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Preparing for USP <232> - A conversation of the upcoming regulation changes with industry experts

The new guidelines set by the United States Pharmacopeia (USP) and the International Conference on Harmonization (ICH) is due for implementation at the beginning of 2018. The USP and ICH have pushed the pharmaceutical and nutraceutical industries to provide accurate, quantifiable results for potentially toxic elements in raw materials, active ingredients, excipients, and finished pharmaceutical products. USP <232> outlines the new limits for over a dozen potentially toxic elements. The upcoming regulations focus on the use of instrumental analytical techniques which previously were not required for pharmaceutical testing under the previous monographs USP <231> which was adopted more than a hundred years ago.

A lot of laboratories are now in the process of implementing new analytical technologies and methods into their analysis to comply with the regulations. This push to become compliant has raised a lot of questions regarding the new regulations. SPEX CertiPrep recently discussed these changes with two industry experts who are often called upon to educate laboratories on the new USP <232>, Anthony DeStefano and Robert Thomas.

1) Thank you both for contributing to our discussion on USP. Can you each tell us more about your history and work with the upcoming USP <232> method?

AD: I came to USP as the Vice President of General Chapters in January, 2008, just as chapters <232> and <233> were being drafted. When the ICH Q3D expert working group began work in the fall of 2009, I was USP's representative to the group. The USP, EP and JP were all observers to the ICH process. I worked with USP staff and the USP expert committees and advisory panels on the elemental impurities chapters until February, 2013.

RT: I became involved with these new USP chapters through my work with the ACS Committee on Reagent Chemicals. Our compendial book of standards contains written monographs for over 1,000 reagent chemicals. We meet twice a year to discuss and draft new, improved and updated methods. We are currently working on the 11th edition, which will be published later this year. ACS has a close working relationship with the USP because the pharmaceutical and dietary supplement industries use many ACS-grade reagent chemicals in their quality testing procedures. In fact, many USP tests refer to using more detailed information described in ACS Reagent Chemicals. As a result, the pharmaceutical industry, in particular, is probably the largest single user of our book. For that reason we share many procedures with the USP and, as a result, we make a concerted effort to align our methods as far as possible with USP methods.

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Participant Bios

Anthony DeStefano

Anthony DeStefano came from a 31 year career at Procter & Gamble focused primarily on analytical and bioanalytical chemistry. He then spent 5 years at the United States Pharmacopeia (USP) where he was Vice President and Senior Vice President of General Chapter and Healthcare Quality Standards. He was the USP representative to the ICH Q3D working group from its inception until February, 2013. He is currently consulting in the areas of bioanalytical, analytical and compendial chemistry.

Robert Thomas

Robert Thomas has worked as an analytical chemist in the field of trace element analysis for over 40 years, including 24 years for an ICP-MS manufacturer and 13 years as a principal of his own consulting company. He has served on the ACS Reagent Chemical Committee for the past 12 years as leader of the elemental impurities task force where he has worked closely with the USP to align heavy metal testing procedures in reagent chemicals with those of pharmaceutical materials. He has authored over 80 publications on trace element analysis and written three textbooks on ICP-MS. He is currently working on his fourth book, which focuses on the new global directives of elemental impurities in pharmaceutical materials. He has a graduate degree in Analytical Chemistry from the University of Wales in the UK and is a Fellow of the Royal Society of Chemistry (FRSC).

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2) Why do you think it took so long (almost 100 years) to reexamine the heavy metal limits and propose a new regulation?

AD: For many years, a non-instrumental approach was the only one available. The ICP-MS technology that made the low levels needed to reach some of the required limits is a relatively recent development. The first article in the Pharmacopeial Forum to advocate for change came via Dr. Blake at Merck in 1995, followed in 2000 by an article by Dr. Wang, also at Merck, detailing the limitations of the old technology. Soon after that article, USP began the process of considering updating the chapter. In addition to the need for the methodology to mature, the other issues that needed to be addressed included what metals are of interest, and what is the appropriate limit for each metal. On the toxicology side, USP established an initial set of limits that served as a starting point for discussions by the international group of ICH toxicologists. Once the ICH Q3D group was formed, it took a considerable amount of deliberation to settle on the limits as Permissible Daily Exposure numbers and to put together a sound risk-based rationale for each of the numbers.

RT: The process for changing the old Chapter <231>, sulfide precipitation test was started in 1995, because the consensus of experts in the field was that it was no longer valid to characterize a group of heavy metals in pharmaceutical material based only on comparison with a qualitative colorimetric sulfide precipitation test using a Pb standard.

One of the many drawbacks of this approach is the assumption that formation of the sulfides in the sample is very similar to the formation of the lead standard solution and is not affected by the sample matrix. However, since many metals behave very differently, the method requires that the visual comparison is performed very quickly after the precipitate has formed. Unfortunately, analysts can differ in their interpretation of the color change, so different analysts, based on their experience, may not consistently read the sample and standard solutions the same every time.

Another limitation of the technique is that the sample preparation procedure, involving ashing at high temperature and acid dissolution of the sample residue, is prone to sample losses particularly for the volatile elements like mercury. The loss of metals is also matrix-dependent and, because the procedures are time consuming and labor intensive, recoveries can vary significantly among different analysts.

3) Tony, one of the first drafts was issued in 2008. We are now looking for implementation in 2018, do you think this was a very long process to get to this point or is it about on par for similar changes to USP methods?

AD: This did take longer than many USP chapters from first draft to implementation. The reasons for this include the fact that <231> was harmonized across the pharmacopeias, the elements and limits needed international agreement, and harmonization (ICH Q3D) didn't begin until after the first USP draft chapter. In addition, this is a big change for the industry, affecting a large number of monographs globally, so the industry needed time to develop the methodology, do the risk assessments and testing necessary, and work through the issues involved with updating their regulatory filing for existing drugs.

4) What were the driving factors that delayed the implementation?

AD: Part of the reason for the long time-frame was the desire for global harmonization of the general chapter (reflected in harmonization with the Q3D guideline). The ICH process did not begin until October, 2009 and effectively ended with the publication of the Q3D guideline in December, 2014. In addition, the Q3D guideline included language stating that implementation for existing products would not be earlier than 36 months after posting of the Q3D document (this language was added after discussion with the pharmacopeias). Since USP can only have one standard, whether for new or existing products, this pushed the earliest implementation date to January, 2018. As a practical matter, the long implementation time was needed for everyone to agree on the metals and limits, and because of the challenges that each company faced in figuring out how to do their own risk assessments (which elements were important for each of their products), figuring out what the levels of metals were for each of their products (either paper exercises or actual method validation and testing), doing all the work necessary (for big companies this could be hundreds of products), and figuring out what to report and how to report on a global basis. There is also the need to assess the levels of elemental impurities in excipients, and for some, such as mined excipients, it takes a lot of data to get a range of impurity levels, since the levels can very depending on things such as the mine and location within the mine. Some of the issues are still being worked out, as witnessed by EMA publishing an implementation guideline in March, 2017. I believe there will be an iterative process between the regulators and industry regarding how much information to report, where to report it in the regulatory filing, and the level of supporting documentation needed to be kept in house to justify the conclusions reached. This process will go on well beyond the January 1, 2018 existing product implementation date.

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5) Rob, what do you believe are the most critical and important changes made by USP <232>?

RT: The process for change has been challenging and sometimes mystifying, based on continued discussions and disagreements between all interested stakeholders including USP, ICH, EMA, EP, and JP. But with the most recent version of Chapter <232>, which is out for review, ALL elemental impurities that could enter the production process from either the raw materials or the manufacturing process are now included and have all been given a classification based on their toxicological impact and delivery method. I don't think this would not have happened if USP had gone on its own.

Chapter <232> has now been fully aligned with ICH Q3D Step 4 Guideline, which specifies limits for 24 elemental impurities in drug products, drug substances, active ingredients, and raw materials defined as maximum permitted daily exposure (PDE) levels in µg/day for the three major drug delivery categories. These impurities may be present naturally, derived from the production catalysts, introduced inadvertently through the manufacturing process, or they could be environmental contaminants in the pharmaceutical raw materials. When elemental impurities are known to have the potential to be present, compliance to the specified levels is a requirement. Additionally due to the ubiquitous nature of arsenic, cadmium, lead, and mercury, those four elements at a minimum, must be monitored. The elemental impurity levels in the drug products, unless otherwise specified in an individual drug product monograph, must show compliance with the limits specified and be made available to the regulatory agency upon request. The full list of elemental impurities is shown in the table below, with Class 1 and 2A elements being the most important to monitor.

Element	Class	Oral PDE µg/day	Parenteral PDE µg/day	Inhalation PDE µg/day
Cd	1	5.0	2.0	2.0
Pb	1	5.0	5.0	5.0
As	1	15	15	2.0
Hg	1	30	3.0	1.0
Co	2A	50	5.0	3.0
V	2A	100	10	1.0
Ni	2A	200	20	5.0
Tl	2B	8.0	8.0	8.0
Au	2B	100	100	1.0
Pd	2B	100	10	1.0
Ir	2B	100	10	1.0
Os	2B	100	10	1.0
Rh	2B	100	10	1.0
Ru	2B	100	10	10
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1.0
Li	3	550	250	25
Sb	3	1,200	90	20
Ba	3	1,400	700	300
Mo	3	3,000	1,500	10
Cu	3	3,000	300	30
Sn	3	6,000	600	60
Cr	3	11,000	1,100	3.0

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6) Rob, from the point of view as a spectroscopist, what do you think are going to be the biggest challenges facing analysts in the lab regarding USP <232>?

RT: There is no question that to meet these PDE levels, the lab must not only have the necessary and appropriate analytical instrumentation, but must also be very familiar with trace element analysis. This choice of which technique to use is covered in USP Chapter <233>, which deals with the analytical procedure, including sample preparation procedure, instrumental method and validation protocols for measuring the elemental impurities using one of two plasma based spectrochemical techniques - Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES); Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), or alternatively any other trace element technique such as flame AA, graphite furnace AA, or X-ray Fluorescence (XRF) as long as it meets the data quality objectives of the method defined in the validation protocol section.

In addition, before any technique is used, it must be confirmed that the overall analytical procedure is appropriate for the instrument being used and the samples being analyzed by following a set of validation protocol performance tests, including: detectability, precision, specificity, accuracy, ruggedness, limit of quantification, and linear range.

Meeting these performance requirements defined in these tests must be demonstrated experimentally using an appropriate system suitability procedure and reference materials. The suitability of the method must be determined by conducting studies with the material under test supplemented/spiked with known concentrations of each target element of interest at the appropriate acceptance target limit concentration (J-Value), before the sample is prepared. Following these validation protocols requires an analyst to have a detailed understanding of analytical plasma spectrochemistry.

7) In looking at the limits for some of the heavy metals, the new limits seem to be possibly a little high compared to other limits set by WHO, EPA, CNHP, APHA (for example: USP mercury (organic) are 30 µg/day where APHA limits to 2 µg/day for adult weight), do you think these limits are low enough, and why or why not?

AD: The USP mercury limit is based on the mercuric inorganic form rather than the organic form which is much more toxic. The methyl mercury limit in the USP is 2 µg/day. The limits were set by a global group of ICH toxicologists, often in collaboration with toxicologists from USP's expert panel. The mercury limit in particular was extensively discussed by USP and ICH toxicologists and after a series of teleconferences they settled on the current number. The numbers may differ a bit from those set by other groups because there are limits for pharmaceuticals. Pharmaceuticals are pharmacologically active, so come with a benefit, which is factored in in some cases as one does a risk/benefit analysis in deciding what the limits should be. Also factored in are the dose limits (limits are based on the maximum daily dose), the weight of the average patient (50 kg for this chapter), and the decision to base the limits on chronic dosing. The numbers would be expected to be different for, say, drinking water, food or materials not used chronically.

RT: It's important to emphasize that Permitted Daily Exposure (PDE) levels are derived from the Provisional Tolerable Weekly Intake (PTWI) that is recommended by the Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) by subtracting the daily exposure (µg/day) to each elemental contaminant from air, food and drinking water. So an average body weight (males and females) of 50 kg, a well established safety factor, is used to calculate the PDE. So if you take the big four heavy metals (Pb, As, Cd, Hg), these PDE data are very similar compared to other global toxicology limits. The only element which is significantly different is Hg, which is worth explaining in a little more detail.

The mercury limits are based upon the inorganic mercuric (2^+) oxidation state. Methyl mercury, the most toxic form, is rarely an issue for pharmaceuticals. Therefore, the limit was established assuming that if mercury was present in the drug compound it would exist as the most common inorganic form. However, if there is a known potential for the material to contain methyl mercury (such as supplements derived from fish or kelp), an appropriate speciation procedure would be required. Chapter <2232> has a separate PDE for methyl mercury (2 µg/day), while Chapter <232> only specifies the inorganic form, as exemplified in the following table.

It's also interesting that the original mercury PDE in Chapter <232> was 15 µg/day, but when they aligned the chapter with the ICH Q3D Step 4 Guidelines, it increased to 30 µg/day. We believe the Japanese Pharmacopeia might have had a voice in influencing this level, because the Japanese population eats a lot more fish and seafood, which is known to contain higher levels of methyl mercury.

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Elemental Contaminant	USP Chapter <2232> PDE Limits (µg/day)	USP Chapter <232> Oral Delivery PDE Limits (µg/day)
Arsenic (inorganic)	15	15
Cadmium	5	5
Lead	5	5
Mercury (total)	15	-
Methyl Mercury (CH_3Hg)	2	-
Mercury (inorganic)	-	30

8) Tony, most of the other limits on heavy metals are calculated in terms of µg/kg of body weight per day, meaning that the limit of each metal is proportional to a child or adult exposure, but, the USP <232> does not have any distinction between exposure to a child versus an adult. What do you think was the rationale on creating the limits without consideration to body weight? Do you think that the rationale is valid?

AD: The ICH Q3D document is a global consensus document. The group needed to settle on this issue early on and decided to base the limits of adult patients weighing 50 kg and taking the drug for many years. Discussions regarding pediatric and geriatric patients are very difficult, in part because global definitions and rules are different in each region. It is up to each company to adjust the PDE based on issues such as age, weight and duration of treatment. Some guidance on how to consider these issues is provided in the ICH Q3D document and accompanying training materials on the ICH website.

9) Speciation for arsenic and mercury are now part of the USP <232>, do you believe that most laboratories will eventually have to implement speciation technologies into their analyses or do you think that in most cases the samples will fall well below the levels and thereby preclude further speciation?

AD: For drugs, it is unlikely that labs will need to do speciation. Methyl mercury is primarily found in fish, so in the pharmacopeia this is handled in the specific monographs where this could occur. Organic arsenic primarily occurs in plants, so this could be an issue for those doing dietary supplements that involve plant based material. This is discussed in more detail in USP <2232>.

RT: I agree, it is unlikely that the PDE limits for As and Hg will be exceeded in pharmaceutical materials or products. However, these elements are of particular concern because of the differing toxicities of their inorganic and organic forms. The arsenic limit is based on the inorganic form, which is the most toxic, and the mercury limit is based on the inorganic form, which, while less toxic than methyl mercury, is the form found in pharmaceutical excipients, manufacturing materials, container-closure systems and drug substances, hence drug products. Arsenic can be measured using a total-arsenic procedure under the assumption that all arsenic contained in the material under test is in the inorganic form. Where the limit is exceeded using a total-arsenic procedure, it should be demonstrated, using a suitable procedure to separate the species, that the inorganic form meets the specification. For mercury, no speciation is needed unless the material can contain methyl mercury (fish or kelp-containing materials or materials where methyl mercury is called out in the monograph). In those cases where methyl mercury may be present, first determine that the methyl mercury level is below 2 µg/day and then determine that the total mercury level is below 30 µg/day.

Chapter <232> does not give any suggestions on how to separate the species, but only that it must be a validated method. However, it is important to emphasize that elemental speciation analysis is not straightforward...it typically requires a chromatographic separation technique (LC, GC, IC) to separate the species and an atomic spectroscopic technique such as ICP-MS to detect the different species. Coupling an HPLC to an ICP-MS is not a trivial task, particularly for a non-experienced operator.

10) That leads us to our next question - What do you see are the biggest challenges facing laboratories as they implement USP <232>?

AD: There are two major challenges that I see. The first is being able to dissolve the sample to achieve a clear solution. Achievement of a clear solution greatly enhances the chances of obtaining accurate and precise results. The second is learning to work with materials at trace levels. The Class 1 and Class 2A elements were selected as important in part because they are present in many places and often at high levels. Not losing material in processing (e.g., evaporation) and not contaminating the sample with metals present in the working environment are both very difficult issues in any trace analysis laboratory.

RT: I believe that having someone with trace elemental analysis experience will be absolutely critical, particularly if it has been determined that ICP-MS is the optimum technique to use (refer to question 4). Understanding the demands of an application is of critical importance when a technique is being purchased, and particularly if there is a minimum amount expertise/experience in house on how best to use it to solve a particular application problem. This would be the likely scenario in a pharmaceutical production laboratory that is being asked to check the elemental impurities of incoming raw materials used to manufacture a drug compound. They will now have to follow the new USP chapters <232> and <233>, which recommends the use of a plasma-based spectroscopic technique to carry out the analysis.

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The expertise of the operator should never be underestimated, because if ICP-MS is being seriously considered, it generally requires an analyst with a higher skill level to develop rugged methodology free of interferences that can eventually be put in the hands of an inexperienced user to operate on a routine basis. This again is a real concern if the technique is being used by novice users who have limited expertise in running analytical instrumentation, which could be the case in the pharmaceutical industry. This fact cannot be overstated.

11) Tony, speaking of instrumental analysis, in the new regulations there is a discussion over something called the J-Value. The concept of J-Value is very confusing to most analysts. Can you explain the concept of J-Value and how it is appropriately applied to the analysis required for USP <232>?

AD: The J-Value concept can be confusing. It is essentially the target limit, on a w/w basis, corrected for the dilution factor needed to get the value measured on to the linear portion of the instrument's calibration curve. So, if one is looking for the J-Value for Pb for a drug with a 10 g/day dose, the target limit is 0.5 µg/g. This will require a dilution factor of about 100 to be in the middle of the range for ICP-MS so J would be (0.5 µg/g)(1000 ng/µg)(1 g/100 mL) = 5 ng/mL. It is important to remember that J is not the value the customer needs. The measured value needs to be converted back to a per gram of original sample basis.

12) Finally Robert, I have had the pleasure of working with both you and Tony during a series of USP <232> webinars this year. You gave a very good presentation regarding the possible pitfalls of instrumental analysis for USP <232>. Can you give is a few important points that an analyst must watch out for in order to be successful in their analyses for USP <232>?

RT: Here are some suggestions that a user might find useful:

- Make sure the right AS technique has been selected, based on the products being evaluated and expertise of the operator
- Find someone with a background in trace element analysis to run the instrument
- Make sure that person understands sample digestion procedures and is comfortable handling concentrated mineral acids using closed vessel digestion techniques, such as microwave digestion
- With regard to the actual analysis:
    - Ensure your dissolution procedure gives you a clear, colorless solution to present to the instrument...all the matrix with the elemental impurities are in solution
    - If using ICP-MS, try to avoid using hydrochloric acid in the sample prep, because it can produce problematic polyatomic interferences, which will impact elements such as As and Se
    - Using concentrated nitric acid on its own is a much better acid to use, if possible
    - The method development procedure has produced a robust method which is free of interferences
    - Collision Reaction Cell (CRC) technology will most likely be required to meet the PDE for all elemental impurities
    - The Chapter <233> drift specification of < 20% will tell you if the correct technique is being used...very important to run first in order to show compliance
    - Make sure you understand how J-Values have been calculated and that the levels in the pharmaceutical raw materials or final products have been calculated back to the original weight of the sample and recommended daily dosage
    - Once the drift spec has been successfully run, all validation protocol tests must be carried our to ensure compliance has been met
    - All spiked additions used in the validation protocol procedures must be made before the sample preparation steps

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