Proposed new USP general chapters <232> and <233> for elemental impurities: The application of ICP-MS for pharmaceutical analysis

White paper

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Abstract

The United States Pharmacopoeia (USP) is in the process of developing a new test for inorganic impurities in pharmaceutical products and their ingredients. The current USP<231> "heavy metals limit test" is widely acknowledged to be inadequate in terms of scope, accuracy, sensitivity, and specificity, and is due to be replaced with new General Chapters USP<232> (Limits) and <233> (Procedures) in 2013. The new methods will address the limitations of the current method, in particular with respect to the list of analytes, sample preparation, retention of volatile analytes, and the use of closed vessel sample digestion and modern instrumental techniques for the accurate recovery and determination of individual analyte concentrations. This White Paper provides the background to the development of the new General Chapters and explains how Agilent's 7700x ICP-MS can address the requirements of the proposed new methods.

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Introduction

The presence of impurities in pharmaceutical samples is a concern, not only because some contaminants are inherently toxic, but also because they may adversely affect drug stability and shelf-life, or may cause unwanted side-effects. As a result, both organic and inorganic (elemental) impurities must be monitored and controlled in raw materials used for drug manufacturing, in intermediates and active pharmaceutical ingredients (APIs), in excipients (stabilizers, fillers, binders, colors, flavors, coatings, and so forth), and in final drug products. Impurities that may be added during the production process, such as catalysts and contaminants from production process equipment, must also be monitored.

In the USA, the limits and procedures used to monitor contaminants (including elemental impurities) in pharmaceutical materials are defined by the United States Pharmacopeia (USP), but the regulatory body responsible for enforcement is the FDA. USP is currently developing new methodology for monitoring inorganic (elemental) impurities in pharmaceutical materials. The proposed new General Chapters USP<232> (Limits) and <233> (Procedures) are due to be implemented in 2013.

The current method used for monitoring inorganic contaminants in pharmaceutical samples is a 100 year-old colorimetric test, defined in USP General Chapter <231>. This method, known as the "heavy metals limit test", is based on precipitation of 10 sulfide-forming elements (Ag, As, Bi, Cd, Cu, Hg, Mo, Pb, Sb and Sn), in a reaction with a reagent such as thioacetamide. The resulting colored precipitate is compared visually to a 10 ppm Pb standard to determine compliance with the heavy metal limit.

Besides the obvious potential bias associated with a subjective visual comparison, USP<231> is a limit test based on the sum of the 10 elements, and so does not give individual concentrations for each individual element. Also it cannot be used for the determination of many elements of interest such as Cr, and the platinum group elements (PGEs) that are commonly used as production catalysts. Moreover, the use of thioacetamide and H_2S is not allowed in many parts of the world.

USP<232> includes a wider range of analytes including catalysts, and the maximum permitted levels are defined according to toxicity, rather than method capability. USP<233> defines sample preparation options including closed vessel microwave digestion, and recommends the use of modern instrumentation, such as multi-element ICP-MS and ICP-OES techniques, instead of the colorimetric test used in USP<231>.

A further acknowledged problem with USP<231> is that the sample preparation method requires ignition of the sample in a furnace at up to 600 °C. Such a high temperature inevitably leads to loss of volatile analytes, including the critical toxic element Hg [1, 2, 3].

In an article published under the "Stimuli to the Revision Process" of the 1st Pharmacopeial Forum (PF) in 1995, Blake noted that "because of the loss of metals during ignition, the validity of test results obtained with the current USP, JP (Japanese Pharmacopoeia) and EP (European Pharmacopoeia) general test procedures is questionable" [1]. In a subsequent article in 2000, Wang proposed the use of a modern instrumental method (ICP-MS) in place of the colorimetric test defined in USP<231>. Wang's article identified some of the limitations of USP<231>, noting that "these methods [USP<231>] 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." [4].

Recognition of these issues has led to a program to replace USP<231> with a new instrumental method that is more reliable, accurate, sensitive, specific, and robust. Three proposed new USP General Chapters relating to elemental impurities are being developed in parallel, USP <232>/<233> and <2232>. USP <2232> is limited to dietary supplements, while USP <232> and <233> deal with pharmaceutical ingredients and products.

Table 1 shows the Permitted Daily Exposure (PDE) limits for the new list of 16 analytes (As, Cd, Hg, Pb, V, Cr, Ni, Mo, Mn, Cu, Pt, Pd, Ru, Rh, Os and Ir) defined in USP<232> [5]. In the most recent (May 2011) revision of USP<232>, the previous Group I and Group II analytes have been combined into a single table, 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 analyte list and limits have been developed based on toxicological data, rather than method capability, and for the first time the list includes catalyst elements (the PGEs Pt, Pd, Ru, Rh, Os and Ir). Catalyst metals must be measured if they may have been added during sample processing.

Table 1. USP<232> analytes and Permitted Daily Exposure (PDE) limits for pharmaceutical products. Limits for Large Volume Parenteral (LVP) medicines are 100 times lower [5].

Element	Daily dose PDE (µg/day)
Cadmium	5
Lead	10
Inorganic arsenic	15
Inorganic mercury	15
Iridium	100
Osmium	100
Palladium	100
Platinum	100
Rhodium	100
Ruthenium	100
Chromium	250
Molybdenum	250
Nickel	250
Vanadium	250
Copper	2500
Manganese	2500

While the required PDE limits defined in USP<232> can be measured easily with either of the instrumental techniques referenced in USP<233> (ICP-OES or ICP-MS) [6], 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.

USP<232> includes a section relating to the elemental form (species) of elements, and notes that As and Hg are of particular concern as some forms 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 analysis 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. Similarly, the Hg limit is based on inorganic Hg (Hg^{2+}), although methyl mercury (MeHg) is the more toxic form. The presence of MeHg in pharmaceuticals is considered unlikely, but it should be separated and measured specifically if samples are derived from material (for example, fish tissue) that may contain the compound in significant amounts.

The PDE limits defined in USP<232> (Table 1) must be adjusted depending on the type of pharmaceutical product and the route of administration. For example drug products delivered by parenteral or inhalational administration must meet a modified PDE that is 10 times lower than the limit for oral administration, while large volume parenteral (LVP) medicines (daily dose greater than 100 mL) must meet a limit 100 times lower than the base PDE.

USP<232> also provides individual component limits for drug substances and excipients, assuming a maximum daily dose of less than or equal to 10 g/day, shown in Table 2. These component limits are applicable to manufacturing quality control, as they allow drug manufacturers to control the concentration of impurities in the raw materials and intermediates used in the final drug product. Table 2 also shows the component concentration limits in a digested solution, and the instrumental detection limits of the 7700x ICP-MS, for comparison.

For any sample requiring digestion or dilution, the

PDE limits must be corrected for the dilution factor applied during sample preparation. For example the individual component limit for Cd is 0.5 µg/g (ppm) for solid drug products and excipients. 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 (the "J" value) of 2 ng/mL (ppb) for Cd. Accurate recovery must be demonstrated at 0.5J (1 ng/mL), suggesting a required detection limit at least 10 times lower than this (0.1 ng/mL) a concentration easily measured using ICP-MS, as shown in Table 2. Component limits for drug products and excipients that would be delivered by parenteral or inhalational administration must be a further 10 times lower than these values, suggesting a required DL of 0.01 ng/mL in the digested sample, still easily within the range of ICP-MS.

Table 2. USP<232> concentration limits for components (drug substances and excipients) of drug products with a maximum daily dose ≤ 10 g/day [5], together with Agilent 7700x ICP-MS Instrumental Detection Limits.
* Component limits for drugs intended for parenteral or inhalational administration are 10 times lower.
† 7700x IDLs are for the preferred isotope of each element and were measured in a matrix of 1% HNO_3 and 1% HCl.

Element	Component concentration limits (µg/g, ppm)*	Concentration limits in solution (ng/mL, ppb) after 250x dilution	7700x instrumental detection limits (ng/mL)†
Cadmium	0.5	2	0.0001
Lead	1	4	0.0002
Inorganic arsenic	1.5	6	0.005
Inorganic mercury	1.5	6	0.001
Iridium	10	40	0.0002
Osmium	10	40	0.0005
Palladium	10	40	0.0001
Platinum	10	40	0.0002
Rhodium	10	40	0.0001
Ruthenium	10	40	0.0002
Chromium	25	100	0.002
Molybdenum	25	100	0.0002
Nickel	25	100	0.002
Vanadium	25	100	0.005
Copper	250	1000	0.002
Manganese	250	1000	0.001

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 (un-solvated), 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 (25:75) [3], 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 [7].

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) [10, 11], and can only be stabilized and measured reliably over an extended period if the digest solution includes a complexing agent such as HCl. USP<233> specifies that samples for analysis by ICP-MS must include an appropriate stabilizer when Hg is to be measured (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.

Instrumentation

One of the primary goals of the proposed new USP General Chapters is to replace the current subjective, colorimetric test (USP<231>) with a modern instrumental analytical method, and ICP-MS and ICP-OES are the instruments referred to in USP<233>. The benefits of ICP-MS in terms of low detection limits for all of the regulated elements have been discussed previously, and the 7700x system is particularly well-suited to the analysis of the variable, high-chloride matrices, which will be typical for digested pharmaceutical samples:

- The 7700 Series provides the highest plasma temperature of any commercial ICP-MS (indicated by the lowest CeO/Ce ratio of around 1%). This delivers improved matrix decomposition and better ionization of poorly ionized elements such as As, Cd, Hg, and the PGEs Os, Ir and Pt. A Peltier-cooled spray chamber is the preferred ICP-MS hardware configuration described in USP<233>, and is standard on all 7700 Series instruments. The 7700x also includes Agilent's unique High Matrix Introduction (HMI) system, which delivers unmatched matrix tolerance through precisely controlled and reproducible aerosol dilution. This technology further increases plasma robustness, giving better ionization and lower levels of interference, while also significantly reducing exposure of the interface and ion lenses to undissociated sample matrix when high dissolved solids samples are analyzed.

- The 7700 includes the third generation Octopole Reaction System (ORS^3), operating in helium (He) mode with kinetic energy discrimination (KED) for interference removal. He mode removes matrix-based polyatomic interferences regardless of sample composition and without the time-consuming sample-specific or analyte-specific optimization that is a characteristic of cell methods that utilize reactive gases [12]. He mode on the 7700 allows samples that contain high and variable amounts of chloride (for example, from HCl in a typical pharmaceutical sample digest) to be run without compromising the detection of elements that can suffer from chloride-based polyatomic overlaps. These elements include ^{51}V (overlap from $^{35}Cl^{16}O$), ^{52}Cr ($^{35}Cl^{16}O^{1}H$), ^{53}Cr ($^{37}Cl^{16}O$), and ^{75}As ($^{40}Ar^{35}Cl$), all of which the 7700 can determine accurately in the presence of % levels of HCl.

- A further benefit of He mode on the 7700 is that it eliminates the polyatomic interferences from all isotopes of each analyte, so secondary or qualifier isotopes are available for analyte confirmation. This is especially useful for pharmaceutical analysis, as USP<233> states that the procedure must be able to unequivocally assess each target element in the presence of other sample components such as other analytes and matrix components. The use of secondary isotopes as qualifier ions for ICP-MS is a well-established and unique capability of He mode [13].

- In cases where an API or other material can be solubilized in an organic solvent, the ICP-MS instrument must be able to tolerate the routine analysis of such solvents. Since the 7700 includes a Peltier-cooled spray chamber as standard, no change to the standard spray chamber is required in order to permit the aspiration of organic solvents. An optional 5th mass flow controller can be added to allow addition of oxygen to the plasma to decompose the organic matrix, and the sample introduction and interface parts are

easily exchanged for solvent-resistant versions. Furthermore, the advanced frequency matching RF generator and recently updated torch design and plasma ignition parameters of the 7700 ensure that the system tolerates volatile organic solvents; even non-water soluble solvents can be run directly.

- The 7700x is easily linked to an Agilent or third party HPLC (liquid chromatography/ion chromatography) system giving a fully integrated system to allow separation of the different 'species' or chemical forms of an element, as required for As and Hg if the 'total' concentration of the element exceeds the PDE.
- A rapid semi-quantitative screening acquisition can also be performed in He mode on the 7700x, allowing unknown samples to be quickly characterized. This mode of operation can also be applied to the determination of any process contaminants or for production failure analysis.

To confirm that He mode on the 7700x was effective at removing the Cl-based interferences derived from the HCl used during digestion, the elements most affected (V and As) were measured in no gas mode as well as the standard He mode. The calibrations in both modes are shown in Figure 1, illustrating the dramatic improvement in detection limits for these interfered elements.

Figures 1a and 1c show the calibrations in no gas mode for V and As respectively, while Figures 1b and 1d show the He mode calibrations for the same two elements. In no gas mode, the Cl-based interferences gave raised background equivalent concentrations (BECs) for both elements (2.49 µg/L for V and 2.55 µg/L for As). He mode provides at least a factor of 100 lower BEC for both V and As (0.023 µg/L for V and 0.005 µg/L for As), due to the effective removal of the ClO and ArCl polyatomics with He mode in the ORS^3 of the 7700.

The reduction of interferences to ng/L (ppt) levels ensures that these potentially interfered elements can be determined reliably at the regulated levels in the range of variable and complex matrices commonly analyzed in pharmaceutical laboratories.

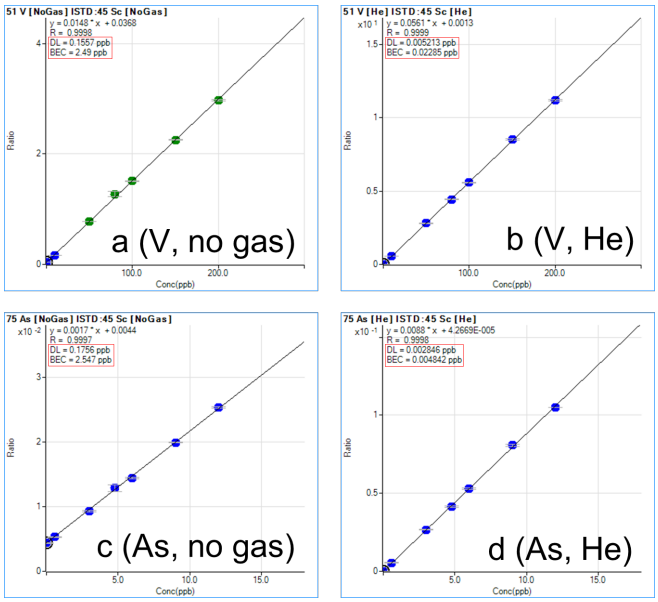

Figure 1. Calibrations for interfered elements V and As in no gas (a and c) and He mode (b and d) showing effective removal of Cl-based interferences in He mode (same cell conditions for all elements)

The method validation requirements of USP<233> depend on the procedure used (one of the specified ICP procedures, or an alternative procedure), and whether it is a limit procedure or a quantitative procedure. Limit procedures must confirm detectability, repeatability, and specificity of the measurement, while quantitative procedures must demonstrate accuracy, precision (repeatability and ruggedness), and specificity.

The system suitability and performance testing validation of the 7700x for both limit and quantitative procedures as defined in USP<233> is described in a separate application note [14].

Regulatory compliance for pharmaceutical manufacturing

Compliance with Federal regulations is a key aspect of sample analysis in pharmaceutical manufacturing. Part 11 in Title 21 of the US Code of Federal Regulations (commonly referred to as 21 CFR Part 11) governs food and drugs in the US, and includes the US Federal guidelines for storing and protecting electronic records and applying electronic signatures. The purpose of these regulations is to ensure the security, integrity and traceability of electronic records, which includes

data, analytical reports and other records (such as daily performance checks) associated with the operation of an analytical instrument.

The 4 areas of compliance related to analytical results are:

- System validation, including design qualification (DQ), manufacturing QC, lifecycle management, installation and operational qualification (IQ/OQ), and performance verification (PV or PQ) for analytical instruments and software.
- Control of access to the workstation for instrument control and data processing (restricted user access with password protection).
- Electronic records control (secure storage, file versioning, audit trail, electronic signatures, and archive/retrieval).
- System operation, suitability testing, procedures, and physical access to the laboratory and records.

The first of these must be demonstrated through the manufacturing quality records and equipment validation certification of the instrument manufacturer. The fourth requires appropriate controls on physical laboratory access, and that system suitability tests (SST) and standard operating procedures (SOP) are documented and followed.

The remaining 2 components are typically implemented through user access control software and an integrated system for managing the electronic records generated during the lab's activities.

In conjunction with the ICP-MS MassHunter User Access Control software for the 7700, Agilent OpenLAB ECM (Enterprise Content Manager) provides a solution that satisfies all the requirements of 21 CFR Part 11. User Access Control provides traceability, while security and integrity are ensured by the server-based file management of OpenLAB ECM. Using a LCDF (location, cabinet, drawer, folder) structure, analytical results and PDF report files are securely stored in checksum protected files. Agilent's flexible, multi-level ICP-MS User Access Control software integrates with Agilent OpenLAB ECM to provide unmatched security, integrity and traceability for ICP-MS data, essential for full compliance with regulatory requirements. Combined with manufacturing quality certification and full installation and operational qualification services (IQ and OQ) for ICP-MS hardware and software, Agilent provides the most complete range of compliance services for regulated laboratories.

Conclusions

The development of new methodology for the preparation and analysis of pharmaceutical samples described in USP<232>/<233> provides an opportunity for pharmaceutical laboratories to update their methodology and instrumentation to address the serious limitations of the current heavy metals limit test (USP<231>). The new General Chapters USP<232> and <233> recommend new sample preparation and stabilization methods, and outline new analytical methods based on modern ICP instrumentation.

The Agilent 7700x ICP-MS provides an ideal analytical capability for USP<232>, with low limits of detection and wide dynamic range (9 orders of magnitude) for all of the regulated elements. This is combined with excellent tolerance (further enhanced with HMI) to the high and variable matrices encountered in pharmaceutical laboratories, effective interference removal, and access to secondary isotopes for confirmation.

The 7700x ICP-MS provides the added capability to perform speciation analysis to separate and quantify different species of elements where toxicity is related to elemental form. The 7700x also provides a rapid screening or semi-quantitative analysis capability to check for other elemental contaminants or for process control. Quality certification, full validation service and integration with Agilent's OpenLAB ECM ensures that the 7700 offers the most complete compliance solution for pharmaceutical manufacturers wishing to implement USP<232>/<233>.

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