



# C43-A2

## Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline—Second Edition

This document provides guidance on establishing uniform practices necessary to produce quality data for quantitation and identification of a drug or drug metabolite using the gas chromatography/mass spectrometry method. Specific quality assurance criteria for maintaining and documenting optimal instrument performance are also presented.

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## Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline—Second Edition

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### Abstract

Clinical and Laboratory Standards Institute document C43-A2—*Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline—Second Edition* is intended to aid the laboratorian in developing appropriate procedures for the use of gas chromatography/mass spectrometry in confirmation analyses. Its primary objective is to establish uniform practices necessary for producing quality data for quantitation and identification of a drug or drug metabolite. To support the scientific basis of the uniform practices, a brief overview of the techniques is provided. Specific quality assurance criteria for maintaining and documenting optimal instrument performance are presented.

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SAMPLE

## Foreword

The detection of a drug in the biological fluid of an individual can have serious professional, financial, and social consequences. As a result, it is often necessary or even required that the detection of a drug using a screening procedure be confirmed using a second method based on a different analytical or physical principle possessing greater sensitivity and specificity than the initial method used for screening. The purpose of the confirmation test is to decrease the probability of false-positive results and to provide additional information and assurance about the identity of the detected compound.

Gas chromatography/mass spectrometry (GC/MS) is widely accepted in both scientific and legal arenas as one of the most powerful analytical techniques for the separation, quantitation, and identification of drug analytes, especially at low concentrations. Technological advances have allowed introduction of bench-top GC/MS instrumentation into forensic and clinical toxicology laboratories. Further advances continue to move state-of-the-art techniques such as gas- and liquid-phase chemical ionization, tandem mass spectrometry (MS/MS), high-resolution mass spectrometry, and high-performance liquid chromatography/mass spectrometry (HPLC/MS) into routine laboratory operation. Appropriate application of these analytical tools requires that the methods used are verified for the purpose and the instruments are operating correctly.

This edition of C43 was revised to clarify concepts and terminology. Some minor content additions were also made, such as MS/MS and time-of-flight (TOF) mass spectrometry.

**NOTE:** The scope of this document is the use of GC/MS in drug confirmation analyses. By definition, it is assumed that the user is attempting to confirm a screening result obtained using another testing method.

## Key Words

Athletic drug testing, clinical toxicology, drugs of abuse, forensic toxicology, gas chromatography, magnetic sector mass spectrometer, mass spectrometry, quadrupole mass spectrometer, tandem mass spectrometry

# Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline—Second Edition

## 1 Scope

This document is intended to aid the laboratorian in developing appropriate procedures for the use of GC/MS in drug *confirmation* analyses. By definition, it is assumed that the user is attempting to confirm a result obtained using a screening method such as immunoassay. It addresses the instrumental and methodological issues in developing a chromatographic mass spectrometric method, routine performance of the analysis, and continued quality assurance.

Guidance documents exist for laboratories involved in regulatory workplace drug-testing programs and are used in laboratories certified by these programs. Additionally, each laboratory needs to consult its own country's regulatory requirements.<sup>a</sup> However, confirmatory assays are also used in settings outside federal workplace drug testing programs, eg, by laboratories engaged in clinical toxicology, other types of forensic testing, and athletic testing. The present guideline was developed to provide assistance in developing GC/MS confirmation tests that are fit for the analytical purpose in each of these areas.

The chain of custody, although an important part of any test result to be submitted to the judicial system, *is not* discussed here. Guidelines for sample collection and screening testing have been published. Refer to CLSI document C52<sup>1</sup> for recommendations on sample collection and screening testing.

## 2 Introduction

GC/MS is generally accepted as the “gold standard” for identification and quantitation of drug analytes. As such, it is frequently used to confirm presumptive positive drug screening tests performed by immunoassay, thin-layer chromatography, HPLC, or GC. The confidence in the ability of GC/MS to provide unequivocal analytical data is based on recognition of its reproducibility, repeatability, specificity, and trace detection capabilities. Although this confidence is well founded, the measurement and identification of trace levels of compounds in biological matrices such as urine, hair, blood, bile, or organ tissue present a unique problem due to the complex and variable nature of the matrices. Because GC/MS confirmation tests are applied in areas of clinical and forensic science other than workplace drug testing, it seems appropriate to establish broader criteria.

In drug analysis, GC/MS is used either to increase confidence in the identification of an unknown compound or to improve the limits of detection or quantitation through increased analytical specificity. Because of this unique combination of identification and quantitation capabilities, GC/MS methods, particularly *confirmation* methods, require a specific set of criteria for verification of methods and for performance verification in routine analysis.

Two broad uses of drug analysis are performed with GC/MS instrumentation. In the first use, the presence or absence of a drug or drug metabolite is determined using concentration thresholds. These thresholds are based on either scientific criteria or administrative needs. When the threshold concentration, threshold ratio of amounts, or other defined parameter is exceeded, the compound is deemed to be present or to be present in nonphysiological amounts. In these cases, the performance of the method and instrument at the threshold has particular importance. The best-known example of the threshold approach was the development of specific administrative threshold concentrations and criteria for identification of five

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<sup>a</sup>In the United States, the Division of Workplace Programs, Substance Abuse and Mental Health Services Administration of the United States Department of Health and Human Services oversees the best-known drug testing program. The US National Laboratory Certification Program provides guidance for laboratories involved in federal workplace drug testing programs.

classes of drugs of abuse for the federal workplace drug-testing program.<sup>2</sup> In the second situation, the technique is used to detect the presence of drugs or metabolites at any documentable concentration. For these nonthreshold compounds, performance criteria for identification may be more important than the ability to quantify.

GC/MS has gained wider use owing to a combination of improvements in instrumentation and software. Laboratories that would not have used the technique in the past have found the small, bench-top models easier to use, affordable, and thus, more appealing. Similar advances in other MS-based techniques such as GC/MS/MS are taking place and will likely move these methods into routine analysis. Thus, there is a need to define uniform practices not only for routine GC/MS methods but also for the application of the more sophisticated approaches.

### 3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.<sup>3</sup> For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious disease, refer to CLSI document M29.<sup>4</sup>

## 4 Terminology

### 4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI’s consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

In C43, the terms accuracy/trueness, bias, precision, sensitivity, and uncertainty were aligned with that of the global community.

All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.

### 4.2 Definitions

**accuracy (measurement)** – closeness of agreement between a measured quantity value and a true quantity value of a measurand (ISO/IEC Guide 99)<sup>5</sup>; **NOTE 1:** The concept ‘measurement accuracy’ is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error (ISO/IEC Guide 99)<sup>5</sup>; **NOTE 2:** The term *measurement accuracy* should not be used for measurement trueness and the term *measurement precision* should not be used for ‘measurement accuracy,’ which, however, is related to both these concepts (ISO/IEC Guide 99)<sup>5</sup>;

## The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI document HS01—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are

- |                                    |  |   |  |
|------------------------------------|--|---|--|
| Documents and Records Organization | Equipment Purchasing and Inventory Process Control | Information Management Occurrence Management Assessment—External and Internal | Process Improvement Customer Service Facilities and Safety |
|------------------------------------|--|---|--|

C43-A2 addresses the QSEs indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents and Records	Organization	Personnel	Equipment	Purchasing and Inventory	Process Control	Information Management	Occurrence Management	Assessment—External and Internal	Process Improvement	Customer Service	Facilities and Safety
					X C24 C52 EP05 EP06 EP09 EP17						M29

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

### Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

C43-A2 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				Examination			Postexamination	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
C52	C52	C52	C52		C52	C52	C52	C52

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

## Related CLSI Reference Materials\*

- C24-A3**      **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.
- C52-A2**      **Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline—Second Edition (2007).** This guideline addresses drug testing in the clinical laboratory, both for clinical and forensic purposes, and pertains to both drugs of abuse and other drugs normally encountered and analyzed by hospital laboratories. The guideline discusses the preanalytical, analytical, and postanalytical considerations for specimen collection, methods of analysis, quality assurance, and the reporting and interpretation of results.
- EP05-A2**      **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.
- EP06-A**      **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (2003).** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance, and for determining and stating a manufacturer's claim for linear range.
- EP09-A2**      **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002).** This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.
- EP17-A**      **Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004).** This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits.
- M29-A3**      **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

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\*CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.



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