

C62

Liquid Chromatography-Mass Spectrometry Methods

This document provides guidance to the clinical laboratorian for the reduction of interlaboratory variance and the evaluation of interferences, assay performance, and other pertinent characteristics of clinical assays. This guideline emphasizes particular areas related to assay development and presents a standardized approach for method verification that is specific to mass spectrometry technology.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

Liquid Chromatography-Mass Spectrometry Methods

William Clarke, PhD, MBA, DABCC
Ross J. Molinaro, PhD, MLS(ASCP)^{CM}, DABCC, FACB
Lorin M. Bachmann, PhD, DABCC, MT(ASCP)
Julianne Cook Botelho, PhD
Zhimin Cao, MD, PhD, DABCC
Deborah French, PhD, DABCC
Seema Garg, MS, MBA
John M. Gawoski, MD
Russell P. Grant, PhD

Andrew N. Hoofnagle, MD, PhD
Bagyalakshmi Iyer, PhD
Vathany Kulasingam, PhD, FCACB
Donald S. Mason, MS
Brian Rappold
Danyel H. Tacker, PhD, DABCC
Steven M. Truscott, PhD, DABCC
Chunli Yu, MD, FACMG
Yusheng Zhu, PhD, DABCC, FACB

Abstract

Clinical and Laboratory Standards Institute guideline C62—*Liquid Chromatography-Mass Spectrometry Methods* provides guidance for the development and verification of liquid chromatography-mass spectrometry (LC-MS) methods in the clinical laboratory. The document is intended to reduce interlaboratory variance for clinical assays through guidance for evaluating interferences, assay performance, and other pertinent characteristics. It emphasizes particular areas related to assay development and presents a standardized approach for method verification that is specific to mass spectrometry (MS) technology. This document is intended for laboratorians responsible for development and verification of MS-based assays, physicians who may use these assays for patient care decisions, external quality assessment programs, and manufacturers of MS instrumentation and reagent kits designed to be paired with a particular mass spectrometer. This document is limited to discussion of LC-MS and is focused on the steps for development of a method, eg, whether the analyte is a drug, hormone, protein, or peptide.

Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods*. 2nd ed. CLSI guideline C62 (ISBN 978-1-68440-154-3 [Print]; ISBN 978-1-68440-155-0 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2022.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org.

If you or your organization is not a member and would like to become one, or to request a copy of the catalog, contact us at:

P: +1.610.688.0100 **F:** +1.610.688.0700 **E:** customerservice@cls.org **W:** www.clsi.org

Copyright ©2022 Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, companion product, or other material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to permissions@clsi.org.

CLSI hereby grants permission to each individual member or purchaser to make a single reproduction of this publication for use in its laboratory procedure manual at a single site. To request permission to use this publication in any other manner, e-mail permissions@clsi.org.

Suggested Citation

CLSI. *Liquid Chromatography-Mass Spectrometry Methods*. 2nd ed. CLSI guideline C62. Clinical and Laboratory Standards Institute; 2022.

Previous Edition:

October 2014

Sample

C62-Ed2

ISBN 978-1-68440-154-3 (Print)

ISBN 978-1-68440-155-0 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 42, Number 16

Contents

Abstract	i
Committee Membership	iii
Foreword	vii
Chapter 1: Scope	1
Chapter 2: Standard Precautions	3
Chapter 3: Terminology	5
3.1 A Note on Terminology	6
3.2 Definitions	7
3.3 Abbreviations and Acronyms	10
Chapter 4: Instrumentation	13
4.1 High-Performance Liquid Chromatography and Ultra-High Performance Liquid Chromatography	14
4.2 Ion Sources for Mass Spectrometry	16
4.3 Mass Analyzers: Quadrupole, Time-of-Flight, and Ion Traps	17
4.4 Instrumentation Performance Parameters	19
Chapter 5: Preexamination Considerations	25
5.1 Analyte of Interest	26
5.2 Internal Standard Selection	28
5.3 Reagents and Sample Preparation	30
Chapter 6: Assay Development	33
6.1 Ion Transitions	34
6.2 High-Performance Liquid Chromatography Stationary Phase	37
6.3 High-Performance Liquid Chromatography Mobile Phase	37
6.4 Examination Variables	39
6.5 Sources of Error	47
6.6 Assay Calibration	50
6.7 Preverification	55
Chapter 7: Assay Verification	61
7.1 Limit of Detection and Lower Limit of the Measuring Interval	62
7.2 Linearity and Dilution	62
7.3 Imprecision	64
7.4 Assay Interferences	65
7.5 Trueness	66

Contents (Continued)

Chapter 8: Quality Assurance and Quality Control of Liquid Chromatography-Mass Spectrometry Assays	69
8.1 Selection of Quality Control Materials	70
8.2 Quality Control Concentration and Frequency	71
8.3 Additional Quality Assurance/Quality Control Samples for Liquid Chromatography-Mass Spectrometry Methods	72
8.4 Evaluation of Quality Control Acceptability	73
8.5 Corrective Action for Failed Quality Control	73
8.6 Quality Control Considerations for Multicomponent or Multiplexed Assays	73
8.7 Periodic Quality Assurance Procedures	74
8.8 Additional Quality Assurance Aspects	76
Chapter 9: Postimplementation Monitoring	77
9.1 Proficiency Testing	78
9.2 System Performance Monitoring	79
Chapter 10: Supplemental Information	83
References	84
The Quality Management System Approach	92

Foreword

The importance of mass spectrometry (MS) in the clinical laboratory is increasing, and this CLSI document was developed in response to the need for increased robustness and harmonization of liquid chromatography-mass spectrometry (LC-MS) methods. Vendors of *in vitro* diagnostic devices often wait until the clinical utility of a particular assay is established and widely accepted before beginning development of a commercial assay. As a result, improvements in patient care can be delayed until a commercial assay is available. These delays can be significant if there are difficulties in the development of the assay. For laboratories with the capability to develop and implement laboratory-developed tests, MS provides an attractive alternative solution based on the ability to rapidly develop an analytically robust assay with excellent analytical sensitivity and specificity. In some cases, it offers an attractive alternative to commercially available assays that have already been developed, but do not offer acceptable performance in the current clinical context. LC-MS technology shows promise as a tool to rapidly develop clinical assays for emerging biomarkers coming from research in proteomics. Advances in instrumentation are likely to enable application of LC-MS technology in routine clinical diagnostic testing.

Despite the significant advantages that can be gained from incorporating MS into the clinical laboratory, considerable challenges exist. Among these is the fact that for some assays, significant laboratory-to-laboratory variability for the same analyte has been observed. There is currently limited assay standardization for MS-based methods, and much of the assay variability can be attributed to the lack of commercially available calibrators; that is, each clinical laboratory must formulate its own calibrators. For example, some sites may use powdered or commercially lyophilized material, some may use organic solvent solutions, and some may even use formulations obtained from their institution's pharmacy to make calibrators. Moreover, the preparation of the calibrators also varies from using solutions made in buffer, from using analyte-free serum or plasma as the matrix, or from using patient specimen remnants as the calibrator matrix—a problem for endogenous analytes. Differences in chromatographic methods from site to site lead to variable matrix effects during analysis. In addition, many laboratories verify their assays using various protocols in accordance with different regulatory or industry standards. This document addresses these issues by providing guidance for the development and verification of LC-MS methods in the clinical laboratory.

This document outlines many important elements for successful implementation of LC-MS technology for clinical analyses. The basic instrument components needed both for chromatography and MS are discussed, along with instrument parameters that must be optimized for development of robust LC-MS methods. In addition, the document contains a discussion of preexamination considerations that must be addressed during the method development process. Various elements of method development are summarized, along with best practice recommendations for addressing those elements during the process. Guidance is provided for verification of an LC-MS method, including a recommendation for preverification evaluation before full method verification. Finally, the document provides guidance for QA, including assay QC and postimplementation monitoring.

Overview of Changes

This guideline was revised in 2022 under the Limited Revision Process and replaces the first edition of the guideline, which was published in 2014. The sole change made in this edition is distinguishing recommended criteria for intralaboratory evaluation from studies required for regulatory submissions.

KEY WORDS

Chromatography

Mass spectrometry

Postimplementation monitoring

Liquid chromatography-mass spectrometry

Method verification

Quality control

Sample

Liquid Chromatography-Mass Spectrometry Methods

1 Scope

This document provides an introduction to, and guidance for, method development, verification, and postimplementation monitoring of quantitative clinical applications using liquid chromatography-mass spectrometry (LC-MS). While LC-MS may also be used for qualitative analyses, the focus of this document is on the use of this technology for quantification of clinical analytes. In addition, while there are commercial and research methods that allow direct injection without chromatography for rapid analyses, this guideline is exclusively focused on liquid chromatography (LC) coupled to mass spectrometry (MS). The purpose of this guideline is to educate both clinical LC-MS practitioners and health care providers (including physicians) who may use these assays for patient care decisions on the benefits and limitations of LC-MS methods used in the clinical laboratory, as well as provide a practical guide for the development and implementation of LC-MS-based clinical applications. It is intended to serve not only as a companion to CLSI document C50,¹ which serves as excellent general guidance for MS in the clinical laboratory, but also to provide an enhanced focus on methods, best practices, and instrumentation related to LC-MS, which is emerging as the most common approach to clinical analyses. This document is also intended to be a resource for instrument manufacturers, manufacturers of LC-MS reagents, regulatory agencies, and educators, as well as individuals responsible for developing laboratory standards and policy.

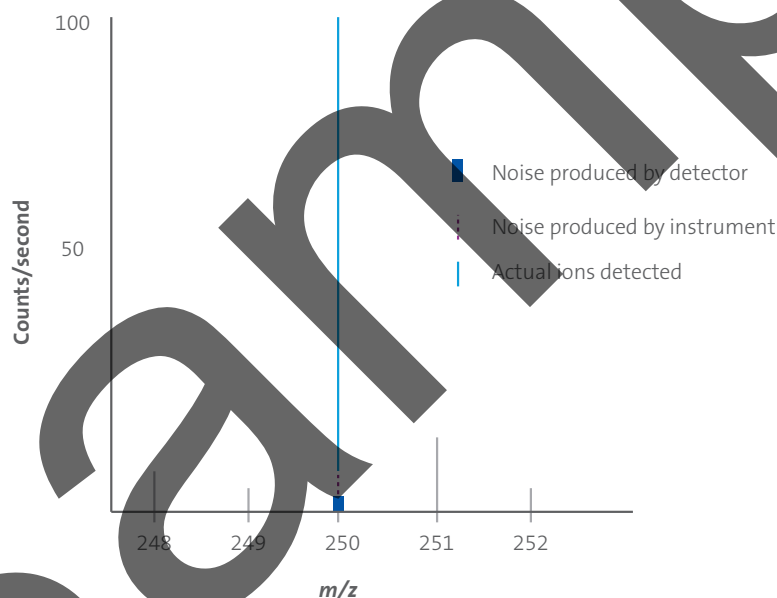
A description of all current clinical applications of LC-MS, as well as all of the pertinent information regarding development and verification of these methods, is beyond the scope of this document. As such, this guideline directs the reader to appropriate existing resources wherever possible. In providing guidance for LC-MS method development, verification, and implementation, this document focuses on:

- Important features of LC-MS instrumentation
- Preexamination factors that can impact assay performance and utility
- Assay calibration
- Analytical variables important in method development
- Assay verification
- QA and QC
- Postimplementation monitoring of clinical methods

result in peak broadening and loss of resolution. Excess tubing, incorrect alignment of tubing with fittings, and increased number of connections and unions will increase dead volume. The length of tubing between the injector and the column is particularly critical and should be minimized as much as possible. Care should be taken when seating fittings and frits, because improper alignment can also cause mobile phase turbulence or mixing errors, resulting in retention time and peak area variation.

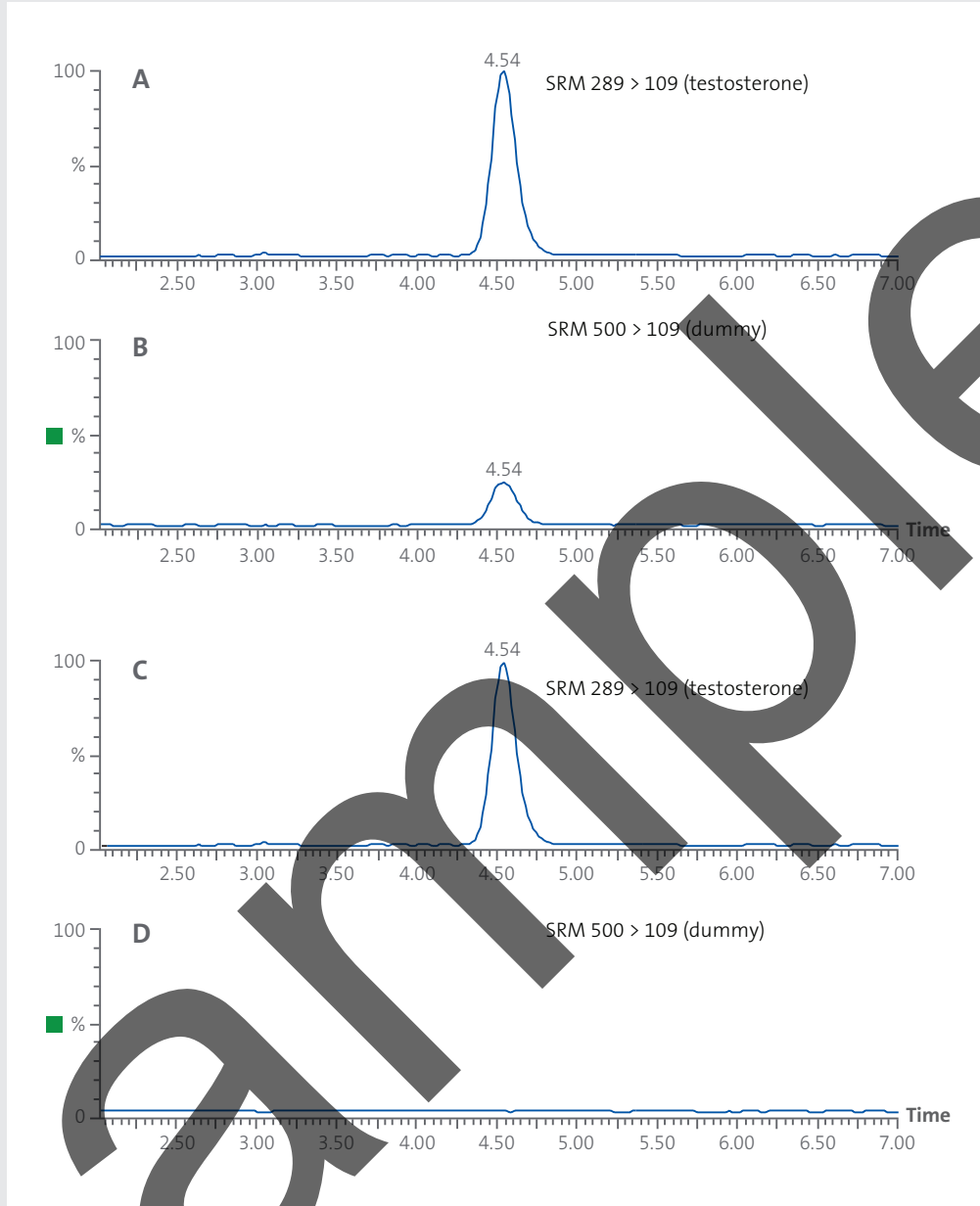
6.4.4 Signal-to-Noise Ratio

A mass spectrometer generates a voltage signal produced by ions striking the detector and depends on many different electronic settings within the instrument. Because the signal depends on different electronic settings, mass calibration and tuning are extremely important to ensuring optimal performance of the instrument, as analytical sensitivity can vary enormously based upon these parameters. An advantage of using SRM mode is that the mass spectrometer spends more time measuring the ions of interest than in full scan mode, enabling analysis with higher sensitivity. Even though operating in SRM mode will generally allow increased absolute signal for the analyte of interest compared to full scan mode, ion transmission through different parts of the mass spectrometer never reaches 100%. Therefore, fewer ions will pass through the second analyzer compared with the first. However, while obtaining the highest absolute signal for an analyte is desirable, it is not the end goal. A common method for measuring signal strength is to determine the S/N ratio of the analyte peak (see Figure 3).



Abbreviation: m/z , mass-to-charge ratio.

Figure 3. Contributions to the Signal Produced at the Detector of a Mass Spectrometer



Abbreviation: SRM, selected reaction monitoring.

Figure 6. Product Ion Cross-Talk. In this example, a 1 ng/mL standard of testosterone was injected onto a chromatographic column. Two SRM transitions were monitored, 289 > 109 (testosterone) and a “dummy” transition 500 > 109. In panels A and B, the dwell time and interchannel delay time were both set to 1 millisecond. The chromatographic peak at 4.54 minutes in panel B (the “dummy” transition) is a result of incomplete clearance of product ions (of testosterone) from the collision cell. In panels C and D, the dwell time and interchannel delay time were both set to 10 milliseconds. Panel D shows the absence of any such product ion cross-talk between testosterone and the “dummy” transition after increasing the dwell and interchannel delay times to 10 milliseconds.

Sample



CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®

PRINT ISBN 978-1-68440-154-3

ELECTRONIC ISBN 978-1-68440-155-0

C62-Ed2