Mass Spectrometry in the Clinical Lab:
Best Practices and Current Applications
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Trends and Challenges in Therapeutic Drug Monitoring: Is LC/MS the Solution?

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Financial Disclosures

- None

Objectives

After this session, the attendee will be able to:

- Summarize some of the technical considerations, logistics, clinical issues, financial and regulatory requirements that are important when determining if TDM tests can or should be performed using mass spectrometry.
- Illustrate the advantages and disadvantages of using LC/MS/MS for TDM tests in the clinical laboratory.
- Assess future opportunities for TDM testing using mass spectrometry and list current clinical applications.

Outline

Trends and Challenges in TDM:Is LC/MS the Solution?

Important Factors to Consider:

1) Analytical/Technical Issues
   - A. Capacity for Growth/Expandable Test Menu
   - B. Required Method Performance Criteria
     - Specificity
     - Sensitivity
   - C. Logistics
     - Labor/Staffing
     - Availability of Standards, QC, Proficiency Testing Material

2) Clinical/Financial Issues
   - A. TAT (Clinical need vs. Market driven)
   - B. Cost/Reimbursement
   - C. Legal/Regulatory issues

3) Clinical Examples

Why is TDM still important?

- Drug Utilization:
  - >3.7 billion prescriptions filled in US 2011
  - From 1999 to 2009, # prescriptions increased 39%, w/ only 9% pop. growth
  - >10% Americans use 5 or more drugs
- Indications:
  - Personalization of dosage
  - Avoidance/detection of toxicity
  - Investigation of non-response
  - Identification of non-compliance

Traditional TDM Targets w/ FDA approved Immunoassays

- Anticonvulsants:
  - Phenytoin
  - Carbamazepine
  - Phenobarbital
  - Primidone
  - Valproic Acid
- Cardioactive drugs:
  - Digoxin
  - Procainamide/NAPA
  - Quinidine
  - Lidocaine
- Antibiotics:
  - Amikacin
  - Gentamicin
  - Tobramycin
  - Vancomycin
- Anti-asthmatic
  - Theophylline
  - Caffeine
- Anti-depressants:
  - Lithium
- Immunosuppressant's:
  - Cyclosporine
  - Tacrolimus
  - Sirolimus
- Anti-neoplastic drugs:
  - Methotrexate
- Analgesics:
  - Acetaminophen
  - Salicylate

TDM Trends: The Move to LC/MS or LC/MS/MS

- Analytical/Technical Considerations:
  1. Future growth and ability to measure evolving targets/new medications
     - Example: Anti-epileptic drugs (AEDs)

Generation	Drug	Year FDA approved
First	Phenobarbital	1912-1979
	Phenytoin	
	Carbamazepine	
	Valproate	
Second	Lamotrigine	1994-2005
	Topiramate	
	Oxcarbazepine	
	Pregabalin	
	Zonisamide	
Third	Lacosamide	2008-present
	Ezogabine (Retigabine)	

Clinical Example #1: Lacosamide

Lacosamide (3rd Generation AED)

- FDA approved for adjunctive therapy for partial seizures
- MOA: enhances the slow inactivation of voltage-gated Na+ channels
- Pharmacokinetic parameters:
  - 100% Bioavailable
  - Tmax: 1-2 hours
  - Elimination half-life: 12-16 hours
  - Protein binding: 90%
  - Minimal drug-drug interaction
- TDM:
  - CNS toxicity associated w/ high drug concentrations
  - Reference interval: 10-20 mg/L
  - Indications for TDM:
    - Hepatic or renal disease
    - Overdose
    - Compliance

Patsalos & Berry, Pharmacotherapy of third-generation AEDs: lacosamide, retigabine, and eslicarbazepine. *Expert Opin. Pharmacother*. 2012;13(5):699-715

Lacosamide continued

- Analytical /Technical Considerations:
  1. Clinical utility and need:
     - >250 requests/month
  2. Instrumentation:
     - No FDA-approved immunoassay available
     - Published HPLC and LC/MS/MS methods
  3. Logistics:
     - Staffing/Instrumentation capacity
     - Accessible materials:
       - Standards: Cerilliant
       - QC: Utak AED II
       - PT: LGC Standards Proficiency Testing

Lacosamide continued

- Clinical/Financial Considerations:
  1. TAT:
     - 2-4 days (send-out)
  2. Cost:
     - Send-out: >$250,000/year
     - In-house: >80% reduction in cost vs. send-out
  3. Legal/Regulatory Issues
     - Laboratory Developed Test (CLIA requirements)
     - No patent issues on TDM method or correlation of blood/serum levels and dosage

TDM Trends: The Move to LC/MS or LC/MS/MS

- Analytical/Technical Considerations:
  1. Future growth and ability to measure evolving targets/new medications
     - Example: Anti-epileptic drugs (AEDs)
  2. Method Performance Requirements:
     - Example:
       - Specificity (Cross-reactivity) for IA vs. MS
       - Functional Sensitivity (LOQ)

Clinical Example #2: Methotrexate TDM Issues

Methotrexate TDM Issues

- Methotrexate is used to treat certain types of cancer of the breast, skin, head and neck, or lung. It is also used to treat severe psoriasis and rheumatoid arthritis
- Jan 2012 FDA approves Voraxaze
  - VORAXAZE (glucarpidase) is indicated for the treatment of toxic plasma methotrexate concentrations (>1 µM/L) in patients with delayed clearance due to impaired renal function.
- Voraxaze Package Insert:
  - WARNINGS AND PRECAUTIONS:

5.2 Monitoring Methotrexate Concentration/Interference with Assay

Methotrexate concentrations within 48 hours following administration of VORAXAZE can only be reliably measured by a chromatographic method. DAMPA (4-deoxy-4-amino-N^{10}-methylpteroic acid) is an inactive metabolite of methotrexate resulting from treatment with VORAXAZE. DAMPA interferes with the measurement of methotrexate concentration using immunoassays resulting in an erroneous measurement which overestimates the methotrexate concentration. Due to the long half-life of DAMPA ($t_{1/2}$ of approximately 9 hours), measurement of methotrexate using immunoassays is unreliable for samples collected within 48 hours following VORAXAZE administration [*see Clinical Pharmacology (12.1)*].

- Other issues with Methotrexate Immunoassay techniques:
  - Low-end precision (0.05 µM/L)

TDM Trends: The Move to LC/MS or LC/MS/MS

- Analytical/Technical Considerations:
  1. Future growth and ability to measure evolving targets/new medications
     - Example: Anti-epileptic drugs (AEDs)
  2. Method Performance Requirements:
     - Example:
       - Specificity (Cross-reactivity) for IA
       - Functional Sensitivity (LOQ)
  3. Logistics:
     - Equipment/Labor/Staffing
     - Availability of Standards, QC, PT material

Availability of Standards/QC Material

1. Source:
   - Commercial vs. Homemade

Commercial	Home-made
Uniform, consistent	Some variability
More expensive	More affordable

Clinical Example #3: Tacrolimus

Instrumentation alone (MS) doesn't solve everything

Clinical Chemistry 57:12
1739–1747 (2011)

Drug Monitoring and Toxicology

The Need for Standardization of Tacrolimus Assays

Daniel M. Levine,[1*] Gregory T. Maine,[2] David A. Armbruster,[2] Christopher Mussell,[3] Christoph Buchholz,[3] Gavin O'Connor,[3] Victoria Tuck,[4] Atholl Johnston,[4] and David W. Holt[4]

- Problem: Tacrolimus, and other immunosuppressants, are measured by a variety of LC/MS and immunoassay-based methods which are all independently calibrated without traceability to an accepted reference LC/MS method or standard tacrolimus reference material.

Misconceptions about Mass Spectrometry

- "Mass spectrometry is always the reference method/gold standard"
- "Mass spectrometry will always give the right answers"
- "Mass spectrometry is accurate / precise"

Mass spectrometry can be all of these, but ONLY if tests are carefully developed, validated, calibrated, QC'd, and have appropriate surveillance.

Isn't LC/MS the GOLD Standard?

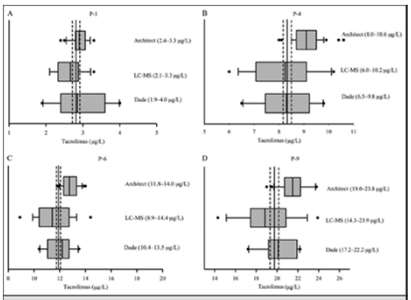


Fig. 1. Box-and-whisker plots of Architect, LC-MS, and Dade Dimension test values for samples P-1 (A), P-4 (B), P-6 (C), and P-9 (D).
Vertical solid line across the plots, reference value determined by EM-IDMS; dashed lines, associated expanded uncertainty; values in parentheses, range of tacrolimus concentration values obtained by each tacrolimus test method.

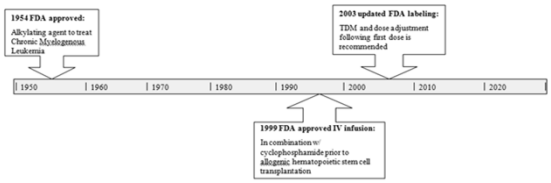
The Move to LC/MS or LC/MS/MS, continued

- Clinical/Financial considerations:
  1. Clinical demand/utility
     - Examples:
       - Busulfan
       - Lenalidomide
       - Lacosamide
       - Dabigatran
       - Leflunomide
       - Posaconazole
       - Thiopurine Metabolites
       - Others

Clinical Example #4: Busulfan

New Formulations, Dosing Regimens, or Guidelines

- Busulfan:



Busulfan

- Alkylating agent used in high doses for bone marrow ablation prior to allogeneic hematopoietic stem cell transplantation (HSCT) for Chronic Myelogenous Leukemia (CML)
- Narrow therapeutic window:
  - High: Toxicity (Hepatic veno-oclusive disease)
  - Low: Increased incidence of graft rejection/relapse
- Pharmacokinetic testing for IV dosing

Dose Adjustment Based on Therapeutic Drug Monitoring

Instructions for measuring the AUC of busulfan at dose 1 (see **Blood Sample Collection for AUC Determination**), and the formula for adjustment of subsequent doses to achieve the desired target AUC (1125 µMxmin), are provided below.

Adjusted dose (mg) = Actual Dose (mg) x Target AUC (µMxmin)/Actual AUC (µMxmin)

For example, if a patient received a dose of 11 mg busulfan and if the corresponding AUC measured was 800 µMxmin, for a target AUC of 1125 µMxmin, the target mg dose would be:

Mg dose = 11 mg x 1125 µMxmin / 800 µMxmin = 15.5 mg

Busulfex dose adjustment may be made using this formula and instructions below.

Blood Sample Collection for AUC Determination:

Calculate the AUC (µMxmin) based on blood samples collected at the following time points:

For dose 1: 2 hr (end of infusion), 4 hr and 6 hr (immediately prior to the next scheduled BUSULFEX administration). Actual sampling times should be recorded.

For doses other than dose 1: Pre-infusion (baseline), 2 hr (end of infusion), 4 hr and 6 hr (immediately prior to the next scheduled BUSULFEX administration).

AUC calculations based on fewer than the three specified samples may result in inaccurate AUC determinations.

For each scheduled blood sample, collect one to three mL of blood into heparinized (Na or Li heparin) Vacutainer® tubes. The blood samples should be placed on wet ice immediately after collection and should be centrifuged (at 4°C) within one hour. The plasma, harvested into appropriate cryovial storage tubes, is to be frozen immediately at -20°C. All plasma samples are to be sent in a frozen state (i.e., on dry ice) to the assay laboratory for the determination of plasma busulfan concentrations.

Calculation of AUC:

BUSULFEX AUC calculations may be made using the following instructions and appropriate standard pharmacokinetic formula:

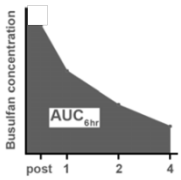
Dose 1 $AUC_{0\text{-infinity}}$ Calculation: $AUC_{0\text{-infinity}} = AUC_{0\text{-last}} + AUC_{\text{extrapolated}}$

where $AUC_{0\text{-last}}$ is to be estimated using the linear trapezoidal rule and AUC extrapolated can be computed by taking the ratio of the busulfan concentration at Hour 6 and the terminal elimination rate constant, λ. The λ must be calculated from the terminal elimination phase of the busulfan concentration vs. time curve. A "0" pre-dose busulfan concentration should be assumed, and used in the calculation of AUC.

If the AUC is assessed subsequent to Dose 1, steady-state AUC_{ss} ($AUC_{0\text{-tau}}$) is to be estimated from the trough, 2 hr, 4 hr and 6 hr concentrations using the linear trapezoidal rule.

Instructions for Dose Administration and Blood Sample Collection for Therapeutic Drug Monitoring

Typical Busulfan Curve



- Determines drug exposure (Area Under the Curve AUC)
- Calculates drug clearance and dose adjustment
- Proven Utility:
  - Improved clinical outcomes (pediatric HSCT recipients)
  - Reduced toxicity
- No FDA-approved Immunoassay; Typically use LC/MS or GC/MS

The Move to LC/MS or LC/MS/MS, continued

- Clinical/Financial considerations:
  1. Clinical demand/utility
  2. TAT needs
     - Market driven vs. Clinical need

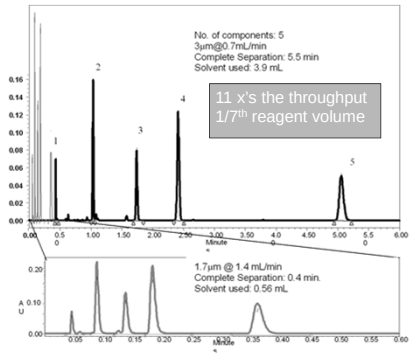
TAT and the Move to LC/MS

- Potential Limitations to Using LC/MS:
  - Batch analysis vs. Random Access
  - May require expensive/time-consuming sample preparation
  - Throughput
- Solutions/Trends to Improve Throughput:
  - UPLC (Ultra Performance Liquid Chromatography)
  - RapidFire
  - LDTD (Laser Diode Thermal Desorption)

Option #1
Conversion from HPLC to UPLC to Increase Throughput

- Why convert HPLC to UPLC?
  - Faster TAT (Cycle time)
  - Higher chromatographic resolution
  - Increased sensitivity
  - Lower injection volume (sample volume)
  - Less reagent/solvent
  - Same robustness as HPLC
  - Overall, increased capacity

Example of an HPLC Assay Converted to UPLC



A UPLC-MS/MS Method for Analyzing Eight Drugs

- Objective:
  - To improve the efficiency and quality of a HPLC-MS/MS method for eight drugs (Cocaine, BE, Coca-ethylene, Diphenhydramine, Methadone, EDDP, Chlorpheniramine, and Dextromethorphan)
- Benefits of converting to a UPLC-MS/MS method:
  - Shorter run time: Saved 4 hours per batch of 30 samples.
  - Better separation of components especially in forensic samples.
  - Less matrix effects which improves the accuracy of results as compared with HPLC-MS/MS.
  - Shorter dwell time in the UPLC-MS/MS method allows for more points across the peaks of the chromatograms ensuring better chromatographic results

By Tim Dahn, Marcie Larson, and Andrea Terrell, Ph.D./DABCC
Presented at the American Association of Clinical Chemistry (AACC) Annual Meeting July 27-31, 2008

Option #2
Using RapidFire® to Improve Throughput

http://www.youtube.com/watch?v=hjSm-p8_Ns4

Option #3
Using LDTD to Improve Throughput

http://www.youtube.com/watch?v=dNnfrfNyeZo

The Move to LC/MS or LC/MS/MS, continued

- Clinical/Financial considerations:
  1. Clinical demand/utility
  2. TAT needs
     - Market driven vs. Clinical need
  3. Cost/Reimbursement

Cost Savings/ROI
Using LC/MS/MS to Bring Tests In-Lab

Send-Out (S/O) Tests	Avg. Monthly Volume	S/O Cost/Test	Monthly S/O Cost	Cost/Test In-Lab	Monthly Cost In-Lab	Monthly Savings	Yearly Savings
Levetiracetam (Keppra)	250	$35.00	$8,750.00	$5.00	$1,250.00	$7,500.00	$90,000.00

The Move to LC/MS or LC/MS/MS, continued

- Clinical/Financial considerations:
  1. Clinical demand/utility
  2. TAT needs
     - Market driven vs. Clinical need
  3. Cost/Reimbursement
  4. Legal/Regulatory issues

Legal/Regulatory Issues

- Important to check for any Intellectual Property or Patent issues on TDM method or correlation of blood/serum levels and dosage.
- Understand how to satisfy CLIA requirements for validating LDTs
  - Sensitivity
  - Specificity
  - Carry-over
  - Accuracy
  - Precision
  - Etc……..

Why Switch to LC/MS for TDM?

Advantages	Disadvantages
• Sensitivity	• High Complexity
• Specificity	• LDT regulations
• Open/Expandable test menu	• R&D tech/expertise
• Long-term cost savings	• Initial cost (need to buy 2)
• Shorter TAT vs. Send-Out	• Staffing
	• Batch tests

Summary

- LC/MS or LC/MS/MS is a viable option for TDM:
- Trends:
  - Labs continue to switch to LC/MS to bring new TDM assays in-house
    - Cost-effective
    - Better TAT
- Challenges:
  - Regulatory: New LDT regulations
  - Personnel/Expertise

Questions & Discussion