

An On-line Tutorial for Bioanalytical Method Validation

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LC-MS/MS has seen enormous growth in clinical laboratories during the last 10-15 years. ¹

- The Neolithic Age(1960-2000):
 - GC-MS
 - Toxicology
- The Bronze Age (past 15 years):
 - LC-MS/MS
 - Small molecules:
 - immunosuppressants (rapamycin)
 - chemotherapeutics (cyclosporins)
 - Multianalyte panels:
 - Steroid Panels
 - Vitamin D
 - Acyl carnitines and amino acids for expanded newborn screening
- The Iron Age (current):
 - Vitamin D and Testosterone
 - Proteins and peptides (Thyroglobulin, IGF-1)
 - MALDI Biotyper : FDA Class II medical device for infectious agent identification



1) <https://www.aacc.org/publications/clin/articles/2015/february/future-of-mass-spec>; Alan Rockwood/FEB.1.2015 /Clinical Laboratory News

Quest Diagnostics LC-MS/MS Based Assays

- Testosterone (Total and free)
- Aldosterone
- Progesterone
- Estrogens
- Pregnenalone
- Cannabinoids
- Tamoxifen
- 25 (OH) Vit D3
- Thyroglobulin
- Steroids Panel
- 5-HIAA
- HVA
- Serotonin
- Amino Acids
- Metanephrines
- Cortisol
- Carbamazepine
- Etc.

LC-MS/MS assays are almost exclusively laboratory Developed Tests (LDTs)

- LDT is an IVD that is intended for clinical use and designed, manufactured and used within a single laboratory.²
- Clinical labs have been using LC-MS almost exclusively for lab-developed tests rather than in combination with FDA-approved assay kits.
- LDTs have so far been regulated by the Centers for Medicare & Medicaid Services under CLIA.
- FDA is now in the process of establishing a framework for regulating the tests as medical devices.³
- Public Workshop - Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs), January 8-9, 2015

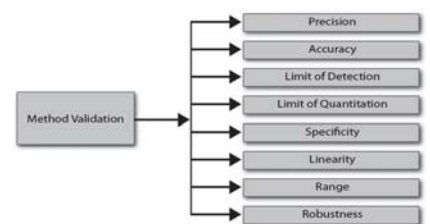
2) 1976 Medical Device Amendments (MDA) of the FD&C Act.
 3) <http://cen.acs.org/articles/93/120/Mass-Spec-Welcome-Clinical-Labs.html?i=1756110830>

The Regulation is coming but the Guidance is already here:

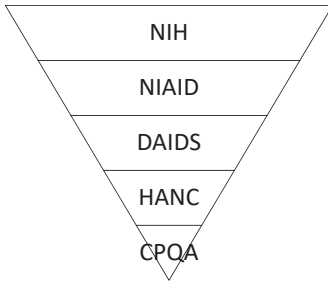
- May 2001/2013: FDA guidance for Bioanalytical Method Validation
 - <http://www.fda.gov/downloads/oc/ohrt/guidancecomplianceregulatoryinformation/guidances/ucm368107.pdf>
- May 2001/2012: CPQA Guidelines for Chromatographic Method Development and Validation based on (and including) FDA Guidelines
 - https://nlaid.usallearning.net/pluginfile.php/11701/mod_resource/content/1/CPQA%20Guidelines%20for%20Chromatographic%20Method%20Development%20and%20Validation.pdf
- November 2005: ICH Validation of Analytical Procedures: Text and Methodology
 - https://www.ich.org/fileadmin/Publiic_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf
- December 2013: LRN CLIA-Compliant Analytical Method Validation Plan and Template
 - https://www.apht.org/about/APHT/publications/Documents/EH_2013Dec_CLIA-Compliant-LRN-C-Method-Validation-Template.pdf
- November 2014: CLSI LC-MS/MS Approved Guidelines
 - <http://clsi.org/blog/2014/11/07/clsi-releases-new-clinical-chemistry-document-liquid-chromatography-mass-spectrometry-methods/>

Common Validation Parameters

- Accuracy
- Precision
- Selectivity/Specificity
 - Interference
 - Matrix effects
- Sensitivity and detection limits
 - LOD
 - LLOQ
 - ULQ
- Precision
 - Intra-day
 - Inter-day
 - Reproducibility
 - Ruggedness
- Stability
 - Sample
 - Analytical



Bioanalytical method validation in the fight against HIV/AIDS



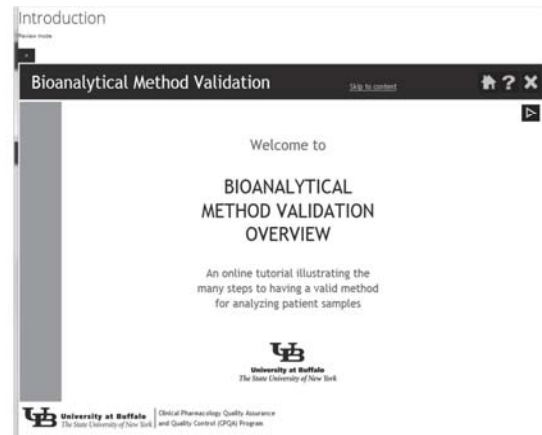
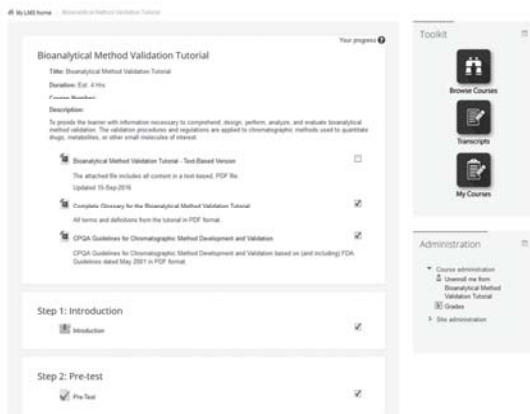
HIV/AIDS Clinical Trials Network and Network Coordination (HANC)



Clinical Pharmacology Quality Assurance and Quality Control Program (CPQA)

- Sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) through DAIDS, 2007 & 2015
- Charged to develop and conduct quality assurance activities for pharmacology laboratories that collaborate in NIAID-sponsored HIV/AIDS networks
- Includes:
 - training and certification for clinical sites and laboratory staff
 - oversight and peer-review of new assay development, validation and implementation
 - proficiency testing program
- Translational Pharmacology Research Core (TPRC)
 - <https://tprc.pharm.buffalo.edu/home/tprc/>
- Frontier Science & Technology Research Foundation, Inc. (FSTRF)
 - <http://www.fstrf.org/>
 - Data Coordinating Center for ACTG, IMPAACT, VQA and CPQA
- HIV/AIDS Clinical Pharmacology Quality Assurance and Quality Control (CPQA) Program
 - <https://www.fstrf.org/apps/cfm/apps/cpg/cpgaDocs/public/index.html>

The Bioanalytical Method Validation Tutorial



Navigation Overview

Print current page (not available on all pages) **Print**

Course Title: Bioanalytical Method Validation

Module Indicator: Introduction

Page Title: Method Validation

Accessibility Option: [icon]

Text-Based Version: [icon]

Progress Page: [icon]

Home: [icon]

Printable Glossary: [icon]

Help: [icon]

Exit Course: [icon]

Jump to a different module by clicking any module title

Copyright Details: [icon]

Method Validation

The Food and Drug Administration Guidance for Industry states that the fundamental parameters for bioanalytical method validation using the Chromatography (Chrom) and Mass Spectrometry (MS) and Mass Spectrometry (MS) include accuracy, precision, sensitivity, selectivity, reproducibility, and stability.

Tip Indicator (additional information)

Back Next

Interactive Element

Location within Module

Method Validation Parameters: Accuracy, Precision, Sensitivity, Selectivity, Reproducibility, Stability

Interaction Directions: [icon]

Directions: Click each term in order to activate.

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Introduction

Bioanalytical Method Validation

Introduction

Course Goal

To provide the learner with information necessary to comprehend, design, perform, analyze, and evaluate bioanalytical method validation. The validation procedures and regulations are applied to chromatographic methods used to quantitate drugs, metabolites, or other small molecules of interest.

Objectives are provided prior to each module.

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Introduction

Bioanalytical Method Validation

Introduction

Modules

The content for this tutorial is divided into seven modules.

Directions: Click a circle on the graphic to read a brief description of the module.

Validation Overview

Stability

Reagents

Calibration

Quality Control and Specificity

Accuracy and Precision

Selectivity

Identifies the necessary analytical samples included within an analysis to provide calibration.

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Introduction

Bioanalytical Method Validation

Introduction

Course Completion

To earn a certificate of completion for this course, you must complete the pre-test, review all the material presented in each module, complete each module's quiz, and complete the post-test. Each quiz score must be 80 percent or higher to count towards the certificate of completion.

You also have the option to review only the course material and not take the quizzes. Just skip the link to take the quiz in the tutorial navigation on the DAIDS Learning Management System (LMS).

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My LMS Home | Bioanalytical Method Validation Tutorial

Your progress

Bioanalytical Method Validation Tutorial

Title: Bioanalytical Method Validation Tutorial

Duration: Est. 4 hrs

Course Number:

Description:

To provide the learner with information necessary to comprehend, design, perform, analyze, and evaluate bioanalytical method validation. The validation procedures and regulations are applied to chromatographic methods used to quantitate drugs, metabolites, or other small molecules of interest.

Bioanalytical Method Validation Tutorial - Text-Based Version

The attached file includes all content in a text-based PDF file updated 15-Sep-2016

Complete Glossary for the Bioanalytical Method Validation Tutorial

All terms and definitions from the tutorial in PDF format.

CPQA Guidelines for Chromatographic Method Development and Validation

CPQA Guidelines for Chromatographic Method Development and Validation based on ICH including FDA Guidelines dated May 2005 in PDF format.

Step 1: Introduction

Introduction [checked]

Step 2: Pre-test

Pre-Test [checked]

Toolkit

Browser Courses

Transcripts

My Courses

Administration

Course administration

Unenroll me from Bioanalytical Method Validation Tutorial

Grades

Site administration

HOME COURSES RESOURCES HELP

DAIDS Learning Management System

You are logged in as Richard Thomas

My LMS Home | Bioanalytical Method Validation Tutorial | Step 3: Pre-test | Pre-Test

Question 6

Special answer

Question 6 of 10

Answer

Analytical methods need to be validated _____

Select one

A. at the beginning of each day's analytical run.

B. before method development begins.

C. just before regulatory site visits.

D. before initial implementation in routine sample testing.

E. whenever a new technician takes over the method.

Submit

Quiz navigation

10 11 12 13

Final attempt

NIH National Institute of Health and Biomedical Research USA.gov

Step 3: Validation Overview

Read content; pass quiz with score of 80% or higher

- Validation Overview presentation
- Validation Overview Quiz
- Clinical Laboratory Improvement Amendments Brochure
A brochure about the Clinical Laboratory Improvement Amendments (CLIA) from the Department of Health and Human Services in PDF format.
- Guidance for Industry - Bioanalytical Method Validation, May 2001
Guidance for Industry on Bioanalytical Method Validation from the U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine in PDF format.
- Statistical Parameter Definitions and Calculations
This document is a reference to calculating mean, standard deviation, %Coefficient of Variation, and %Deviation as used throughout the tutorial.

Step 4: Reagents

Read content; pass quiz with score of 80% or higher

- Reagents - presentation
- Reagents Quiz
- CPQA Certificate of Authenticity Example
Sample Certificate of Authenticity (COA) from the CPQA.

Step 5: Calibration

Read content; pass quiz with score of 80% or higher

- Calibration - presentation
- Calibration Quiz

Step 6: Quality Control

Read content; pass quiz with score of 80% or higher

- Quality Control - presentation
- Quality Control Quiz

Step 7: Accuracy and Precision

Read content; pass quiz with score of 80% or higher

- Accuracy and Precision - presentation
- Accuracy and Precision Quiz

Step 8: Selectivity

Read content; pass quiz with score of 80% or higher

- Selectivity - presentation
- Selectivity Quiz
- Testing for Matrix Effects Mass Spectrometry Reference Sheets
This reference sheet contains a copy of the data presented in the Selectivity Module in PDF format.

Calibration - presentation


Bioanalytical Method Validation

Calibration

Calibration describes the relationship between analytical instrument response and theoretical concentration. The FDA requires a minimum of six calibration standards be included in each analysis performed.

Directions: Click the test tube to the right to access the Calibration Activity.

Attention Google Chrome users: Please hold down the Shift key when clicking this icon. This will ensure it appears in a new window rather than on a new tab.

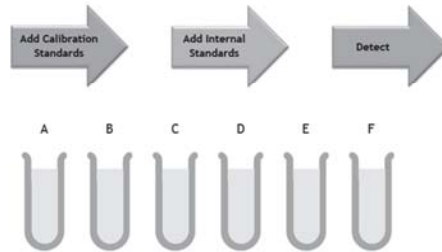


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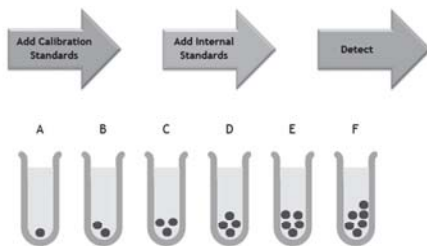
Calibration Standards Preparation

Six tubes appear below to prepare calibration standards of 100, 200, 300, 400, 500, and 600 ng/mL matrix. Click each arrow from left to right to prepare, process, and detect the calibration standards.



Calibration Standards Preparation

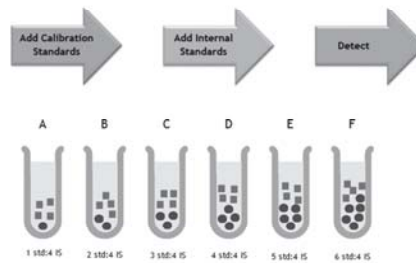
Six tubes appear below to prepare calibration standards of 100, 200, 300, 400, 500, and 600 ng/mL matrix. Click each arrow from left to right to prepare, process, and detect the calibration standards.



● = Calibration Standard (std)

Calibration Standards Preparation

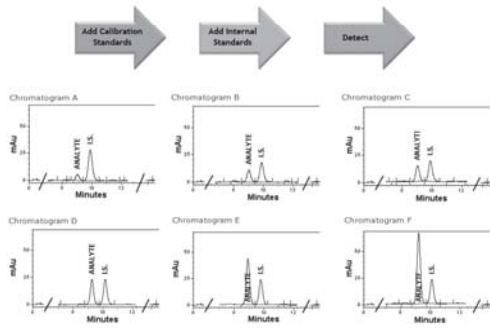
Six tubes appear below to prepare calibration standards of 100, 200, 300, 400, 500, and 600 ng/mL matrix. Click each arrow from left to right to prepare, process, and detect the calibration standards.



● = Calibration Standard (std)
■ = Internal Standard (IS)

Calibration Standards Preparation

Six tubes appear below to prepare calibration standards of 100, 200, 300, 400, 500, and 600 ng/ml matrix. Click each arrow from left to right to prepare, process, and detect the calibration standards.



Step 9: Stability
Read content; pass quiz with score of 80% or higher

Stability - presentation
 Stability Quiz

Step 10: Post Test
 Post Test

Certificate of Completion

Tip:

- Please refresh/load this page after each step for the system to mark it as complete.
- Once you click on "Certificate of Completion" below, you will no longer see this course listed on your "My home" page under "My Courses".
- To access completed courses, click on the "Transcripts" link in the Toolkit in the right column.

Certificate of Completion

Not available unless:

- You achieve a required score in Validation Overview Quiz
- You achieve a required score in Reagents Quiz
- You achieve a required score in Calibration Quiz
- You achieve a required score in Quality Control Quiz
- You achieve a required score in Accuracy and Precision Quiz
- You achieve a required score in Selectivity Quiz
- You achieve a required score in Stability Quiz
- The activity Post-Test is marked complete.

Calibration - presentation

Bioanalytical Method Validation

Calibration

Calibration describes the relationship between analytical instrument response and theoretical concentration. The FDA requires a minimum of six calibration standards be included in each analysis performed.

Directions: Click the text tube to the right to access the Calibration Activity.

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Summary Outcomes (2/2/2017)

	Count						
Started*	57						
Completed	29						
	%						
Avg. Pre-test	63.13						
Avg. Post-test	85.19						
Educational Level	2-yr	4-yr	MS	PHD	MD	Other	
% of completed	7	62	25	4	0	2	
Network	ACTG	HPTN	IMPAACT	INSIGHT	MTN	VTN	Educational
% of Completed	24	21	4	4	10	10	49
Evaluation (%)	Overall	Utility	Organization	Level			
Very Satisfied	39	56	28	45			
Satisfied	61	39	72	50			
Unsatisfied	0	0	0	0			
No Opinion	0	5	0	5			

* Includes demonstrations and trials

Accessing the Bioanalytical Method Validation Course on the DAIDS LMS

Go to the DAIDS Learning Portal (<http://www.daidslearningportal.com>).

Request an account if you do not have one. Click Go to LMS when you have your credentials.

DAIDS Learning Portal

Welcome, Guest | Sign in or Request account

Click here after you receive your account credentials.

Learning Management System

The Learning Management System (LMS) offers online training courses and lets sites assign, track, and monitor the completion of required training.

Go to LMS
Find a Course
Get Help

Registering your students

DAIDS Learning Portal — Multiple Account Request Form

Please send the completed form to the DAIDS Training Support Team at daidsrns-support@westat.com.

Name of SITE:	Your Institution		
SITE ID:	Your DAIDS LMS Account #		
Contact:	Your Name		

First Name (*required)	Last Name (*required)	Email Address (*required)	Role (*required)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

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Introduction

Please note

Bioanalytical Method Validation Skip to content

Home ? X

◀ ▶

Intellectual Property Claim

This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract number H6427220080019C, titled, "Clinical Pharmacology Quality Assurance and Quality Control."

The contractor, The Research Foundation on behalf of the State University of New York, University at Buffalo, retains intellectual property rights to the content herein.

Please contact CPQASupport@strf.org with any questions regarding the content, its use and/or use dissemination.

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