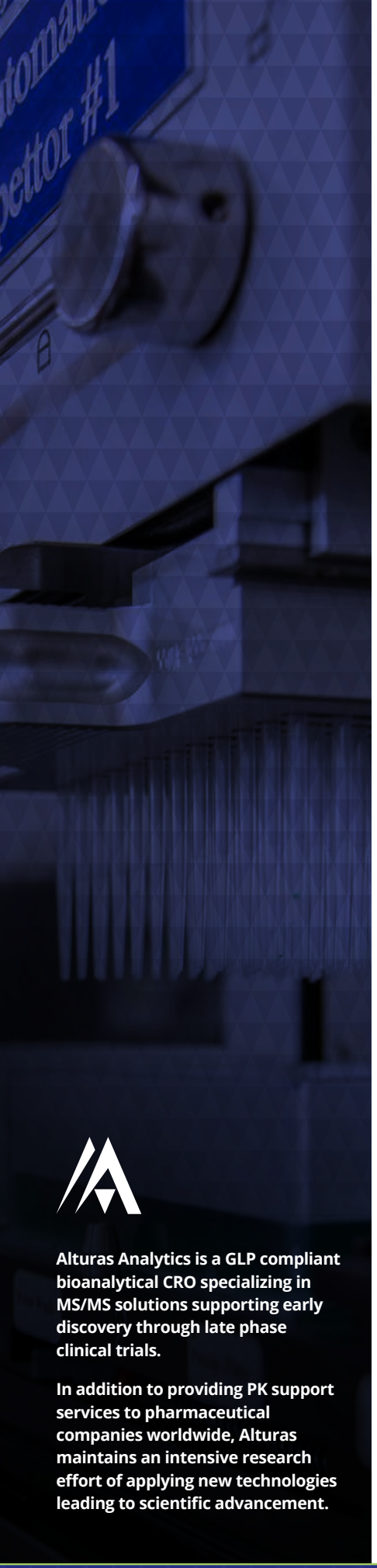
CHECK STATUS



CONCLUSIONS

As a result of developing Holmes over several years, the following key benefits have been recognized by Alturas Analytics:

- Cost savings over off-the-shelf, full ELN systems
- Barcoding providing instant traceability
- Minimized storage space (no paper records)
- Customization meeting evolving needs
- Electronic pipette verification
- 21 CFR Part 11 compliance
- Electronic access to all batch and study records
- Ensured legibility of records
- Elimination of transcription and manual calculation errors
- Direct electronic connection to balances



The "Alturas Way": Focused on MS/MS Bioanalysis and Pharmacokinetic Services

When choosing a bioanalytical services provider, you must consider the choices in the context of a rapidly changing and complex industry. Alturas Analytics is recognized as a pioneer and technology leader of MS/MS bioanalysis with a reputation built on performance history, personalized service and creative solutions to some of industry's greatest challenges.

"As a privately owned company, we focus on fostering lasting relationships with our sponsors while having the flexibility to accommodate unique needs as they arise."

- Robin Woods, President

Founded in 2000, Alturas remains privately owned, and operates with a commitment focused on the needs of our Sponsors. Frequent status updates and immediate access to technical experts who understand the urgency of each project are standards built into our routine workflow. Results are delivered on time with scientific integrity that exceeds industry standards.

This is the "Alturas Way."

BIOANALYTICAL SERVICES

- Method Development and Validation
- Regulated and Non-Regulated Sample Analysis
- Biomolecule and Small Molecule Quantitation in Any Matrix
- Antibody Drug Conjugates (ADC) and Other Conjugates
- Quantitative Biomarker Analysis
- Plasma Protein Binding Determinations
- Microsample Analysis Techniques
- SEND Compliant Datasets
- Pharmacokinetic and Toxicokinetic Analysis
- Expedited Services Offered

QUALITY SYSTEMS

- Compliant with FDA and ICH GLPs and Guidances
- Onsite QA and IT Departments
- Watson LIMS™ Data Management and Security System
- 21 CFR Part 11 Compliant
- Validated Analyst® Software
- Validated Phoenix® WinNonlin®
- Holmes Electronic Lab Notebook
- Custom Laboratory Integration System for a Connected Workflow

RESEARCH

- Capacity Reserved for Research
- New Bioanalytical Techniques Including:
  - Microflow LC-MS/MS
  - Immunocapture
  - PicoFuze™
  - Translucent Matrices: Tears, Cerebrospinal Fluid, Synovial Fluid
- Robotic Applications

FACILITIES & EQUIPMENT

- ~30,000 Sq. Ft. Purpose-Built Facility
- Sciex Triple Quadrupole LC-MS/MS 6500+/6500/5500/4000 Systems
- Thermo Scientific™ TSQ™ 9000 GC-MS/MS Systems
- Shimadzu HPLCs
- Waters AQUITY UPLC® M-Class Systems
- HTDialysis Micro-Equilibrium Devices

Alturas Analytics is a GLP compliant bioanalytical CRO specializing in MS/MS solutions supporting early discovery through late phase clinical trials.

In addition to providing PK support services to pharmaceutical companies worldwide, Alturas maintains an intensive research effort of applying new technologies leading to scientific advancement.