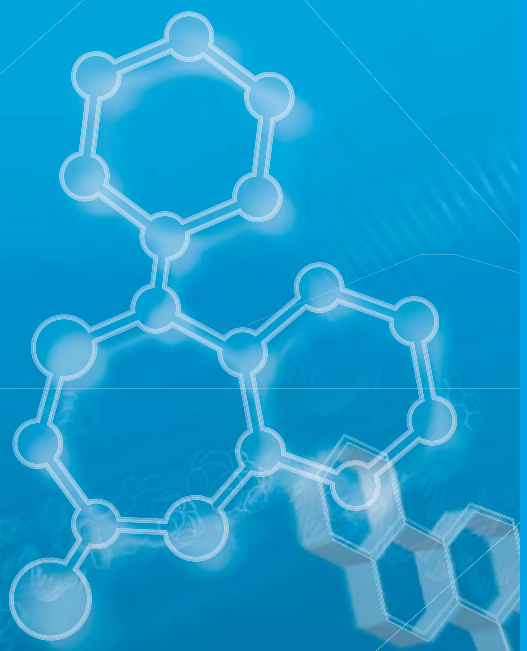
ELEMENTAL IMPURITY ANALYSIS IN REGULATED PHARMACEUTICAL LABORATORIES

A Primer

The Measure of Confidence



Agilent Technologies

Table of Contents

Preface

The United States Food and Drug Administration (FDA) and equivalent international health care agencies require harmful impurities in drug products to be controlled and removed to the extent possible. Pharmacopeias have set binding limits for metal residues in pharmaceutical products and also developed methods for control. For example, since 1905 the United States Pharmacopeia (USP) has defined a procedure established as General Chapter <231>, known as the Heavy Metal Limit Test. However, this current pharmacopeia procedure is based on old methodology that lacks the sensitivity, specificity, and recovery to properly monitor the levels of elements that should be controlled based on high toxicity. This is changing. Regulatory bodies are in the process of developing mandatory guidelines that require control of more elements at lower levels, and new pharmacopeia methods will be based on modern instrumentation rather than on chemistry-based methods.

Analysis in pharmaceutical development and quality control laboratories is highly regulated, with a high impact on the way analyses are conducted. Working in regulated environments has specific requirements for the management and operation of laboratories. For example, equipment needs to be qualified and methods and systems need to be validated. Routine operation must follow written procedures, and each analysis step and result needs to be well-documented. Computer systems and electronic records require special attention to ensure security, availability and integrity of analytical data.

With the change from chemistry-based testing to instrumental analysis, a new group of people will be faced with FDA and other regulations. They include not only instrument manufacturers and suppliers, and technicians operating and maintaining the instruments, but also IT departments, as all suggested instruments are computer-controlled.

This primer 'Elemental Impurity Analysis in Regulated Pharmaceutical Laboratories' gives an overview of regulatory requirements, and guides analysts, laboratory managers, IT administrators, quality assurance managers and validation professionals through the entire process from instrument qualification, through method and system validation, to ensuring compliance of electronic records.

The concept, examples, templates and recommended procedures are based on our more than 20 years' multinational experience and incorporate information from validation and qualification practices applied at Agilent Technologies and Labcompliance. Readers of this primer will learn how to speed up their validation and qualification process, thereby avoiding troublesome reworking and gaining confidence for audits and inspections.

Typically, regulations and quality standards are around for a long time without significant changes. Guidelines developed by regulatory and industry task forces are published more frequently. Interpretations, inspection and enforcement practices experience most frequent changes. What is state-of-the-art today may not be appropriate tomorrow. Therefore, a timely update of all information is important and only possible using online information tools, such as the Internet. To take this fact into account, we recommend these websites, which offer regular updates related to compliance in laboratories:

- www.fda.gov
  Website of the US Food and Drug Administration, detailing regulations and guidelines for the biopharmaceutical industry.
- www.ema.europa.eu
  Website of the European Medicines Agency.
- www.ich.org
  Website of the International Conference for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).
- www.picscheme.org
  Website of the Pharmaceutical Inspection Co-Operation Scheme.
- www.usp.org
  Website of the United States Pharmacopeia.
- www.who.org
  Website of the World Health Organization.
- www.agilent.com/chem/icpms
  Website with updates on ICP-MS.
- www.labcompliance.com
  A website with tutorials, many references and regular updates related to all quality and compliance issues in laboratories.

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1. Introduction

For the purpose of this primer, elemental impurities are defined as elements that are found in the environment or that are used or introduced in the manufacture of drug substances or excipients. They have to be monitored in pharmaceutical laboratories for mainly two reasons:

- Metals known to be toxic have to be controlled during the entire manufacturing process from testing of source material to quality control of finished drugs.
- Metals can affect the stability of a formulation and catalyze degradation of drug substances.

Background

Metal impurities have long been monitored in food and drug articles. The current Heavy Metals Limit Test, method USP <231> is stated in pharmacopeias. The method is more than 100 years old and is based on reaction of the elements with thioacetamide to form sulfides. The intensity of the colored sulfide precipitate is compared with a lead standard. The assumption is that each of the specified elements reacts 100% or in the same way as the lead standard.

In the past decade or so it has been recognized that the performance of the sulfide precipitation method is not adequate for the purpose of controlling the levels of potentially toxic elements, and needs to be replaced by modern instrumental methods.

Inorganic metal impurities in drugs can originate from different sources. For example, from:

- Raw material (plants, animal proteins).
- Excipients (stabilizers, coatings, binders, release agents, flavors, colors).
- Contaminants introduced during the manufacturing process of active pharmaceutical ingredients (APIs) and drug products. For example, by leaching from pipes, vessels and other equipment.
- Metals deliberately added as catalysts.
- Leachable metallic impurities in packaging material.

Some of the metals are also used as active drug ingredients rather than as contaminants to exert a beneficial effect, or they are necessary as minerals or trace elements. For example, the platinum compounds cis-platin, carboplatin and oxaliplatin are widely used in cancer therapy, aluminum is used in antacids, zinc is part of insulin suspensions and iron is used for treatment or prevention of anaemia.

Content of the Primer

After a literature overview, the primer will discuss regulations as they apply during the drug life from research and development to manufacturing. Next, it will provide more detailed information about regulatory requirements for laboratories. Section 3 will guide readers through compliance requirements along the sample and data flow, from sampling to archiving of test reports and other documents. Section 4 will focus on qualification of analytical instruments and validation of analytical methods, software and complete systems. Specifically it will discuss verification of compendial procedures and transfer of analytical methods. Section 5 will provide information on what is necessary to manage electronic records for compliance with various FDA and international regulations.

Sections 6 and 7 will focus on applications of elemental impurity analysis in the pharmaceutical industry. While Section 6 will describe new USP procedures for related impurities in drugs and drug substances, Section 7 will give an overview of other important applications.

Resources

While the scope of this primer is to give an overview on compliance related to elemental impurities, there are many resources available where readers can get more details. They come from regulatory agencies, joint industry/agency task forces and from private authors. Regulatory and other official documents will be discussed in the next section.

There are many publications from private authors that are published as traditional journal papers, online articles and traditional text books. For online articles, readers are referred to well-known search engines. This section gives an overview of some resources published by private authors or organizations:

- Blake [1] reported in the paper "Harmonization on the USP, EP and Japan heavy metals testing procedure" about severe limitations of the traditional colorimetric method.
- Wang *et. al.* [2] recognized in the 1990s the limitations of the traditional colorimetric method for modern analyses, for example sensitivity, selectivity and accuracy. The team identified limitations of the traditional heavy metal procedure compared to ICP-MS for analysis of elemental traces in pharmaceutical material.

- Lewen and co-workers [3] followed up with a detailed assessment of how the traditional procedure compared to ICP-MS for elements as specified in different pharmacopeias.
- Lorenz and co-workers [4] described the importance of knowledge of organometallics (speciation) in directing synthetic purification decisions. The team also demonstrated how the combination of HPLC-ICP-MS can be used for selective and quantitative measurement of organometallics in APIs.
- Lira and co-workers [5] described the use of ICP-OES for the testing of heavy metals in excipients as a replacement for the colorimetric method.
- DeStefano and co-workers [6] explained the toxicological and regulatory basis for the selection of elements and limits of elemental impurities limits specified in USP <232>.
- Fliszar *et. al.* [7] described the analysis of metal leachables from pharmaceutical packaging materials with ICP-OES.
- Li and co-workers [8] and Xia and team [9] used ICP-MS for determination of toxic heavy metals in traditional Chinese medicine.
- Hussain and co-workers [10] gave an example of how to verify performance of an ICP-MS system for USP <232> and <233> compliance, based on the requirements defined in the May/June 2011 revision of USP <232>/<233>.
- Agilent Technologies has published two primers about validation and compliance for pharmaceutical laboratories: Analytical Instrument Qualification and System Validation [11] and Validation of Analytical Methods [12]. They are quite useful to get a good understanding about compliance and validation in laboratories. Agilent has also published a primer on ICP-MS, one of the two USP recommended instruments for elemental impurities in pharmaceutical products [13].
- Huber has authored a validation reference book for the analytical laboratory [14]. It covers all validation aspects of an analytical laboratory including equipment, analytical methods, reference compounds and personnel qualification.
- The Good Automated Manufacturing Practices Forum (GAMP) has developed guidelines for computer validation. The most recent version was released in 2008 [15]. These guides have been specifically developed for computer systems in general, and because of their importance have also been used for validation of computerized laboratory systems.

2. Regulations and Compliance for Elemental Impurities

The pharmaceutical industry is among those subject to the highest level of regulation. Drug development and manufacturing are controlled by government agencies through a set of laws, regulations and guidance documents in all industrialized countries and in an increasing number of developing countries. Most important underlying regulations are the so-called GxP regulations consisting of good laboratory practices (GLP), good clinical practices (GCP) and good manufacturing practices (GMP). In addition there are special regulations for product labeling, the use of computers in a regulated environment and for marketing authorization.

The main purpose of regulations is to ensure quality, safety and efficacy of drugs. For marketing authorization of new drugs, agencies evaluate study data and determine if the benefit of the drug is higher than the risk through insufficient drug safety. Regulations for the pharmaceutical industry in general follow modern quality system principles with a high focus on data accuracy, reliability and integrity.

Qualification of instruments and validation of analytical methods and systems is a requirement of the FDA and equivalent international agencies. No or inadequate qualification can result in regulatory actions, such as shipment stops of drugs and APIs. The rationale behind this assumption is that analytical test results obtained with no or inadequately qualified instruments and validated methods can be wrong. Because of the importance of compliance, this section is dedicated to regulations and regulatory guidance.

The section describes the roles of US FDA and European health agencies, and lists the most important documents. It also describes the tasks and documents produced or controlled by other organizations and task forces with high impact on the pharmaceutical industry, such as PIC/S, ICH and pharmacopeias.

The United States

In the United States, pharmaceutical development and manufacturing is regulated by the Food and Drug Administration (FDA). The goal of FDA activities is to protect public health by assuring the safety, efficacy, and quality of human and veterinary drugs and biological products. Besides drugs the FDA also controls food, tobacco, medical devices and cosmetics. The FDA derives its statutory power from the Federal Food, Drug and Cosmetic (FD&C) Act. The act has its origin in the Pure Food and Drugs Act from 1906, a law that prohibited interstate commerce of adulterated and misbranded food and drugs.

The first version of the FD&C was passed by Congress as early as 1938. This law, for the first time, required companies to prove the safety of new drugs before putting them on the market. It also added the regulation of cosmetics and therapeutic devices, and included general updates to improve consumer protection.

Amendments in1962 required that all drugs be proven effective as well as safe, and gave the FDA the authority to regulate prescription drug advertising. The Medical Device Amendment of 1976 gave the FDA authority to ensure the safety and effectiveness of medical devices, including diagnostic products.

Laws are quite general and usually don't state details of implementation and enforcement. For enforcement of laws, Federal Agencies such as the FDA promulgate rules or regulations. These are published as the Code of Federal Regulation (CFR) in the Federal Register, and inform the public and industry how the laws are implemented specifically.

Typically, regulations are not detailed enough for the industry and FDA inspectors to define the requirements for implementation and enforcement. Therefore, the FDA has developed inspection and industry guidances on a large number of topics. They are available on the Internet for use by FDA staff and the industry (www.fda.gov; search for FDA Guidance). The guidances provide assistance to the regulated industry by clarifying requirements imposed by Congress or issued in regulations by the FDA, and by explaining how industry may comply with these statutory and regulatory requirements. They also provide specific review and enforcement approaches to ensure that the FDA's investigators implement the agency's mandate in an effective, fair and consistent manner. While laws and regulations are mandatory for the industry, guidance documents are not. Industry can decide to use alternatives to comply with regulations.

Important FDA guidances related to elemental impurities in pharmaceutical laboratories are:

- Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production [16]
- Part 11, Electronic Records; Electronic Signatures — Scope and Applications [17].

FDA regulations and guidances are quite static and typically don't change for several years. More dynamic than regulations are inspection and enforcement practices. Information can be found in the FDA's inspection documents, such as warning letters, establishment inspection reports (EIRs) and 483 form inspectional observations. Most critical are the FDA's warning letters. They are sent to companies in case of serious regulatory violations. Companies are expected to respond within 15 business days. If there is no response or if the response is inadequate, the FDA will take further actions that may cause delay of new product approvals, import alerts and denials, or product recalls. Since March 2003, warning letters are reviewed by higher-level FDA officials and reflect FDA's current thinking.

Warning letters are published on the FDA website: www.fda.gov/ICECI/EnforcementActions/WarningLetters. The only problem is that there are thousands of them and they mostly relate to marketing and labeling of drugs, so it is difficult to find the ones that are of interest for laboratories. Interesting are sites that only publish warning letters related to GxP issues. For example, www.fdawarningletter.com has many quotes related to qualification of instruments, validation of methods and validation of analytical systems.

Europe

Drugs in Europe are evaluated for marketing authorization through the European Medicines Agency (previously EMEA, now EMA). It is a decentralized agency of the European Union, with headquarters in London. Their main responsibility is the protection and promotion of public health. The scientific opinions of the EMA for medicines for human use are prepared by the Committee for Medicinal Products for Human Use (CHMP). As in the US, in Europe a drug must have a marketing authorization before it can be distributed. Marketing authorization can be applied for through a:

- Centralized procedure,
- Mutually recognized procedure, or
- National procedure.

The most common procedure is the centralized procedure. The national procedure is mainly used when marketing authorization is only applied for in a single country. When marketing has been approved in a single country, the applicant can nominate this country as a reference state, and using the mutual recognition procedure apply for approval in other countries. Unless there is a safety risk, states for which the mutual recognition is intended will accept the marketing authorization.

All marketing authorizations for biopharmaceuticals have to use the centralized procedure. For this procedure, product evaluation is made by the EMA. Within the EMA, the CHMP performs the actual assessment of the application and issues a scientific opinion. Based on this opinion, the EU decides if it will grant marketing authorization. GMP requirements for drugs, or so-called medicinal products in Europe, are laid down in the EU guide: The Rules Governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practices Medicinal Products for Human and Veterinary Use [18].

The most important document related to elemental impurities is the "EMA Guideline on the Specification Limits for Residues of Metal Catalyst or Metal Reagents" [19]. The document recommends maximum acceptable concentration limits for the control of 14 metal catalysts or metal reagents that may be present in pharmaceutical substances or in drug products.

International Conference for Harmonization (ICH)

The ICH was initiated in 1990 to bring together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industries in the three regions to discuss scientific and technical aspects of product registration.

The ICH publishes guidelines that are either signed into law by member countries, for example in Europe, or recommended as guidelines by national authorities, for example by the US FDA.

The most important ICH documents related to elemental impurities are the:

- **ICH Q3 Series**
  The series provides guidance on acceptance limits for impurities in drug substances. Most interesting are a concept paper and a business plan on Q3D: Impurities — Guideline for Metal Impurities. The guideline will provide a global policy for limiting metal impurities qualitatively and quantitatively in drug products and ingredients [20, 21].
- **Q2(R1), Validation of Analytical Procedures: Definitions and Methodology [22]**
  This guidance is the international standard for setting parameters and procedures for the validation of analytical methods

Pharmaceutical Inspection Convention/ Co-Operation Scheme (PIC/S)

PIC/S is one of the most important organizations in the area of global harmonization of GMP regulations and inspections. Its mission is to lead the international development, implementation and maintenance of harmonized Good Manufacturing Practice (GMP) standards and quality systems of inspectorates in the field of medicinal products. This is to be achieved by developing and promoting harmonized GMP standards and guidance documents; training competent authorities, in particular inspectors; assessing (and re-assessing) inspectorates; and facilitating the co-operation and networking for competent authorities and international organizations. As of January 2012 there are 40 participating authorities in PIC/S, including health agencies from all EU member countries, Australia, Singapore, Canada and the US FDA. Several more organizations have applied for PIC/S membership. Most likely, new member countries that don't have their own GMP regulations will comply with the PIC/S GMPs, which are very similar to the EU GMP Guide. For example, Switzerland, Singapore and Australia have declared PIC/S GMP Guides as their national GMP regulation.

The most important document related to GMP for pharmaceutical laboratories is the guide: Inspection of Pharmaceutical Quality Control Laboratories [23]. The main purpose of the document is to provide guidance for GMP inspectors to assist in training and preparing for inspections, but it is also quite useful for regulated users in preparation for GMP inspections.

Pharmacopeias

Pharmacopeias develop methodology for specific applications and general chapters on different analytical aspects for regulated pharmaceutical industry.

United States Pharmacopeia

According to Section 501 of the Federal Food Drug and Cosmetic Act, USP methodologies constitute legal standards. For marketing authorization, manufacturers should meet USP standards for the drug substance, for excipients and for the drug product, if available. USP has developed several general chapters with impact on elemental impurities analysis:

- **Chapter <1058> on "Analytical Instrument Qualification" [24]**
  This chapter provides a framework for the qualification of analytical instruments. It covers the complete process from writing specifications through installation to initial and on-going testing and maintenance.
- **Chapter <1224> on "Transfer of Analytical Procedures"**
  Describes four different options and elements for controlled method transfer [25].
- **Chapter <1225> on "Validation of Compendial Methods"**
  Defines parameters and tests for validation of compendial (defined) methods. The recommendations are also useful for laboratories developing and validating their own methods [26].
- **Chapter <1226> on "Verification of Compendial Methods"**
  This chapter has been written for laboratories implementing compendial and standard methods. The recommendations are also useful for laboratories implementing validated methods from any other laboratory [27].
- **Chapters <231>, <232>, <233>, <2232> [28–31]**
  These chapters are all related to analysis of elemental impurities. More details can be found in Section 6 of this primer.

The United States Pharmacopeia also develops and provides standards and certified reference materials that can be used as quality control samples in routine analysis and for validating the accuracy of analytical methods.

European Pharmacopeia

The European Pharmacopeia provides several general chapters for the analysis of elemental impurities in pharmaceutical substances using modern instrumentation:

- Chapter 2.2.23: Atomic Absorption Spectrometry (AAS), including flame and graphic furnace AAS (GF-AAS).
- Chapter 2.2.22 Atomic Absorption Spectrometry.
- Chapter 2.2.57 Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).
- Chapter 2.2.58 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Chinese Pharmacopeia

The Chinese Pharmacopeia Appendix IX provides safety levels and several procedures for heavy metals in traditional Chinese Medicines (TCMs):

- Limit tests based on the colorimetric method.
- Quantitative tests based on atomic absorption spectrometry and inductively coupled plasma-mass spectrometry.

FDA's 21 CFR Part 11 and the EU GMP Annex 11 — Regulations for Electronic Records and Signatures

In 1997, the United States Food and Drug Administration (FDA) issued a regulation that provides criteria for acceptance by the FDA of electronic records, electronic signatures and handwritten signatures [32]. With this regulation, entitled Rule 21 CFR Part 11, electronic records can be equivalent to paper records and handwritten signatures. The rule applies to all industry segments regulated by the FDA that includes Good Laboratory Practice (GLP), Good Clinical Practice (GCP) and current Good Manufacturing Practice (cGMP). Similar requirements for Europe have been published in Chapter 4 of the EU GMP with updates in 2011 [33], and in 2011 in the Annex 11 to the EU GMP directives [34]. Section 5 of this primer will be dedicated to requirements of Part 11 and Annex 11.

Development, Registration and Marketing of Medicinal Products

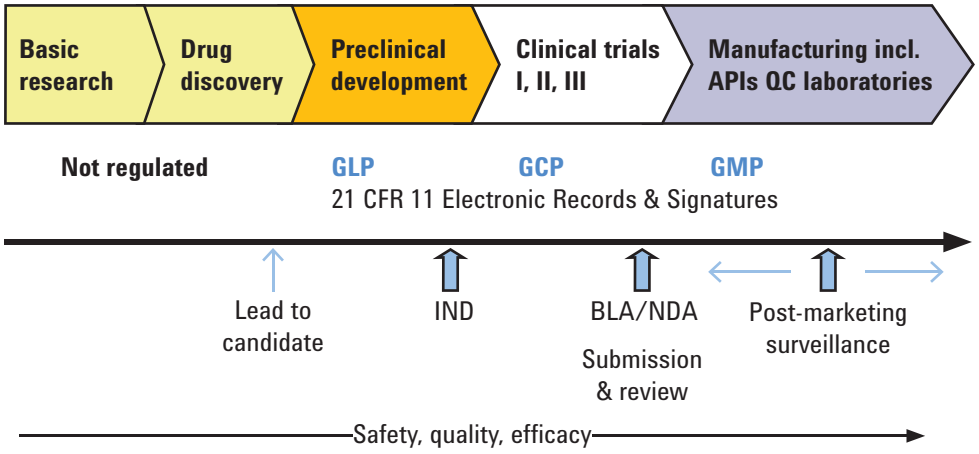
The drug discovery, development and marketing authorization process is a long process that typically takes more than 10 years. The process can be divided into several phases as shown in Figure 1. It starts with basic research and discovery activities, the results of which are then used to define efficacy targets for the potential drug.

Once a target compound has been identified to be a potential drug candidate, it goes through preclinical studies for initial safety tests. They are regulated through Good Laboratory Practice regulations. Clinical trials are regulated through Good Clinical Practice regulations, and the manufacturing process through current Good Manufacturing Practice regulations. Quality control laboratories are also regulated by GMP, as well as the manufacturing process of drug substances or active pharmaceutical ingredients (APIs). At the end of the preclinical studies the drug producer submits an Investigational New Drug (IND) Application, and at the end of the clinical trials, a New Drug Application (NDA) or new Biological License Application (BLA). The applications are reviewed by the FDA to decide if the drug can move to the next phase.

Once the drug has been registered and is available on the market, health agencies regularly control compliance with GMP regulations through testing of products on the market and through inspections of manufacturing establishments. In case of noncompliance, agencies take enforcement actions. Examples are sending the company management a warning letter and shipment stop of products for companies in the US, or an import alert for non-US based companies.

Throughout the development, the principles of the three pillars of GxPs are:

- Safety, to assure the maximum achievable protection against adverse events relative to the benefit obtained by the drug.
- Quality, to assure high technical product excellence.
- Efficacy, to demonstrate the product effectiveness.



GLP = Good Laboratory Practices
GMP = Good Manufacturing Practices
GCP = Good Clinical Practices

GxP = GLP + GCP + GMP = Predicate Rules
IND = Investigational New Drug
BLA = Biologic License Application
NDA = New Drug Application

Figure 1. Regulations along drug development and manufacturing

3. Requirements for Laboratories

It is important that data measured in biopharmaceutical laboratories are reliable and accurate, to ensure that only safe and efficient drugs are authorized for marketing and released for product shipment. Therefore, biopharmaceutical product development and QC laboratories have to follow GxP regulations to demonstrate quality of data.

This section describes the GxP requirements for pharmaceutical laboratories. When reading through the section, scientists and professional analysts may consider many of the requirements to be common sense and that there should be no need for formal compliance. However, in a regulated world it is not enough to understand what should be done, and it is even not enough to implement the requirements. Most important is to document what has been implemented, as an inspector must consider everything not documented as not having been done.

While, in prinicple, the requirements listed apply to all phases of drug development and manufacturing, an incremental approach should be used for implementation along the phases from preclinical studies to finished drug QC laboratories. For example, in clinical Phase I it may be sufficient to create a document that describes why an analytical method is suitable for its intended use. On the other hand, in Phase III the statements must always be supported by experiments. While in GMP environments all requirements listed in this section should be fulfilled, this is not always necessary for earlier phases.

Requirements for laboratories can be divided in two categories:

- **General quality system requirements**
  Apply to all regulated activities within a company. For example, control of documents, internal audits and qualification of personnel. They are typically called management requirements.
- **Laboratory specific requirements**
  Apply to specific situations in a laboratory. For example, validation of analytical methods, sampling, product testing and review, and approval of test reports.

Compliance Along the Sample and Data Workflow

The overall impact of regulations on a pharmaceutical laboratory can be best illustrated by looking at the whole sample/data workflow, as shown in Figure 2. The upper part shows general quality assurance requirements that are applicable to regulated laboratories. The lower part of the figure shows a typical laboratory workflow of samples and test data, together with key requirements. The middle part shows requirements that are applicable to the entire sample or data workflow.

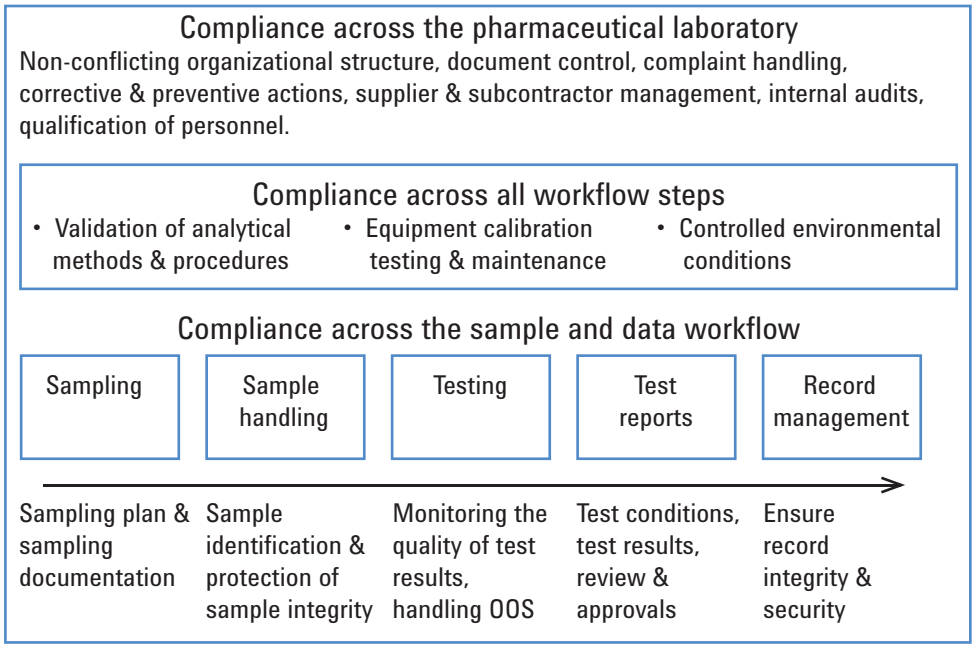


Figure 2. Compliance requirements for pharmaceutical laboratories

Quality Assurance and Compliance Across the Entire Pharmaceutical Laboratory

Pharmaceutical laboratories are expected to follow quality assurance practices that are commonly accepted in regulated industries. They include:

- **Documentation control**
  GxPs require that regulated documents are controlled from creation and approval to distribution, archiving and disposal. Typical documentation includes: policies, quality plans, master plans, standard operating procedures, and records, such as analytical test records and training records.
- **Organizational structure and responsibilities**
  Organizational structures should be regulated so that departments with conflicting interests do not adversely influence quality and compliance of data. For example, finance and the QA department should operate independently from laboratory activities. Tasks and responsibilities should be defined for each job.
- **Qualification of personnel**
  Personnel should be qualified for the assigned task. Qualification can come from education, experience in the job and from formal training. The effectiveness of training should be verified and documented.
- **Facilities and environments**
  The laboratory should have procedures to ensure that its facilities and environmental conditions do not adversely affect or invalidate sample handling, instrumentation, instrument calibration and qualification, and analytical testing.
- **Internal audits**
  Internal audits are a key element of any quality system. Their objective is to evaluate activities and existing documentation, to check whether these meet predetermined internal and/or external standards and/or regulations or customer requirements.

Compliance Across All Workflow Steps

Some required activities are applicable for all workflow steps. They are listed in the middle section of Figure 2. They include:

- **Validation of analytical methods and procedures**
  GxPs require analytical methods and procedures to be validated to demonstrate suitability for their intended use. The ultimate objective of the method validation process is to provide evidence that the method does what it is intended to do, accurately, reliably and reproducibly. Typical method characteristics to be validated are: precision of amounts, reproducibility, specificity, linearity, accuracy, robustness, limit of quantitation and limit of detection. Section 4 of this primer covers these requirements in more detail.
- **Equipment calibration and qualification**
  All equipment that may have an impact on regulated activities should be qualified and/or calibrated. The objective of equipment calibration and qualification is to provide evidence that the equipment is and remains suitable for its intended use. Equipment to be calibrated or qualified includes hardware, software such as Microsoft™ Excel™ spreadsheets, and complete computerized systems consisting of hardware and software.
- **Equipment maintenance**
  Equipment should be well-maintained to ensure proper on-going performance. Procedures should be in place for regular preventive maintenance of hardware to detect and fix problems before they can have a negative impact on analytical data.
- **Controlled environmental conditions**
  Environmental conditions such as temperature and humidity should be controlled and monitored to ensure that they do not adversely affect the performance of equipment and material. Environmental condition specifications are typically provided by suppliers of equipment and material.

Compliance for Individual Workflow Steps

All workflow steps as shown in the lower section of Figure 2 have specific requirements. They include:

- **Sampling**
  Sampling of substances, materials or products for subsequent testing should follow a well-documented procedure. Inspectors want to see a sampling plan with a description of the sampling system, how and when sampling is performed and by whom. Sampling data should be recorded, specifically sampling procedure used, location, the identification of the person who took the sample, equipment used for sampling and environmental conditions, if relevant.
- **Handling of test items**
  Laboratories should ensure proper identification and protection of samples from the time the sample is taken until its disposal. Receipt, protection, storage, processing, retention and disposal should be described in a procedure. The procedure should include provisions for protection against deterioration, loss or damage during transportation, handling, labeling and storage.
- **Testing**
  Procedures for testing should ensure that only validated methods are used, that the equipment is qualified and that sufficient system suitability test runs are conducted. Specifications and acceptance criteria should be defined for the sample to be tested. Procedures and parameters for testing should be documented.
- **Handling out-of-specification test results**
  GMPs require that an investigation is conducted whenever a test result is observed that falls outside the previously specified acceptance criteria. This includes laboratory testing during the manufacture of APIs and raw materials, and testing of finished products to the extent that cGMP regulations apply.
- **Data validation and reporting of results**
  Test results should be signed by the analyst, and reviewed and approved by a second person. A reviewer can be, for example, the analyst's supervisor or a member of QA staff.
- **Record management**
  All records associated with testing should be archived. These records include certificates of analysis (CoA), instrument and method parameters, periodic performance check reports, supporting information such as chromatograms and spectra, and equipment qualification records. The archiving period is defined by individual regulations and can range from 6 to over 15 years. Controls should be in place to ensure security, integrity and availability of the records during the entire archiving period.

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4. Validation of Analytical Equipment, Methods and Systems

GxPs require that analytical instruments and methods used in the generation, measurement, and evaluation of analytical data are suitable for their intended use. This means instruments should be well-designed, qualified, calibrated or checked to ensure compliance with predetermined specifications. It also means that analytical methods and procedures should be validated and systems should be checked for suitability during on-going use. This section presents recommendations for instrument qualification and maintenance including computer system validation, method validation and system testing. Before we go into the details, we will explain how the different activities relate to each other and contribute to the overall quality of data.

Components of Analytical Data Quality

USP Chapter <1058> [24] starts with explaining the four critical components involved in the generation of reliable and consistent high quality analytical data. Figure 3 shows these components as layered activities within a quality triangle, with analytical instrument qualification forming the base for high quality data.

Whether you validate methods or systems, verify the suitability of a system for its intended use or analyze quality control samples, you should always qualify the instrument first. It is the basis of all other components. It is the collection of documented evidence to demonstrate that an instrument performs suitably for its intended purpose and that it is properly maintained and calibrated. If the instrument is not well-qualified, weeks can be spent to validate an analytical method without success until a determination is made that the instrument did not meet required and previously defined performance specifications.

After you have qualified the instrument, you validate analytical methods on qualified instruments. This should prove that the method works as intended. This validation is independent of any specific instrument. If you want to use the method on instruments from different vendors, you should validate the method on each of those instruments.

Then you can combine any specific instrument with a specific method and run system suitability tests. This ensures that the complete system meets the analyst's expectations under the specific conditions of the tests.

The highest level of testing is the analysis of quality control samples. You analyze well-characterized standards or samples with known amounts and compare the analytical results with the correct, certified, or known amounts. Again a prerequisite to generating valid quality control data is to use qualified instruments and validated methods.

USP <233> requires running reference standards as quality control samples before and after sample runs [30]. Only when quality control or system suitability criteria are met, can test samples be analyzed. For more details on USP Chapter <233>, see Section 6 of this primer.

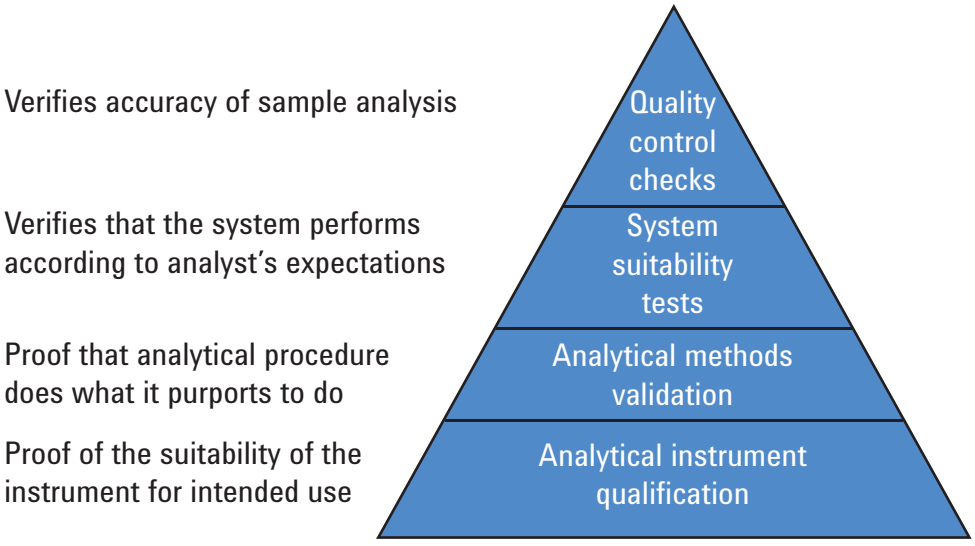

Figure 3. Components of analytical data quality

Equipment

Analytical equipment should be qualified and well-maintained by following written procedures. The standard process for analytical instruments has been defined in the USP Chapter <1058> Analytical Instrument Qualification [24]. This section will give a brief overview on the USP process for qualification and maintenance. More detailed information is available in the primer from Agilent Technologies: Analytical Instrument Qualification and System Validation [11].

Analytical instrument qualification according to USP <1058>

Equipment qualification and validation of computerized systems cover the entire life of a product. It starts when somebody has a need for a specific product, and ends when the equipment is retired. Because of the length of time and complexity, the process has been broken down into shorter phases, so-called life cycle phases. Several life cycle models have been described for qualification and validation. USP chose the 4Q model, which is widely used in pharmaceutical laboratories. The process is illustrated in Figure 4.

The entire qualification process is broken down into four qualification phases: design qualification (DQ), installation qualification (IQ), operational qualification and performance qualification (PQ). The whole process for a specific project is outlined in the qualification plan and the results are summarized in a qualification report.

In the DQ phase the user writes requirement specifications for the equipment. This includes all functions the instrument should have and the performance specifications the equipment should meet as required for the intended application. Next the user compares his/her specifications with the vendor's specification sheet. As long as the vendor's specifications are equal to or better than what the user requires, the design is qualified for the intended use.

Also included in the DQ phase is a formal vendor assessment. This can be made based on experience with the vendor, through a mail audit or through a direct audit.

The instrument selected is purchased and delivered to the laboratory. During the IQ phase the shipment is compared with the purchase order for completeness, and the vendor's installation instructions are executed. This may also include checking if the laboratory conforms to the vendor's environmental specifications, for example humidity and room temperature. Computerized systems are configured according to configuration specifications as defined in the DQ phase. At the end of the installation process, the IQ protocol is completed by recording the vendor, model, serial number and other relevant information.

After the IQ phase has been completed, the instrument is tested against the functional and performance specifications as defined in the requirement specifications document. These OQ tests can be performed by a vendor representative or by the user. In any case, a user representative has to sign the OQ document. The instrument's OQ is repeated at regular intervals and whenever a major change occurs, such as moving an instrument to a different laoratory.

Requalification frequency depends on the instrument itself, the recommendations from equipment manufacturers, laboratory experience and the extent of use. Typically, a complete spectrometry system OQ performance test should be done every 6 to12 months.

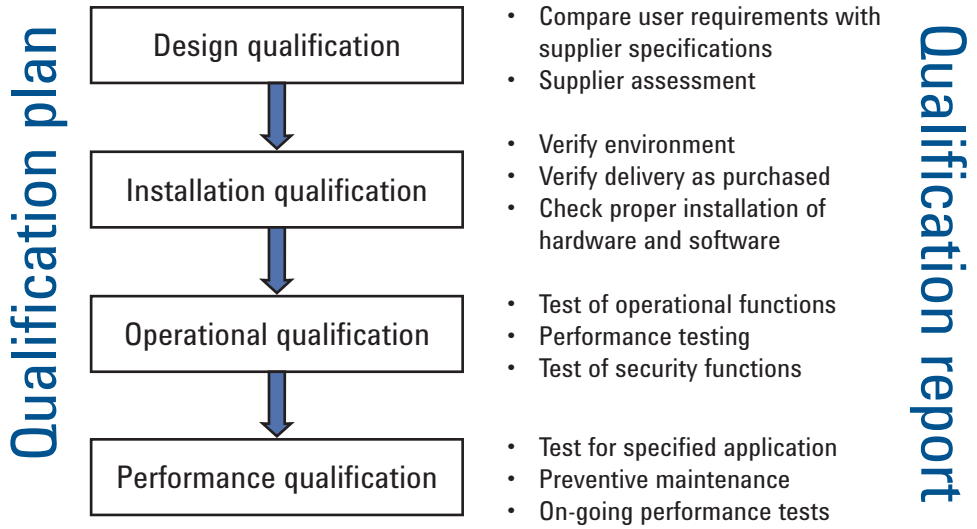

Figure 4. Analytical instrument qualification according to the 4Q model

Also, in between the regular OQ's the instrument's performance should be checked on a day-to-day basis or whenever the instrument is used. This PQ test should be application-specific. According to USP <1058>, system suitability tests and quality control samples can substitute regular PQ tests.

Validation of software and computer systems

Software and computer systems used in GxP regulated environments should be validated. The effort required to validate a computer system can be higher than for instrument hardware. Depending on the system, the costs for software validation and computer system validation may be up to 50% or more of the costs for the software itself, with an increasing trend. The main reason is that software offers more and more functionality, and systems may be configured by the user or on behalf of the user. All software functions with high impact on drug or API quality should be validated. This does not mean correct functionality should always be tested in the user's laboratory, but as a minimum, all functions should be specified in the DQ phase and the need for testing should be evaluated in the OQ phase.

The industry standard for computer system validation is the document from Good Automated Manufacturing Practice: A Risk-Based Approach for Compliant GxP Computerized Systems, Version 5 [15].

Like equipment hardware, computer system validation also follows a life cycle approach. The major differences are:

- More focus should be put on qualification of suppliers. Suppliers should provide documented evidence that the development followed a documented process and that the software has been validated as part of this process (through a Certificate of Software Validation).
- Whereas for hardware equipment qualification all specifications are verified in the user's environment, this is not required for software. It is enough to verify a relatively small set of key software functions and to perform a complete system test. What and how much is tested should be based on a justified and documented risk assessment.
- Many times users customize computer systems, for example through specific access rights to systems, data and functions or when setting network configurations. Users should include these configurations in the requirement specifications document and should verify that the functions work properly.

More information on validation and examples of software and laboratory computer system validation is included in Reference 11.

(Preventive) maintenance

Analytical instruments should be well-maintained to ensure proper on-going performance. Procedures should be in place for regular preventive maintenance of hardware to detect and fix problems before they can have a negative impact on analytical data. The procedure should describe:

- When maintenance should be done.
- How it should be done.
- What should be requalified after maintenance is done. For example, a PQ test should always be performed after instrument maintenance.
- How to document maintenance activities.

Planned maintenance activities should follow a documented instrument maintenance plan. Some vendors offer maintenance contracts with services for preventive maintenance at scheduled time intervals. A set of diagnostic procedures is performed and critical parts are replaced to ensure on-going reliable system uptime.

Unplanned activities that are necessary in addition to the planned activities should be formally requested by the user of the instrument or by the person who is responsible for the instrument. The reason for the requested maintenance should be entered, as well as priority. All maintenance activities should be documented in the instrument's log book.

Handling defective and nonqualified instruments

Defective and nonqualified instruments should be either removed from the laboratory area or when this is inconvenient, for example for large or permanently-installed instrument systems, clearly labeled as being defective or not qualified. Procedures should be available for most common problems. Such procedures should also include information if and what type of requalification is required after the repair.

Equipment records and other documents

Written records should be maintained of all inspection, maintenance, testing, calibrating and/or qualification/ validation operations. These records, containing the date of operation, should describe whether the maintenance operations followed written SOPs.

Written records should be kept of repairs performed on equipment as a result of failure or malfunction. Such records should document the nature of the defect, how and when the defect was discovered and any remedial action taken in response to the defect. Remedial action should include a review of any potential effects on data generated before the defect was discovered. All such records should be entered in an equipment log book. The log book should

be maintained for the same retention period as the analytical data generated by the equipment.

Validation of Analytical Methods and Procedures

GxPs require analytical methods to be validated to demonstrate their suitability for the intended use. All methods used to check the quality, efficacy and safety of drugs should be validated. The ultimate objective of the method validation process is to provide evidence that the method does what it is intended to do, accurately, reliably and reproducibly.

Regulatory agencies and other official organizations have developed several documents on analytical method validation. For example, USP has a general chapter on "Validation of Compendial Methods" [26]. The global reference document for validation of analytical methods is the ICH Q2(R1) guide "Validation of Analytical Procedures" [22].

ICH validation parameters for target applications

The ICH Q2 and USP Chapter <1225> specify validation parameters for each intended application. They have been taken as inputs for the testing of alternatives to the compendial procedures, as described in USP Chapter <233>. For details, see Section 6 of this primer.

ICH Q2 validation parameters are listed in Table 1. Robustness is not included in this list, but ICH expects tests to be done during method development. The FDA and other agencies expect that related robustness tests are included in the method validation package.

Table 1. ICH validation characteristics. (For a detailed description of terminology and methodology, see Reference 12).

Analytical task	Impurity testing			
	Identification	Quantitative	Limit tests	Assay
Accuracy	No	Yes	No	Yes
Precision				
Repeatability	No	Yes	No	Yes
Intermediate precision	No	Yes	No	Yes
Reproducibility	No	Yes	No	No
Specificity	Yes	Yes	Yes	Yes
Limit of detection	No	No	Yes	No
Limit of quantitation	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

The concept of the ICH approach is that it is not always necessary to validate all analytical parameters as listed in Table 1. For example, if the method is to be used for qualitative trace level analysis, there is no need to test and validate the method's limit of quantification or the linearity over the full dynamic range of the equipment. The extent of validation also depends on the life cycle phase of the drug. While agencies expect full validation in clinical Phase III and for drug manufacturing control, most time-consuming tests, such as intermediate precision, reproducibility and ruggedness most likely are not necessary in preclinical studies and for Phase I clinical studies. However, a statement is expected, explaining why the manufacturer believes that the method is suitable for its intended use.

Strategies for method validation

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format.

Just like equipment qualification and computer system validation, method validation also is not a one-off event. It starts when somebody wants to implement a new method in a laboratory and ends when the method is no longer used. Because of the length of time and complexity, the process is broken down into phases. The process is illustrated in Figure 5.

First we develop a validation plan including owners, responsibilities and deliverables. Next the scope of the method is defined. This includes the target compounds with their concentration ranges, the sample matrix, the specific equipment that should be used and the location where the method should be used for sample analysis. Once we know what should be analyzed, performance characteristics, performance tests and acceptance criteria are defined. Then test protocols are developed with all experimental details and tests executed according to the test protocols. Tests results are compared with acceptance criteria. As a last step, procedures are developed to use the method routinely and to verify on-going system performance at the time of analysis. Tests may include system suitability testing and/or the analysis of quality control samples. All experimental conditions and validation results are documented in a validation report.

Verification of compendial methods

Laboratories working in regulated environments are recommended to use official methods, such as those developed and validated by recognized organizations such as the American Society for Testing and Materials (ASTM), or the USP. For example, the US Food, Drug & Cosmetic

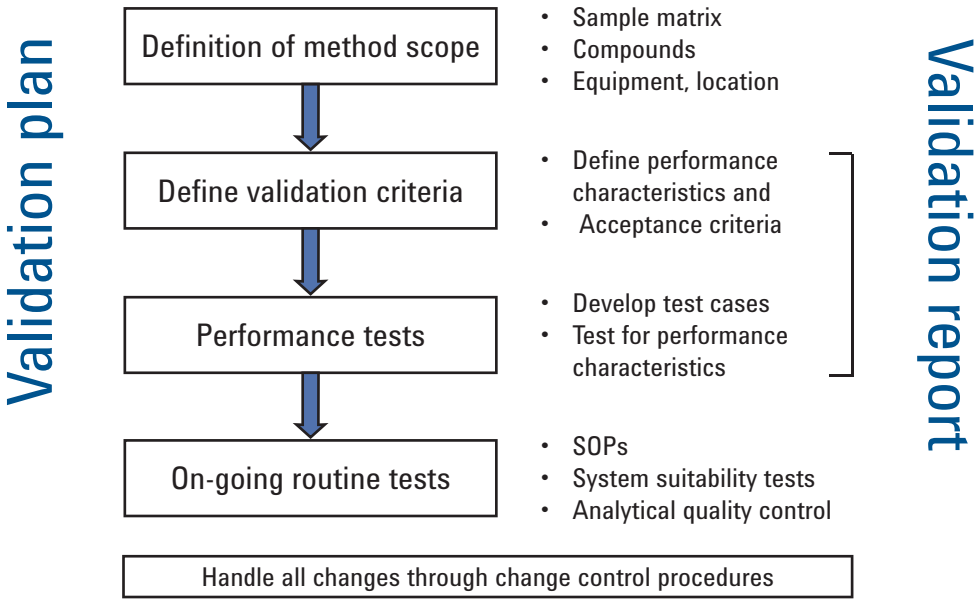

Figure 5. Method validation process

Act requires FDA regulated industries to use compendial methods or demonstrate equivalency. These methods are validated, so many analysts believe that the method can be used as it is without any further validation, verification or testing done in the laboratory. This is a wrong assumption. The FDA GMP regulation states in 21 CFR 211:194 a: "If the method employed is in the current revision of the United States Pharmacopoeia, or in other recognized standard references, or is detailed in an approved new drug application and the referenced method is not modified, a statement indicating the method and reference will suffice. The suitability of all testing methods used shall be verified under actual condition of use".

This makes it clear that official methods do not need to be validated as long as they are unchanged, but the laboratory should demonstrate that it is capable of successfully running the method. This is also the recommendation stated in USP <233>. The chapter recommends that even a new compendial instrument method should be qualified to demonstrate suitability of the method through validation experiments as described for alternative procedures. For on-going routine use, system suitability tests are then sufficient.

What and how much to test should be based on a justified and documented risk assessment. Criteria are described in the USP Chapter <1226>:

- The level of training and experience of the user.
- Associated equipment or instrumentation.
- The specific procedural steps.
- The material to be tested (for example impurity profile).
- The drug substance's synthetic route.
- The effect of the matrix on the recovery of impurities.
- Appropriateness of detector signal response.

Like the validation of methods developed in-house, the evaluation and verification of compendial methods should also follow a documented process, for example a validation plan or an SOP. Results should be documented in the validation protocol.

Transfer of analytical methods

When validated methods are transferred between laboratories, the receiving laboratory should demonstrate that it can successfully run the method. Typical instances of method transfers occur from the Research and Development (R&D) laboratory to Quality Control (QC), from Site A to Site B when a product line is moved from a sponsor company to a contract laboratory, or from Company X to Company Y when a product is purchased by another company. USP has published a general chapter <1224> with the title "Transfer of Analytical Procedures" [25].

The key recommendations are:

- The transferring laboratory defines one or more well-characterized samples, and documents method parameters and acceptance criteria, for example for accuracy of the method. The sample(s) should cover the complete range as specified when the method was originally validated.
- The samples are analyzed in the receiving laboratory and the results are compared with the acceptance criteria.

- Depending on the criticality of the analysis and on the complexity of the method, one or two validation tests should be repeated. For example, limit of quantification for quantitative impurity analysis.

Method transfer should follow a documented process, for example a transfer plan or an SOP.

5. Managing Electronic Records for FDA Part 11 and EU Annex 11 Compliance

Spectrometric instruments for elemental impurity analysis are connected to computer systems with specific application software that controls instrument parameters, acquires signal and spectral data, converts the original digital data into meaningful test results (such as concentrations), and finally prints results and stores and archives instrument and method parameters, original digital data and processed data for the required retention period. The United States and the European Union have regulations for managing computers and electronic records and signatures. The US regulation is the FDA's 21 CFR Part 11 [32] supported by the industry guidance document "Scope and Applications" [17]. The equivalent regulations in Europe are Chapter 4 of the EU GMPs [33], which deals with documentation, and Annex 11 to the EU GMPs [34] with requirements for managing computer systems. 21 CFR Part 11 applies to all computer systems used in an FDA-regulated environment; the cited EU documents apply to systems and environments regulated by European GMPs.

The objective of all these documents is to make sure that electronic records and signatures are as trustworthy and as reliable as paper records and handwritten signatures, and that the use of computer systems does not adversely impact the product quality and quality assurance when compared to manual systems.

Because of the importance of this topic, a section of this primer is dedicated to computer system and electronic record management. It will cover all important requirements as defined in references that are relevant to computerized systems used in pharmaceutical development and QC laboratories.

Regulatory Requirements and Recommendations

For requirements and recommendation, we refer to US FDA Part 11 [32], to the supporting guidance "Scope and Applications" [17] and to EU GMP Annex 11 [34].

Risk assessment

Risk management should be applied according to EU Annex 11 throughout the life cycle of the computerized system, taking into account patient safety, data integrity and product quality. As part of a risk management system, decisions on the extent of validation and data integrity controls should be based on a justified and documented risk assessment of the computerized system.

Recommendations are:

- Determine the risk level of the computerized system: high, medium, or low. Criteria are: impact of the system on data integrity, (medicinal) product quality and patient safety.
- Apply the appropriate type and extent of compliance activities according to the defined category, for example for the extent of validation and the frequency of revalidation, whether electronic audit trail should be implemented or not and the frequency of backup.

System validation

Computer systems used to generate, maintain and archive electronic records should be validated to ensure accuracy and reliability of the records.

Recommendations are:

- Follow the life cycle model for validation as described in Section 4 of this primer.
- Apply the concept of risk-based validation.
- Check Annex 11 Chapter 4 [33] for the type of documentation that should be generated and available during inspections.

Data accuracy

Systems exchanging data electronically with other systems should include appropriate built-in checks for the correct and secure entry and processing of data, in order to minimize the risks. For critical data entered manually, there should be a check on the accuracy of the data. This check may be done by a second operator or by validated electronic means.

Recommendations are:

- Validate the accuracy of data transfer between computer systems. This can be done during operational or performance qualification.
- Use software functionality, if available, to check the plausibility and accuracy of manual data entries.
- If there is no software functionality to validate manual data entries, for high risk data verify the accuracy through a second person.

Limited access to authorized users and authority checks

Procedures and technical controls should be in place to limit access to systems and data to authorized individuals. Suitable methods of preventing unauthorized entry to the system include the use of keys, pass cards, personal codes with passwords, biometrics, and restricted access to computer equipment and data storage areas.

The system should conduct authority checks to ensure that only authorized individuals can use the system, electronically sign a record, alter a record or perform the operation. Systems should be designed to record the unique identity of operators entering, changing, confirming or deleting data including date and time.

Recommendations are:

- Develop policies for generation, distribution, use and maintenance of passwords.
- Develop procedures for limited access to the system to individuals, for example through user ID and password. Each employee should have his/her unique user ID and should select his/her own unique password.
- Develop procedures for limiting access to the system operational functions and data.
- Make sure that the installed software can be used to implement your company's password policies and procedure.
- Configure the system to implement your company's password policy.
- Configure the system to implement your company's procedure for limited access to systems and data.
- Verify correct implementation of password policy and procedures for limiting access to the system and data.
- Establish a list of authorized users.

Raw data and copies of records

Many documents exist in hybrid forms, that is, some elements as electronic and others as paper-based. Regulated users should define which data are to be used as raw data. At least, all data on which quality decisions are based should be defined as raw data. When copies of records are made, the copies should be accurate and complete or should provide the content and meaning of the original record.

Recommendations are:

- For each application, define what raw data are. For example, original electronic records, intermediate processed data or computer printouts.
- For spectrometric systems, define original electronic records as raw data.
- Keep electronic raw data for review and copying by the agency after printing results.
- Contact the agency if there are any questions regarding the ability of the agency to perform this review and copying of electronic records.

Protection of records

Records should be protected to enable their accurate and ready retrieval throughout the required records retention period. Data should be secured by both physical and electronic means against loss or damage. Stored data should be checked for accessibility, readability and accuracy. Access to data should be ensured throughout the retention period. Regular backups of all relevant data should be done. Integrity and accuracy of backup data and the ability to restore the data should be checked during validation, and monitored periodically.

Recommendations are:

- Follow your company's procedure for retention of electronic records.
- Check the required retention period for your records according to requirements as specified in relevant GxP regulations.
- Migrate the electronic records when the systems are upgraded or replaced by new ones. Ask the vendor for validated file conversion routines.
- Regularly check availability and integrity of the electronic records during the entire archiving period.
- Make a backup of electronic records.
- Validate backup and restore procedures.

Computer-generated time-stamped audit trails

Secure, computer-generated, time-stamped audit trails should be used to independently record the date and time of operator entries and actions that create, modify or delete critical electronic records. Record changes should not obscure previously recorded information, that is, the modified record should be saved as a new version of the record. The audit trail documentation should be retained for a period at least as long as that required for the subject electronic records, and should be available for agency review and copying. The audit trail documentation should be regularly reviewed. For records supporting batch release, it should be possible to generate printouts indicating if any of the data has been changed since the original entry.

Recommendations are:

- Include electronic audit trail in the user requirement specification for computerized spectrometric systems.
- Specify the requirements for the audit trail to include: what was changed, who made the change, when the change was made by date and time and as an option, the reason for the change.
- Make sure that audit trail documentation is available in human-readable form.
- Make sure that changed records do not replace or obscure original records.
- Regularly review electronic audit trail documentation.
- Verify correct functioning of the electronic audit trail.
- Retain the audit trail documentation for as long as the required retention period for subject records.
- Use software that can recognize changed records based on printouts. If such software is not available, implement a manual procedure.

Electronic signatures

Part 11 and Annex 11 allow the signing of records electronically. Part 11 has more specific requirements for the execution of electronic signatures. Information associated with the electronic signature should include:

- The printed name of the signer.
- The date and time when the signature was executed.
- The meaning of the signature. For example, review, approval, responsibility and ownership.
- Handwritten and electronic signatures should be permanently linked to their retrospective electronic records.

Recommendations are:

- Decide and document the decision whether to sign records electronically or through handwriting.
- If electronic signatures are to be used, include the required software functions in the system's user requirement specifications document.
- If electronic signatures are to be used, send a letter to the FDA that your company will be using electronic signatures (only applies to US FDA regulated industries).
- Train personnel on the meaning and accountability of electronic signatures.
- Configure the system for use with electronic signatures.
- Validate correct functioning of electronic signatures.

Periodic evaluation

Computerized systems should be periodically evaluated to confirm that they remain in a valid state.

Recommendations are:

- Develop and implement procedures to periodically review computerized systems.
- Set the time frequency to twice per year.
- Include in the evaluation the current range or functionality, deviation records, incidents, problems, upgrade history, reliability, security and validation reports, especially the results of regular performance qualifications.

Requirements for Instrumentation and Supplier Support

Users of computerized systems need help from their suppliers to comply with relevant regulations and guidelines. Requirements are related to instrumentation and support.

Instrumentation

Suppliers should provide instruments and systems that have all the necessary functionality to enable users to comply. User firms should not rely only on a supplier's blank statement that software or systems are Part 11 or FDA compliant or certified. Users should define and document requirements and verify that software conforms to the requirements. However, selecting and installing the system does not automatically mean that the system operates in a compliant manner. Users need to define the procedures that should be implemented, followed and enforced. For example, operators need to be formally trained, and the systems may have to be configured to meet requirements, for example to set up and validate access control to systems, data and tasks.

System requirements include:

- Limiting system access to authorized individuals following the user firm's password policies, for example password expiry period and automated logout after a specified number of unsuccessful logons.
- Authority checks to ensure access privileges to data, applications and tasks according to the user firm's procedures.
- Recording and archiving of raw data and processed data as defined by the user firm.

- Recording and archiving of instrument and method parameters.
- Secure backup of data according to the user firm's backup policies and procedures.
- Link authorized users to records generated by that user.
- Electronic audit trail with information on what was changed, by whom and when (by date and time-stamp).
- Optional audit trail entry for the reason of the change.
- Audit trail tables should not be changeable by the user.
- Audit trail function should log changes to raw data, processed data and method and instrument parameters.
- Previously recorded information should not be obscured.
- Changed records should be recognized based on printouts (for example by revision number).
- Audit trail tables should be reviewable.
- Audit trail tables should be printable.
- Data should be protectable against incidental and accidental deletion.
- Verification of file accuracy during storage and transfer, for example through check-sum routines.
- When electronic signatures are used, the software should, at the time of signing, display the user's printed name and the user should be requested to re-enter his/her user ID and password. In addition, the user should be able to enter a reason for the signature, for example review or approval.
- Handwritten and electronic signatures should be linked to records for which they are intended.

Support

Supplier support requirements include:

- The supplier should provide documented evidence that the system has been developed in accordance with an appropriate quality management system. This includes evidence that the software is developed, delivered and supported by qualified personnel. It also means that the supplier has an efficient revision and version control system in place for software and documentation.
- If requested by the user, the supplier should consent to be audited by the user.
- If requested by the user, the supplier should enter a formal agreement with the user about system delivery and support. The agreement should include a clear statement of the responsibilities.
- The supplier should provide up-to-date system documentation.
- The supplier should alert users in case of software defects and errors with a high impact on data accuracy and integrity.
- The supplier should support the system through phone or onsite support.
- The supplier should provide the user with a list of software or system functions.
- The supplier should provide training on system operation.
- The supplier should provide services for installation.
- The supplier should provide services for installation qualification and operational qualification, and requalification.

6. Elemental Impurity Analysis According to USP <232>/<233>/<2232>

The measurement of elemental impurities, previously called heavy metals, in pharmaceutical materials has a long history. This section describes how new chapters with new methodologies based on modern instruments will replace the traditional procedure. It also describes how the elements and limits have been selected and finally will discuss two compendial procedures, their verification process and validation of alternative procedures

Development of the New USP Chapters for Elemental Impurities

History and limitations of USP Chapter <231>

Elemental impurities have been regulated for many decades in environmental material and food but also in medicinal products. While drug regulations are set and controlled by national healthcare agencies, methodologies for analysis are developed by pharmacopeias. For example, Pharmacopeias in the United States, Europe and Japan have developed chapters for elemental analysis in drugs and drug substances. The elements defined in the current pharmacopeia methods are arsenic (As), cadmium (Cd), copper (Cu), tin (Sn), antimony (Sb), lead (Pb), bismuth (Bi), silver (Ag), mercury (Hg) and molybdenum (Mo). Methodology is similar in all pharmacopeias and is based on precipitation of metal sulfides from a weak acid media. The intensity of the black or brown precipitate formed in the test solution is compared with a 10 ppm lead sulfide reference standard. In the US the method is published as USP General Chapter <231>: Heavy Metals Analysis [28]. The methodology has been in place for over 100 years. Despite significant limitations it has survived for such a long time because of some practical advantages, such as the fact that it does not require extensive investment for instruments and highly trained personnel.

Over the last 20 years the need for new methods became obvious because of some significant disadvantages of the colorimetric method. For example, the method:

- Relies on subjective visual examination of the sample solutions compared to a lead standard solution.
- Can only be used for elements that form colored sulfide precipitates.
- Is not selective, which means it cannot distinguish between elements with high and low toxicity.
- Requires large sample amounts for low detection levels (for example a minimum of 2 g sample is required for a detection limit of 10 ppm).
- Is time-consuming and labor intensive.
- Requires sample preparation for solids with ashing of the sample in a furnace at 600 °C, leading to the loss of volatile elements.
- The loss of volatile elements is element-specific, which also means that the procedure does not provide real quantitative results. For example, mercury in solid samples is not recovered at all.

Development of superior methodology

Already in 1995, Blake [1] noted that loss of metals was a significant problem associated with the current procedure: “Because of the loss of metals during ignition, the validity of test results obtained with the current USP, JP and EP general test procedures is questionable”.

Wang and co-workers made this even more clear in a paper published in 2000 [2]: “Although still widely accepted and used in the pharmaceutical industry, these methods based on the intensity of the color of sulfide precipitation are non-specific, insensitive, time-consuming, labor intensive, and more often than hoped, yield low recoveries or no recoveries at all”. In the same paper the authors presented a method based on ICP-MS that did not have such limitations.

Lewen and co-workers [3] developed and optimized the ICP-MS method for the 10 elements as listed in USP <231> plus palladium, platinum, selenium and ruthenium. They found that the ICP-MS analysis and the associated closed-vessel acid digestion sample preparation method “provides good sensitivity, requires minimal sample size, affords minimal elemental interferences and readily provides a means to perform rapid and automated multi-elemental analyses”. They also noted that detection limits in the sub-ppb level allow the analyst to run less concentrated sample solutions, thereby eliminating the need for large sample sizes and minimizing the potential effects that the sample matrix may have on the result. They used 40 times less sample than the compendial method (0.025 versus 1 g) and used solutions containing only 1 mg/mL of the sample matrix.

Lewen and co-workers also compared the recoveries obtained from the colorimetric test versus the ICP-MS method. They found that with the colorimetric method the recoveries for selenium, tin, antimony, ruthenium and mercury were all significantly less than 10%, with mercury showing no recovery at all because of its high volatility.

Silver was the only element with a recovery of more than 50%.

The publication also noted that recovery rates not only depend on the type of element but also on the sample matrix and because it is so labor intensive also on the operator's work practices. This makes any reliable quantification impossible.

Planning for new chapters

Shortly after the publication of the previously mentioned experiments, USP formed Ad Hoc Advisory Panels on Heavy Metals and Metal Impurities, consisting of staff from USP, FDA and industry. The teams were given the task of proposing a new general chapter with a method that:

- Is based on modern instrumentation (for example inductively coupled plasma or atomic absorption spectroscopy).
- Is sufficiently selective, sensitive, robust and affordable.
- Can handle realistic toxicological limits, and report results for individual metals, not the sum of all test metals.
- Is applicable for drugs, drug substances, food and dietary supplements.
- Is affordable.

In addition the team was asked to work with other pharmacopeias and ICH, with the objective that the method has the potential to get global acceptance.

The Metal Impurity Advisory Panel made the following recommendations:

- Always test for the toxic elements arsenic, cadmium, lead and mercury in APIs and excipients.
- In addition include 11 elements as used as catalysts and listed in EMA Metal Catalysts Guidance, with the exception of iron and zinc.
- Establish multiple options for limit calculation following the model of USP <467> for Volatile Organic Solvents.
- Initially develop three new general chapters and update USP General Notices with implementation strategy.
- Coincide the effective date with the one for the EMA Metal Catalyst Guide.

After several preliminary publications and conference meetings the first proposal for new chapters came in the January 2010 issue of Pharmacopeial Forum (PF) and was updated with minor changes in May 2011 and again in a Second Supplement published in June 2012 [29, 30], which contains further revisions expected to be close to the final methods. The USP invited comments on the draft chapters from experts in industry and academia. USP also made an effort to involve the FDA in the revision process, to avoid any disagreement on interpretation between the FDA and industry.

According to the panel's recommendation, USP plans to document the transition from <231> to the new chapters in the Pharmacopeia's General Notices section. Currently there are about 1000 USP monographs that require elemental impurity analysis according to USP <231>. After the proposed implementation date those monographs will be required to follow the new chapters. Rather than change each individual monograph, USP will have a statement in the General Notice section that from April 2014, <231> methodology will have to be replaced by <233>, and unless otherwise specified in the monograph the elements and limits of <232> will apply.

Chapter overview

The panel proposed to substitute Chapter <231> with initially three chapters, <232>, <233> and <2232> with a potential fourth chapter a few years later. The chapters are supported by already existing chapters <730> on Plasma Spectrochemistry, <1058> on Analytical instrument Qualification, and <1225> and <1226> on analytical method validation (see Figure 6).

Chapter <232> defines the elements and the limits for the amounts of elemental impurities in drug products.

Chapter <233> describes instrumentation, sample preparation and analytical methods for two compendial test procedures and their verification in user laboratories. It also describes validation procedures for alternative methods and tests and acceptance criteria for system suitability testing.

The proposed Chapter <2232> is planned for the analysis of elemental contaminations in dietary supplements. This chapter has been updated in May 2012 [31].

Selection of Elements and Limits

The elements and limits defined in USP <232> have been selected based on toxicity rather than on method capability. Only six of the 10 metals required by <231> are included: Cd, Pb, As, Hg, Cu and Mo. In addition the six platinum group elements (PGEs), typically used as catalysts (Ru, Rh, Pd, Os, Ir, Pt), and three additional elements (Cr, Ni and V) have been added to the list. DeStefano and Zaidi [6] described in much detail the toxicological and regulatory basis for the elemental impurity limits. For the selection, USP took inputs from the rationale for reference doses (RfDs) published by the US Environmental Protection

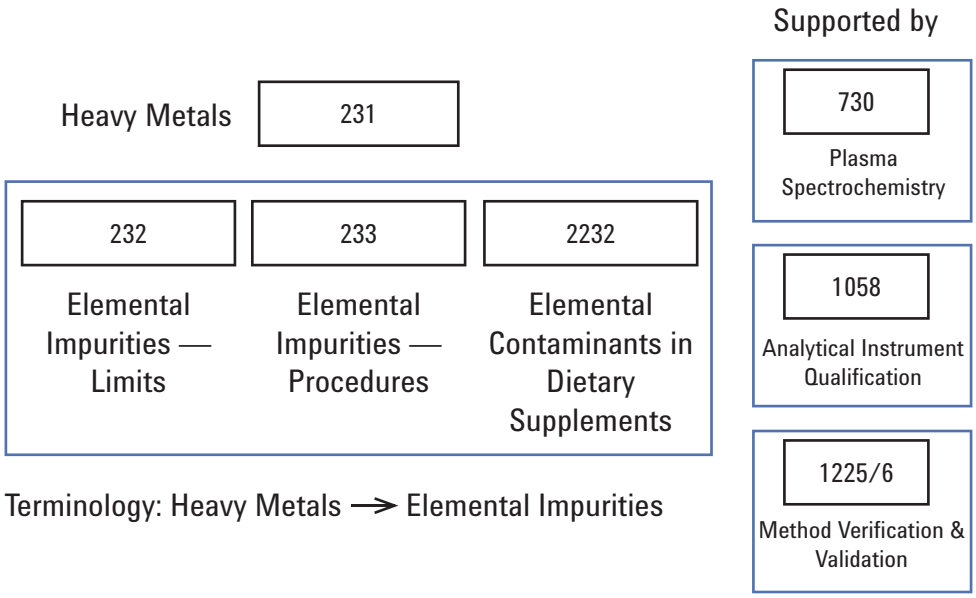

Figure 6. Overview of USP chapters for elemental impurity analysis

Agency (EPA) [35] as well as from the EMA Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents [19].

Main selection criteria have been:

- Human toxicity associated with exposure to the metal.
- Likelihood of presence of the metal in the article to be tested.
- Other sources of exposure to the metal.
- Additive toxicity from different metals.
- Special populations at increased risk for toxicity.

Analogous to USP Chapter <467> and ICH Q3C (R4) on limits of residual metals, the so-called PDE (permissable daily exposure) is used for the calculation of the concentration limits defined in <232>. This is also in line with EMA's Residues of Metal Catalysts guidance. The PDE is defined as "the pharmaceutically maximum acceptable exposure to a metal on a chronic basis that is unlikely to produce any adverse health effect".

The PDE limits are consistent with initial deliberations of the ICH Q3D expert group [20]. If the recommendation from ICH related to selection and limits of elements change for the final Q3D guide, USP will manage the changes through chapter updates via the existing USP revision process.

Table 2 shows the permitted daily exposure (PDE) limits for 15 elements as defined in USP <232> [29] from June 2012. The previous Group I and Group II analytes from the first draft were combined into a single table in the May 2011 revision, but the more toxic elements As, Cd, Hg and Pb, sometimes referred to as the 'Big Four' are controlled at much lower levels than the other analytes, and must be measured in all samples. The PDE limit for As has been lowered again (to 1.5 µg/day) in the June 2012 revision, reflecting the level of concern over these four toxic and ubiquitous elements. The need for measurement of the other 11 elements (Mn was included up to the May 2011 revision, but has been removed in the latest draft) should be based on a justified and documented risk assessment. The criterion is the likelihood that the impurities are present based on the manufacturing process (for example if the element was used as a process catalyst), and inputs from suppliers of drug components.

When drugs are administered parenterally and the total volume exceeds 100 mL/day, a limit is set on the absolute level that may occur in any component of the solution. This is referred to as the large volume parenteral limit (LVP). Limits are also shown in Table 2.

The toxicity of an elemental impurity is related to the extent to which a patient is exposed to it, or the pharmaceutical dosage form. USP <232> has defined three different limits based on routes of administration, or in other words, how the drug enters the patient's body. Exposure factors are different for drugs administered parenterally, for example injectables and implants, and by inhalational exposure, for example aerosols, inhalers and gases. All other routes of exposure, including solid and liquid oral administration, mucosal and topical, have the same (higher) limit. The reason is that absorption by the body is expected to be higher for injectables/inhalables than for oral solid and liquid administration. This means that the limit is lower for parenteral and inhalational administration than it is for oral administration.

Table 2. Limits of elemental impurities in drug products for oral administration (parenteral and inhalational drug limits are typically much lower; see Reference 29) and for large volume parenterals. PDE = Permissible daily exposure on a 50 kg person. LVP = Large volume parenteral (daily dose greater than 100 mL). * Cr is not considered a safety concern for pharmaceutical products and the daily dose limit of 25 µg/day applies only to drugs intended for inhalational administration.

Element	Oral dose PDE (µg/day)	LVP component limit (µg/g)
Cadmium	25	0.25
Lead	5	0.5
Inorganic arsenic	1.5	0.15
Inorganic mercury	15	0.15
Iridium	100	1.0
Osmium	100	1.0
Palladium	100	1.0
Platinum	100	1.0
Rhodium	100	1.0
Ruthenium	100	1.0
Chromium	- *	- *
Molybdenum	100	1.0
Nickel	500	5.0
Vanadium	100	1.0
Copper	1000	25

The new Chapter <232> lists limits for drug components for different routes of administration (see Table 3). These limits are not legally binding but recommended default values for use with discussions between suppliers of materials and drug manufacturers. As for drug products, the recommended limits are listed for three different routes of administration: oral (and mucosal or topical); parenteral; and inhalational.

USP <232> describes different options to evaluate whether test results are above or below the limit: the drug product option, the summation option and the LVP option for a daily dose of more than 100 mL parenteral drugs.

The drug product option measures the amounts of each element in the drug product and compares results scaled to maximum daily dose with the modified daily dose PDE as shown in Table 2. The measured amount of each elemental impurity scaled to the daily dose must not be more than the specified PDE.

The summation option measures impurities in drug components (raw materials, excipients and APIs) and calculates the elemental impurities in drug products based on amount of impurities in drug components and percentage of each component in the drug product.

Table 3. Recommended default limits for drug components. Default concentration limits for drug substances and excipients used for drug products with maximum daily dose of ≤ 10 g/day. * Cr is not considered a safety concern for pharmaceutical products and the daily dose limit of 25 µg/day applies only to drugs intended for inhalational administration.

Element	For oral drug products (µg/g)	For parenteral drug products (µg/g)	For inhalational drug products (µg/g)
Cadmium	2.5	0.25	0.15
Lead	0.5	0.5	0.5
Inorganic arsenic	0.15	0.15	0.15
Inorganic mercury	1.5	0.15	0.15
Iridium	10	1.0	0.15
Osmium	10	1.0	0.15
Palladium	10	1.0	0.15
Platinum	10	1.0	0.15
Rhodium	10	1.0	0.15
Ruthenium	100	10	1.5
Chromium	- *	- *	2.5
Molybdenum	10	1.0	25
Nickel	50	5.0	0.15
Vanadium	100	10	30
Copper	100	10	7

For orally administrated drugs and parenterals/inhalationals with a daily dose of <10 mL either the drug product or summation option can be used. For parenteral drugs with daily dose between 10 and 100 mL, only the summation option can be used.

For LVPs the amount must be controlled through the individual components used to produce the product. The amounts of elemental impurities present in each component must be less than the LVP component limit in Table 2.

For the summation option as well as for the LVP option the pharmaceutical manufacturer should verify that no elements are introduced to drugs during the manufacturing process.

Instrumentation and Procedures

Chapter <233> describes instrumentation, sample preparation and analytical methods for two compendial test procedures and their verification in user laboratories. It also defines target elements that have to be tested as elements with the potential of being present in the material under test. Target elements must include lead, mercury, arsenic and cadmium in all samples. PGEs that may have been added during the manufacturing of APIs or drug products

should also be tested. USP Chapter <233> also describes the criteria and procedures for validation of noncompendial procedures as an alternative to the proposed compendial procedures.

USP <233> does not require the determination of the oxidation state, organic complex or other chemical form of each element, which is termed speciation. However, knowledge of the chemical form of the element may be important because toxicity may be dependent on the form of the element. Arsenic and mercury are of particular interest because of significantly different toxicities of their inorganic and complexed organic forms. The arsenic limits defined in <232> are based on the inorganic form, which is the most toxic. Arsenic can be measured conventionally as total-arsenic under the assumption that if the total As is below the regulatory limit, the material must contain less than the limit for inorganic As, even if all the As in the material is present in the inorganic forms (arsenite (As(III)) and arsenate (As(V)). If the total amount of As exceeds the specified PDE, speciation analysis is required to quantify the different forms.

Similarly, the mercury limits are based upon the inorganic Hg(II) oxidation state. The methylmercury form, which is more toxic, is rarely an issue for pharmaceutical products, although until recently Hg (as Thimerosal or Thiomersal) was added to many vaccines as an anti-bacterial agent. A test for methylmercury would only be required for material that has the potential to contain methylmercury, for example material derived from fish, if the total mercury exceeds the PDE limit. For speciation procedures the chapter refers to USP monographs and literature. For example, Lorentz and co-workers [4] described a method based on HPLC-ICP-MS for speciation and quantification of individual metallic and organometallic compounds in APIs.

Instrumentation

The USP Metal Impurity Panel looked at different instruments with the potential to meet all requests as discussed before. Instruments included:

- Flame atomic absorption spectrophotometry (FAAS)
- Graphite-furnace atomic absorption spectrophotometry (GFAAS)
- Inductively coupled plasma-atomic emission spectroscopy (optical emission spectroscopy, ICP-OES)
- Inductively coupled plasma-mass spectrometry (ICP-MS)
- Atomic fluorescence spectrometry, and
- X-ray fluorescence spectrometry.

The instruments vary widely in features and performance but also in cost, both initial investment and on-going costs. Generally the more costly instruments are also better in functionality and performance.

Desirable features and performance criteria include:

- High number of elements that can be analyzed.
- Low level of interference or overlap/contribution from other elements (specificity).
- Little time required for multiple element analysis.
- High automation capability.
- Wide linearity and dynamic range.
- High tolerance of different matrices.
- Low sample volume required to conform to USP <232> requirements.
- Ability to conveniently interface with other techniques, for example HPLC for speciation.

ICP-OES and ICP-MS are the only instruments that can determine all of the specified elements in a single multi-element measurement. ICP-MS clearly has the best performance in terms of sensitivity and linearity.

After looking at advantages and disadvantages of the different instruments, the USP panel proposed ICP-OES and ICP-MS as preferred instruments. USP <233> uses both instrument categories for compendial procedures, together with a microwave assisted digestion in closed vessel for sample preparation. Alternative methods can be used as limit procedures and as quantitative procedures, as long as they fully meet the criteria outlined in <232>. This has to be demonstrated through extensive validation experiments as described in <233>.

Whenever budget can be made available it is recommended to go for one of the compendial methods: ICP-MS or ICP-OES. Besides superior features and comparable or better performance in all categories, one should not underestimate the fact that these are the compendial methods. When samples have to be sent to the FDA for random confirmation analysis, FDA will use compendial procedures with the expectation that the results are identical to the results from the drug manufacturer. In case of any deviation, generally the compendial procedure is considered to be correct.

Other arguments that justify instrumentation with fast multi-elemental analysis are:

- There is an increasing trend to acquire APIs and excipients from multiple suppliers in different parts of the world, with little and sometimes unreliable information from suppliers. However, when it is unknown if and which impurities are present, a

broader screen is required, not least because USP <233> includes the requirement that manufacturers demonstrate "unequivocal" identification of the analytes listed, when measured in the presence of other elements and matrix components. In order for a producer to comply with this requirement, it is apparent that they should possess some means of characterizing all of the elemental content of a given sample, using a technique such as screening analysis by ICP-MS, in order to eliminate the possibility of unidentified and unexpected interferences.

- Different suppliers may use different manufacturing processes generating different and unknown impurity profiles.

The most obvious difference between ICP-OES and ICP-MS is that ICP-MS has much greater linear range and much lower limits of detection and quantitation.

While the required PDE limits defined in USP <232> can be measured by direct analysis using either of the instrumental techniques referenced in USP <233> (ICP-OES or ICP-MS), many novel drugs are based on increasingly sophisticated and costly APIs, which may only be available in very small amounts. The large dilution associated with the preparation of these mg-scale sample weights means that instrumentation with the lowest possible detection limits may be essential. Low limits of detection and linear calibrations over a wide dynamic range (9 orders in the case of the Agilent 7700 Series) are highly valued characteristics of ICP-MS. Low limits of detection are particularly important for some of the potentially toxic trace elements that must be controlled at the lowest levels according to USP <232>, notably As, Cd, Hg and Pb.

One should also take into account that the PDE limits defined in USP <232> (Table 2) must be adjusted depending on the type of pharmaceutical product and the route of administration. For example, drug products delivered by parenteral administration must meet a modified PDE that is typically 10 times lower than the limit for oral administration, while large volume parenteral (LVP) medicines (daily dose greater than 100 mL) must meet limits typically 100 times lower than the oral dose PDE.

Furthermore it should be considered that PDE limits must be corrected for the dilution factor applied during sample preparation. For example, the individual component limit for Cd is 2.5 µg/g (ppm) for solid drug products and excipients for oral administration. A dilution factor of 250 times during sample digestion (for example 0.2 g digested and diluted to a final volume of 50 mL) would give a PDE limit in the sample digest of 10 ng/mL (ppb) for Cd. Accurate recovery must be demonstrated at half the target limit — 0.5J (5 ng/mL), suggesting a required detection limit at least 10 times lower than this (0.5 ng/mL), a concentration easily measured using ICP-MS, as shown in Table 6. Component limits for drug products and excipients that would be delivered by parenteral or inhalational administration are at least 10 times lower than these values, suggesting a required DL of less than 0.05 ng/mL in the digested sample, still easily within the range of ICP-MS.

Sample preparation

A wide range of possible samples may be analyzed using USP <232>/<233>, so it is not practical for the method to provide a detailed sample preparation approach that would be suitable for all sample types. Some pharmaceutical samples can be analyzed directly (unsolvated), while others can be prepared using simple dilution or solubilization in an aqueous solvent (such as water or dilute acid) or a suitable organic solvent (such as 2-butoxyethanol:water, DMSO or DGME). Methods that utilize a simple dilution or solubilization in an aqueous or organic solvent must take account of chemical stability and, in the case of organic solvents, variable volatility of the compounds present in the sample. For many APIs, dilution in an organic solvent is the preferred approach, in which case it may be necessary to include some means of stabilizing the analytes to avoid variable recovery due to the presence of more or less volatile species compared to the calibration standard.

Many raw materials, excipients, intermediates, APIs and final products will be insoluble in any of the commonly-used aqueous or organic solvents, and so will require acid digestion. USP <233> specifies the use of "strong acids" for digestion of such insoluble samples, although it is left to the individual laboratory to develop and validate the acid composition and digestion method that gives acceptable recovery and sample stability for their samples. Nevertheless, there are some general points that will apply to most sample types that require digestion.

The list of elements in USP <232> includes Hg and the PGEs. These elements are chemically unstable at low concentrations in an oxidizing matrix such as nitric acid (HNO_3) or nitric/peroxide (HNO_3/H_2O_2), and can only be stabilized and measured reliably over an extended period if the digest solution includes a complexing agent such as HCl. While USP <233> does not specifically state that samples for analysis by ICP-MS must include an appropriate stabilizer when Hg is to be measured, this element is known to require the additional of a complexing agent such as HCl to ensure chemical stability (Hg is a required analyte in all samples measured under the revised general chapters).

Pharmaceutical products may be a complex combination of the API, plus fillers, binders, colorings and coatings. These coatings may be organic polymers that are formulated to resist acid attack in the stomach, and thereby control the point at which the drug substance is released in the

small intestine. Given the range of sample types and their variable and complex matrices, it is likely that microwave digestion will typically be employed in order to ensure complete digestion of pharmaceutical samples, and closed vessel microwave digestion is the preferred digestion technique referred to in USP <233> for solid samples. Closed vessel digestion also eliminates any issues of loss of volatile elements such as Hg, which is a problem with USP <231> as already discussed.

An example of a closed-vessel microwave digestion approach is shown in Table 4.

Table 4. Example of closed-vessel microwave digestion method for pharmaceutical samples

Digestion conditions	
Microwave oven make and model	Milestone Ethos
Rotor type	High pressure, quartz inserts
Rotor capacity	10 vials of ~20 mL sample volume
Digestion	
Sample weight	0.2 g
HNO_3	1 mL
HCl	0.25 mL
H_2O_2	0.5 mL
De-ionized water	3.5 mL
Oven program	
Pre-digestion (room temperature)	15 min
Ramp (to 1200 W, 150 °C)	15 min
Hold (at 1200 W, 150 °C)	10 min
Cool down	15 min
Final dilution	
De-ionized water	To 50 mL
Total dilution factor	250x

Compendial (Instrumental) Procedures

Chapter <233> describes two referred procedures for quantitative analyses that are suitable for simultaneous quantification of elemental impurities. Procedure 1 is based on inductively coupled plasma-optical emission spectroscopy (ICP-OES), and Procedure 2 on inductively coupled plasma-mass spectrometry (ICP-MS).

The chapter includes step-by-step instructions for the execution of the method. In preparation for and during sample analysis, general principles of GxP and quality assurance as outlined in Section 3 of this primer should be applied. Specifically:

- Before an instrument is used the first time for Procedure 1 or 2, it should be qualified to demonstrate that the instrument conforms to the specifications as required and documented for the user's applications. We recommend following the process as defined by USP <1058> and described in Section 4 of this primer. This also would include validation of the computer system.
- Before an instrument is used the first time for a specific sample, the appropriateness of the procedure for the instrument should be verified by meeting the Alternative Procedure validation requirements as defined in Chapter <233>.
- Make sure that the materials such as reagents used for the preparation of the standardization solution have adequate quality. For example, solutions should be free of elements in accordance with USP Chapter <730>.
- Make sure and document that the (certified) reference material used for the standard solution has adequate quality.
- Make sure that operators are trained on the instrument and procedure, and that the training is documented.
- Set up the instrument parameters according to manufacturer's recommendations.

Recommended steps for method execution are the same for both procedures.

1. Prepare standard solution 1: 2J of Target Elements in Matched Matrix. The acid concentration should be similar to that of the sample solution.

(J is the concentration (w/w) of the elements of interest at the target limit, appropriately diluted to the working range of the instrument.)

2. Prepare standard solution 2: 0.5J of Target Elements in Matched Matrix.

3. Prepare sample stock solution (for mercury we suggest to add a stabilizer such as HCl, as discussed above).

4. Prepare sample solution through dilution to a final concentration of not more than (NMT) 2J.

5. Prepare Blank (Matched Matrix).

6. Set up ICP-MS or ICP-OES according to manufacturer recommendations.

7. Run Standard solutions 1, 2 and Blank for calibration.

8. Run the appropriately diluted sample solution and evaluate the quantitative amounts.

9. Run Standard 1 again to measure drift.

System suitability results are calculated from results in Steps 7 to 9.

Suitability requirements

10. Drift: Compare results from standard solution 1 (2J) before and after the analysis of the sample solutions.

11. Suitability criteria: deviation NMT 20% of each target.

Performance verification for ICP-MS compendial method

Examples of the data sets generated during the performance verification of an ICP-MS for quantitative procedures are described in the following section (based on the May/June 2011 revision of USP <232>/<233>), although the performance verification requirements for limit procedures follow essentially the same process and limits. The performance verification data based on the May/June 2011 revision of USP <232>/<233> is presented in full in Reference 10.

The instrument used was an Agilent 7700x ICP-MS, and the operating conditions for this instrument are shown in Table 5.

Table 5. ICP-MS operating conditions used for pharmaceutical samples

ICP-MS operating conditions	
Instrument	Agilent 7700x
Plasma mode	Normal, robust
RF forward power (W)	1550
Sampling depth (mm)	8
Carrier gas flow rate (L/min)	0.95
Dilution gas flow rate (L/min)	0.15
Spray chamber temperature (°C)	2
Extraction lens 1 (V)	0
Kinetic energy discrimination (V)	4
He cell gas flow rate (mL/min)	4

The list of analytes regulated under the new draft General Chapters USP <232>/<233> includes several elements that are not chemically stable at low concentrations in the HNO_3–based sample preparation that has traditionally been recommended for ICP-MS analysis. Alternative acids (HCl and H_2SO_4) that might have been used to stabilize HNO_3-insoluble elements have been avoided in ICP-MS, due to their contribution to Cl- and S-based background interferences in the spectrum.

However, modern ICP-MS instruments such as the 7700x are equipped with collision/reaction cell (CRC) technology that can reduce such interferences to negligible levels, so 0.5% HCl is now used routinely to prepare samples for ICP-MS analysis. The addition of HCl ensures that most elements remain stable for extended periods. In the case of the elements defined in USP <232>, the most critical of these elements is mercury (Hg), but all the platinum group elements (PGEs) also require HCl to ensure chemical stability at low concentrations. The use of helium (He) cell gas mode allows for very simple method development, as the same instrument operating conditions can be used for all analytes.

The calibrations in Figure 7 show As, Cd, Hg, Pb, Pd and Pt in the 2% HNO_3/0.5% HCl sample preparation solution discussed above, measured in He mode on the 7700x. These calibrations demonstrate limits of detection of 1ng/L or below, and good sensitivity and linearity for elements (Hg, Pd and Pt) that require stabilization in HCl.

The detection limit performance of ICP-MS is further illustrated in the method performance figures of merit for the Agilent 7700x shown in Table 6. The method detection limits shown in this table were calculated from 3σ of replicate unspiked digested GelCap samples (n = 10 external replicates). 1J actual control limit values are based on a 250x dilution (for example 0.2 g in 50 mL). Note that the data presented in Table 6 includes figures for more than one isotope of most elements; the primary (or preferred) isotope, and a secondary (or qualifier) isotope. The qualifier ion approach is common in organic mass spectrometry, and is a useful way for ICP-MS analysts to address the USP <233> requirement for "unequivocal" identification of the target analytes. Further details can be found in Reference 10.

USP <233> defines a "system suitability check" that requires the selected analytical method to demonstrate drift of not more than 20% for a 2J standard measured before and after the batch of samples. Table 7 illustrates that the 7700x stability was significantly better than the required performance, at around 2 or 3% drift for most analytes.

Accurate spike recovery (within 70% and 150% of the true value) must be demonstrated at several spike levels between 0.5x and 1.5x the Target Concentration (between 0.5J and 1.5J). Table 8 illustrates both the accurate spike recovery obtained on the 7700x (easily within the 70% to 150% limit) and good precision in the low single % range, easily within the required acceptance criteria of not more than 20%RSD at spike levels of 0.5J and 1.5J.

Quantitative procedures must also demonstrate acceptable performance for a "ruggedness" test (a test that is not required for limit procedures). This necessitates running the repeatability test either on different days, using different instruments or performed by different analysts.

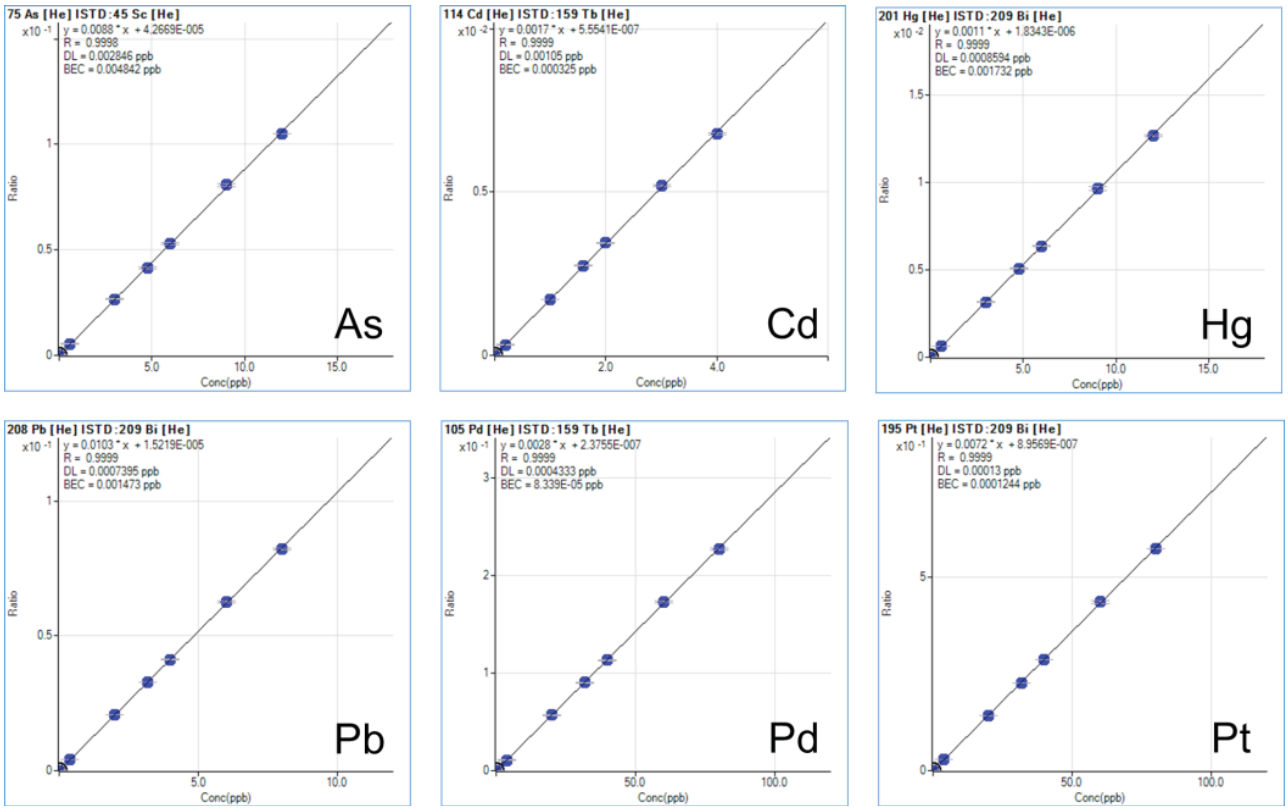

Figure 7. Example ICP-MS calibrations for analytes regulated by USP <232>/<233>

The data obtained using the Agilent 7700x ICP-MS (for GelCap samples spiked at 0.5J (worst case), presented in full in Reference 10) was again easily within the required performance of not more than 25% difference between the two sets of samples, with most elements varying by around 5% or less, and all less than 10% difference.

Overall, ICP-MS system performance verification delivered data that was easily within the method requirements for accuracy, stability and spike recovery, and detection limits were all several orders of magnitude lower than the levels at which the trace elements are controlled. This provides the reassurance that the 7700x will be able to meet the regulatory requirements for pharmaceutical materials regulated under USP methods, even if required control limits are reduced significantly in the future.

A further benefit of the simple helium cell gas mode used as standard on the 7700x is that it supports the USP <233> requirement for "unequivocal" identification and quantification of analytes, as He mode removes potential interferences on all isotopes of the analytes, making "qualifier" or "confirmatory" isotopes available for confirmation of the result reported at the primary isotope.

Modern ICP-MS systems such as the 7700x also provide a full mass spectrum screening capability, are tolerant of all commonly-used organic solvents, and can be linked to a chromatography system to provide integrated separation and analysis of the different forms or species of As and Hg, as required under USP <232> if the total level of these elements exceeds the prescribed limit. These additional capabilities are described in Section 7.

Table 6. ICP-MS detection limits compared to method requirements (based on May/June 2011 revision). J values assume 250x dilution factor.
* MDL calculated from the measurement of 10 unspiked GelCap samples.

Mass	Element	Cell mode	ISTD	Integration time (s)	Daily dose PDE (ug/day)	Component limits (ug/g)	1J actual values (ng/mL)	MDL* (ng/mL)
51	V	He	Sc	0.5	250	25	100	0.162
52	Cr	He	Sc	0.5	250	25	100	0.176
53	Cr	He	Sc	0.1	250	25	100	0.261
55	Mn	He	Sc	0.5	2500	250	1000	1.694
60	Ni	He	Sc	0.5	250	25	100	0.359
62	Ni	He	Sc	0.5	250	25	100	0.339
63	Cu	He	Sc	0.5	2500	250	1000	1.333
65	Cu	He	Sc	0.5	2500	250	1000	1.114
75	As	He	Sc	1	15	1.5	6	0.015
95	Mo	He	Tb	0.5	250	25	100	0.180
97	Mo	He	Tb	0.5	250	25	100	0.183
101	Ru	He	Tb	0.5	100	10	40	0.063
103	Rh	He	Tb	0.5	100	10	40	0.070
105	Pd	He	Tb	0.5	100	10	40	0.063
111	Cd	He	Tb	0.75	5	0.5	2	0.005
114	Cd	He	Tb	0.75	5	0.5	2	0.004
188	Os	He	Bi	0.5	100	10	40	0.274
189	Os	He	Bi	0.5	100	10	40	0.270
191	Ir	He	Bi	0.5	100	10	40	0.065
193	Ir	He	Bi	0.5	100	10	40	0.062
194	Pt	He	Bi	0.5	100	10	40	0.064
195	Pt	He	Bi	0.5	100	10	40	0.066
200	Hg	He	Bi	2	15	1.5	6	0.059
201	Hg	He	Bi	2	15	1.5	6	0.060
202	Hg	He	Bi	2	15	1.5	6	0.061
206	Pb	He	Bi	0.5	10	1	4	0.013
207	Pb	He	Bi	0.5	10	1	4	0.014
208	Pb	He	Bi	0.5	10	1	4	0.011

Table 7. ICP-MS system suitability (drift) performance before and after GelCap sample batch

Mass	Element	2J actual values	Measured mean (n = 6)	% RSD	Drift (%)	Limit
51	V	200	202.3	0.6	-0.3	20%
52	Cr	200	202.0	0.6	-0.5	20%
53	Cr	200	202.9	0.9	-0.5	20%
55	Mn	2000	2025.8	1.2	2.6	20%
60	Ni	200	202.3	0.7	-0.9	20%
62	Ni	200	201.9	0.8	-1.5	20%
63	Cu	2000	2105.4	2.8	7.0	20%
65	Cu	2000	2112.4	3.1	7.5	20%
75	As	12	12.2	0.8	-1.7	20%
95	Mo	200	202.2	0.5	-0.5	20%
97	Mo	200	202.2	0.6	-0.5	20%
101	Ru	80	80.6	0.9	2.1	20%
103	Rh	80	80.3	0.9	2.1	20%
105	Pd	80	80.3	0.8	1.5	20%
111	Cd	4	3.9	0.8	-0.1	20%
114	Cd	4	4.0	0.6	0.0	20%
188	Os	80	78.3	1.3	-2.9	20%
189	Os	80	78.4	1.2	-2.6	20%
191	Ir	80	81.6	1.5	3.6	20%
193	Ir	80	81.7	1.4	3.2	20%
194	Pt	80	82.0	1.6	3.7	20%
195	Pt	80	82.1	1.6	4.0	20%
200	Hg	12	12.2	1.3	3.1	20%
201	Hg	12	12.2	1.6	3.6	20%
202	Hg	12	12.2	1.5	3.2	20%
206	Pb	8	8.0	0.6	0.9	20%
207	Pb	8	8.0	0.6	1.1	20%
208	Pb	8	8.0	0.6	1.2	20%

Table 8. ICP-MS spike recovery at 0.5J and 1.5J in GelCap samples. Limit — 20%RSD at spike levels of 0.5J and 1.5J.

Mass	Element	Sample at 0.5J		Recovery		Sample at 1.5J		Recovery	
		Actual	Mean	%	%RSD	Actual	Mean	%	%RSD
51	V	50	52.84	106	1.6	150	157.4	105	1.6
52	Cr	50	52.63	105	2.3	150	155.9	104	1.4
53	Cr	50	52.74	106	2.2	150	157.2	105	1.6
55	Mn	500	524.0	105	1.7	1500	1696	113	1.1
60	Ni	50	52.96	106	1.9	150	155.9	104	1.5
62	Ni	50	52.72	105	1.9	150	156.1	104	1.5
63	Cu	500	523.9	105	1.7	1500	1733	116	1.4
65	Cu	500	524.0	105	1.2	1500	1727	115	1.4
75	As	3	3.21	107	3.9	9	9.53	106	3.2
95	Mo	50	52.61	105	1.8	150	157.5	105	1.5
97	Mo	50	52.65	105	1.6	150	157.1	105	1.4
101	Ru	20	20.75	104	2.0	60	62.64	104	1.2
103	Rh	20	20.91	105	2.0	60	62.57	104	1.2
105	Pd	20	20.77	104	2.2	60	62.19	104	1.2
111	Cd	1	1.03	103	2.7	3	3.04	101	1.2
114	Cd	1	1.04	104	2.5	3	3.08	103	1.3
188	Os	20	17.15	86	1.8	60	52.51	88	1.3
189	Os	20	17.17	86	1.6	60	52.63	88	1.2
191	Ir	20	20.56	103	1.6	60	63.33	106	1.2
193	Ir	20	20.63	103	1.9	60	63.42	106	1.1
194	Pt	20	20.63	103	1.8	60	63.77	106	1.2
195	Pt	20	20.64	103	1.6	60	63.87	107	1.1
200	Hg	3	3.09	103	2.0	9	9.51	106	1.3
201	Hg	3	3.09	103	2.3	9	9.47	105	1.0
202	Hg	3	3.08	103	1.9	9	9.47	105	1.3
206	Pb	2	2.08	104	1.9	6	6.21	104	1.5
207	Pb	2	2.08	104	1.9	6	6.22	104	1.4
208	Pb	2	2.08	103	2.1	6	6.20	103	1.1

Validation of Alternative Procedures

USP allows the use of alternative procedures, through the Pharmacopeia's General Notice. It is also stated specifically for elemental impurities in Chapter <233>: "Any alternative procedure that has been validated and meets the acceptance criteria that follow is considered to be equivalent to the compendial procedures for the purposes of this test." In addition, the suitability of the method for the system should also be verified through system suitability tests as described on the previous pages. Chapter <233> presents two types of validation procedures.

Limit procedures

Limit procedures should be tested for detectability, short-term precision (repeatability) and specificity. Parameters, test methodology and acceptance criteria are summarized in Table 9. Details can be found in USP Chapter <233> [30].

Table 9. Validation of limit procedures for alternative (non-instrumental) methods

Parameter	Test	Acceptance criteria
Detectability (1)	Response comparison of spiked sample with standard	Spiked sample response is greater than standard response
Detectability (2)	Response of sample spiked at 80% of target level	Response is less than sample spiked at 100% of limit value
Specificity	Measure response of other components that may be present	The procedure must be able to unequivocally assess each Target Element in the presence of components that may be expected to be present

Quantitative procedures

Quantitative procedures should be tested for accuracy, short-term precision (repeatability), ruggedness (intermediate precision) and specificity. Parameters, test methodology and acceptance criteria are summarized in Table 10. Limit of quantitation, range and linearity are demonstrated indirectly by meeting the accuracy requirements over all specified amounts.

Table 10. Validation of quantitative procedures for alternative (non-instrumental) methods

Parameter	Test	Acceptance criteria
Accuracy	Comparison of spiked sample with standards between 0.5J and 1.5J	70–150% of recovery
Precision (repeatability)	Analysis of six individual blank samples spiked at 1.0J	RSD <20% (n = 6)
Precision (intermediate)	Repeatability test performed by: separate analyst, different system, over two different days (only one such test is required)	RSD <25% (n = 12)
Specificity	Measure response of other components that may be present	The procedure must be able to unequivocally assess each Target Element in the presence of components that may be expected to be present
LOQ, range, linearity	None	Accuracy met

7. Extending Applications in Pharmaceutical Laboratories

Analysis of Organic Solvents

Many APIs are soluble only in organic solvents such as DMSO, DGME or 2-butoxyethanol in water. Routine analysis of organic solvents is therefore a requirement for many pharmaceutical laboratories, and the limitations of ICP-OES for low-level analysis in organic solvents may lead some laboratories to favor ICP-MS for these analyses.

Developments on the Agilent 7700x ICP-MS provide simple, routine analysis of most organic solvents, including those commonly used in pharmaceutical laboratories.

A new organics torch with a 1.5 mm internal diameter has recently been developed for the 7700, providing higher sensitivity than the 1 mm torch previously used while maintaining plasma tolerance to volatile solvents.

In addition to the new torch design, a new revision of ICP-MS MassHunter software and modified firmware in the 7700 provide optimized flow rates and timings for the carrier, make-up and option gas parameters during the ignition sequence. This greatly increases the tolerance of the plasma to solvents and allows the plasma to be ignited reliably with very volatile organic solvents.

Measurement of Elemental Forms or Species

USP <232> contains a section relating to the elemental form (species) of elements, and notes that As and Hg are of particular concern, as some forms of these elements are much more toxic than others. The PDE for As is based on inorganic As and, if the total As concentration exceeds the limit, the sample must be re-analyzed using a procedure that allows the different As species to be separated and quantified.

This is required because inorganic As is much more toxic than the common organic forms, such as arsenobetaine, so speciation is necessary to separate the different chemical forms and confirm that the level of inorganic As — the sum of arsenite (As(III)) and arsenate (As(V)) — is below the limit. The Agilent 7700 ICP-MS is easily coupled with HPLC allowing routine separation of the arsenic species relevant to USP <232>, as illustrated in Figure 8.

The chromatogram and calibration plots for As(III) and As(V) by LC-ICP-MS with the Agilent 1260 LC and Agilent 7700x ICP-MS illustrate the fast (less than 12 minutes) and complete separation of both As(III) and As(V) from the organic As species.

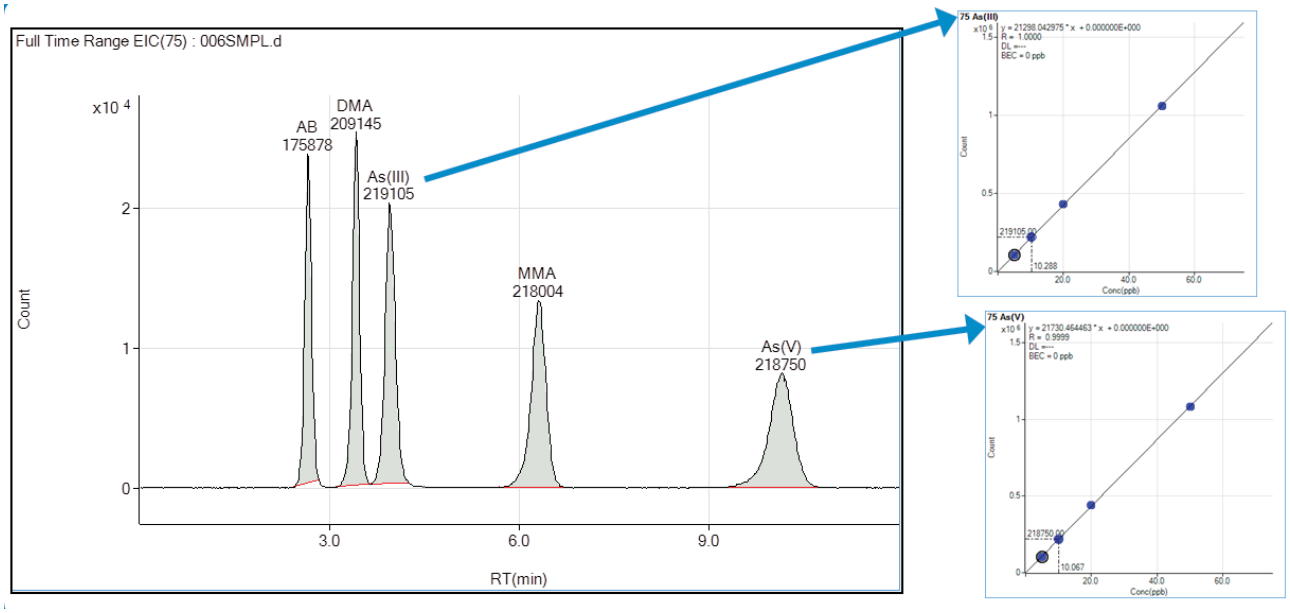

Figure 8. Chromatogram and calibrations showing determination of inorganic arsenic (As(III) and As(V)) by LC-ICP-MS

Full Mass Screening or Semi Quantitative Analysis

In addition to low level and reliable (interference-free) analysis of all 16 regulated elements in the proposed new USP <232> method, the 7700x also provides a unique screening capability in combination with helium (He) cell mode. Since He mode removes the polyatomic interferences from all analytes, regardless of the sample matrix, He mode screening provides a simple, easily interpreted spectrum, giving a comprehensive elemental composition from a single rapid scan, as illustrated in Figure 9.

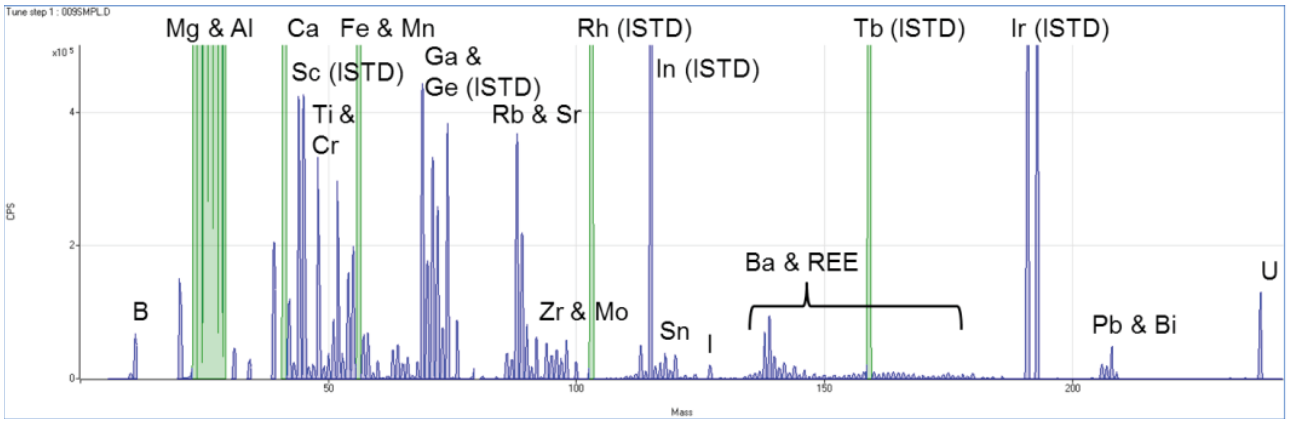

Figure 9. Example of full mass range 'screening' or semiquantitative measurement of a natural plant material measured using ICP-MS

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Glossary

API	Active pharmaceutical ingredient
CFR	(US) Code of Federal Regulations
cGMP	Current good manufacturing practice
DQ	Design qualification
EMA	European Medicines Agency (previously EMEA)
EP	European Pharmacopeia
EU	European Union
FDA	Food and Drug Administration
GAMP	Good automated manufacturing practice
GCP	Good clinical practice
GLP	Good laboratory practice
GMP	Good manufacturing practice
GxP	Good practices **x** stands for clinical. laboratory or manufacturing
ICH	International Conference for Harmonization
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectroscopy
IQ	Installation qualification
JP	Japanese Pharmacopeia
LVP	Large volume parenterals
NOEL	No-observed effect level
OOS	Out of specification
OQ	Operational qualification
PDE	Permitted daily exposure
PF	Pharmacopeial forum
PGEs	Platinum group elements (Pt, Pd, Ru, Rh, Os, Ir)
PGMs	Platinum group metals (Pt, Pd, Ru, Rh, Os, Ir)
PIC/S	Pharmaceutical Inspection Convention/ Cooperation Scheme
PQ	Performance qualification
QA	Quality assurance
QC	Quality control
SOP	Standard operating procedure
SST	System suitability testing
USP	United States Pharmacopeia

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