Preparing Your Lab for USP Chapters <232> and <233>

Pharma & Biopharma Tours | 2016
Simon Nelms
Introduction to USP Chapters <232> and <233>

• Concerned with testing of elemental impurities in pharmaceutical products

• New USP Chapters introduced to replace <231>
  • <232> Elemental Impurities – Limits
  • <233> Elemental Impurities – Procedure
  • <2232> Elemental Contaminants in Dietary Supplements

• Chapter 232 sets out the limits for 15 elements
  • ‘Big Four’ arsenic, cadmium, lead and mercury – must test for these
  • Remainder are commonly used as catalysts – must test if thought to be present

• Chapter 233 describes two analytical procedures:
  • Procedure 1 – ICP-OES
  • Procedure 2 – ICP-MS
  • Acceptance criteria for alternative procedures
USP has...
• Deferred introduction of both chapters in May 2013
• Both chapters have undergone revision to be aligned with ICH Q3D
• Both chapters became official in August 2015, and implemented (for new pharmaceutical products) in December 2015
• Both chapters will be implemented for all existing pharmaceutical products on January 1st 2018 in alignment with ICH Q3D

ICH Q3D...
• Step 4 ICH guideline issued on December 16th, 2014
• Final implementation (Step 5) set for January 1st 2018

Other regulatory bodies like the European Medicines Agency (EMA)...
• Delayed implementation dates for compliance for e.g. marketed products
Instrumentation

ICP-OES
Procedure 1

ICP-MS
Procedure 2
ICP – Optical Emission and Mass Spectrometry

Sample introduction and Plasma

ICP-Optical Emission Spectroscopy

ICP-Mass Spectrometry

Data processing
Schematic of an ICP-OES

POP window
Purged optical pathway
Plasma Generation
Optical tank
Torch box
Schematic of an ICP-MS

- Detector
- Quadrupole mass filter
- Collision cell
- Removal of neutral gas molecules
- Interface
- Plasma Generation
- Torch
Interference Removal by Collision/Reaction Cell

- **Collision/Reaction Cell**
  - A multipole (e.g. flatapole) enclosed in a chamber
  - Controlled flow of gas into the cell (usually pure He)
  - Interaction of ions with the gas mainly by collisions
  - Preferred approach: Kinetic Energy Discrimination (KED)
    - This filters out unwanted interference signals on the basis that these ions have lower KE than the isotopes with which they interfere
  - If reactive gas used, reactions occur
    - All cells are reaction cells

\[ M^+ + \text{ArX}^+ \]
Requirements to Operate an ICP-OES or ICP-MS

- **Power**
  - ICP-OES: Typically single phase 30 A
  - ICP-MS

- **Gases**
  - ICP-OES: Argon (grade 4.6 or better)
  - ICP-MS: Argon (grade 4.6 or better) + He (CCT - grade 5.0 or better)

- **Extraction**
  - ICP-OES: Typically 5 to 15 m/s
  - ICP-MS

Analysis time for both instruments = typically 2 to 3 mins per sample
Purchase Decision of an Instrument

Expected amount of analysis
- ✓ Sample Throughput
- ✓ Automation

Instrument performance
- ✓ Detection Limits
- ✓ Measurement range

Cost of ownership
- ✓ Installation Requirements
- ✓ Bench space requirements

Operator skills
- ✓ Automation
- ✓ Automated software routines

ICP-OES or ICP-MS?
Typical Instrument Implementation Process

- Purchase of an instrument
  - ICP-OES or ICP-MS

- System Delivery and Installation
  - Installation- and Operation Qualification (IQ/OQ)

- Method Development
  - Direct analysis, summation?
  - Generation of SOP

- Method Validation and Implementation into Routine Analysis

- Enforcement of new Regulations by FDA, EP etc.
Validation, Software and FDA Compliance

- **System qualification includes:**
  - Specification Qualification (SQ)
  - Design qualification (DQ)
  - Installation and operational qualification (IQ/OQ)
  - Performance qualification (PQ)
  - Periodic Requalification (RQ)

- **System qualification covers:**
  - Instrument Hardware and Software

- **Compliance with federal regulations is critical**
  - Part 11 in Title 21 of the US code of Federal Regulations (21 CFR Part 11)
    - Governs food and drugs in the United States
    - Includes Federal guidelines for storing and protecting electronic records
    - Must contain electronic signatures etc.
The Thermo Scientific™ iCAP™ Qualification Kit

• Available for ICP-OES and ICP-MS
• Contains all necessary documentation for IQ/OQ .... And more!

- Description of all necessary steps for fast and reliable implementation of a system in your lab
- Manuals and Application Notes for a quick start
- Documentation for IQ/OQ, Preventive Maintenance and Requalification (RQ) carried out by a service engineer
- Best Practices Guide, useful information provided by Application Chemists for new users
Work flow for Metals Analysis

- **Samples**
  - Solvent or microwave digestion

- **Sample preparation**
  - As prescribed in <232>/<233>

- **Method development**
  - ICP-OES or ICP-MS

- **Validation and security**
  - For compliance

- **Data analysis and reporting**
  - LIMS/network connectivity

- **Implementation of QA/QC**

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For compliance
Sample Preparation

- A typical drug can be described as an API (active pharmaceutical ingredient) + an excipient.
- Common excipients are:
  - Binders e.g. xanthan gum
  - Glidants and lubricants e.g. magnesium stearate
  - Disintegrants e.g. crospolividone (E1202)
  - Sweeteners e.g. sucrose
  - Flavourings e.g. fruit
  - Pigments e.g. titanium dioxide
  - Preservatives e.g. methylparaben
  - Coating e.g. shellac or gelatine

- For USP <233> three sample preparation options:
  1. Direct Aqueous
     - Dissolution in an aqueous matrix
     - Not all excipients soluble e.g. TiO$_2$
  2. Direct Organic
     - Dissolution in an organic matrix
     - Not all excipients soluble e.g. Magnesium stearate
     - Example with DMSO and ICP-OES
  3. Indirect Solution
     - Closed vessel digestion
     - See ICP-MS section for an example
     - **Most universal method**
Method Validation Criteria

- **Accuracy**
  - Closeness of the result to the true value

- **Precision**
  - Degree of agreement between individual tests of multiple samples

- **Specificity**
  - Unequivocal assessment of the analyte in presence of other compounds

- **Ruggedness**
  - Capacity to be unaffected by small but deliberate changes in the procedure

- **LoD, LoQ, Range, Linearity**
  - Analytical figures of merit
• #1 Calibrate the system, analyze a sample containing 0.5J and 1.5J three times each

• Definition of J:

• Example:
  • Oral administration;
  • Dose: 10g/day

Concentration (w/w) of the Target limit, appropriately diluted to the working range of the instrument

<table>
<thead>
<tr>
<th></th>
<th>As</th>
<th>Cd</th>
<th>Hg</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target Value [µg/day]</td>
<td>1.5</td>
<td>25</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Target limit [µg/g]</td>
<td>0.15</td>
<td>2.5</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Dilution factor for ICP-MS</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>J [ng/g]</td>
<td>0.15</td>
<td>2.5</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.5 J [ng/g]</td>
<td>0.075</td>
<td>1.25</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>1.5 J [ng/g]</td>
<td>0.225</td>
<td>1.875</td>
<td>1.125</td>
<td>0.375</td>
</tr>
</tbody>
</table>
#1 Calibrate the system, analyze a sample containing 0.5J and 1.5J three times each

- Determined spike recovery 70-150% (Accuracy)
  Test implemented in the Qtegra software QC repertoire

- Calculation of LoD/LoQ, Range and Linearity
  Qtegra ISDS automatically generates LOD data and correlation coefficient

- Specificity indicated by correct spike recovery result
- Alternative: Monitor two isotopes for one element
Required Tests from <233>

• # 2 Calibrate the system, analyze 6 times a sample spiked with 1J
  → Precision; RSD between all measurements not more than 20%

• # 3 Repeat #2 (6 times J) on either:
  - Two different days
  - Two different analysts
  - Two different systems
    → Intermediate precision, ruggedness; RSD between all not more than 25%

*Total: 24 analyses + 1 for unspiked sample*
The key benefits of ICP-OES

• Easy to use, learn & maintain
• Fast multi-element capability
• Robust plasma and flexibility for complex sample matrices

• Ability to analyse multiple matrix types in a single method
  → Axial and radial plasma observation
  → Robust RF generator
  → Easy handling of organic solvents using Organics Kit

<table>
<thead>
<tr>
<th>As, Cd, Hg and Pb</th>
<th>‘The big four’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr, Cu, Mn, Mo, Ni, Pd, Pt, V, Os, Rh, Ru, Ir</td>
<td>Common catalysts</td>
</tr>
</tbody>
</table>
Analysis of Two Over-the-Counter Medicines

• Preparing samples in DMSO
  • DMSO (dimethyl sulfoxide) is a very strong solvent
  • Less toxic than DMF (dimethylformamide)
  • High-boiling point

• Drawbacks of using DMSO
  • Require silicone pump tubing
  • O-rings on spray-chamber require changing more often
  • Will not dissolve all excipients
    • For example: silica, titanium dioxide
Analysis of Two Over-the-Counter Medicines contd.

- Two over-the-counter medicines were tested according to USP <233>
  - Drug 1 – anti-inflammatory
  - Drug 2 – antihistamine
- 0.5 g of dehydrated sample was dissolved in 25 g of DMSO
  - J defined as the w/w concentration of analyte at Target Limit after dilution
  - Target Limit > MDL; recoveries tested at the 0.5J and 1.5J

<table>
<thead>
<tr>
<th>Elements</th>
<th>0.5 J (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>125</td>
</tr>
<tr>
<td>Lead</td>
<td>25</td>
</tr>
<tr>
<td>Inorganic As</td>
<td>7.5</td>
</tr>
<tr>
<td>Inorganic Hg</td>
<td>75</td>
</tr>
<tr>
<td>Iridium</td>
<td>500</td>
</tr>
<tr>
<td>Osmium</td>
<td>500</td>
</tr>
<tr>
<td>Palladium</td>
<td>500</td>
</tr>
<tr>
<td>Platinum</td>
<td>500</td>
</tr>
<tr>
<td>Rhodium</td>
<td>500</td>
</tr>
<tr>
<td>Ruthenium</td>
<td>500</td>
</tr>
<tr>
<td>Molybdenenum</td>
<td>500</td>
</tr>
<tr>
<td>Nickel</td>
<td>2500</td>
</tr>
<tr>
<td>Vanadium</td>
<td>500</td>
</tr>
</tbody>
</table>
Analysis of Two Over-the-Counter Medicines contd.

- Precision
  - Determined by analyzing six individual samples
  - Samples spiked at J
  - USP acceptance criteria < 20%

<table>
<thead>
<tr>
<th>Elements</th>
<th>Drug 1 Run 1 µg/L</th>
<th>Drug 1 Run 2 µg/L</th>
<th>Drug 1 Run 3 µg/L</th>
<th>Drug 1 Run 4 µg/L</th>
<th>Drug 1 Run 5 µg/L</th>
<th>Drug 1 Run 6 µg/L</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>232.4</td>
<td>232.7</td>
<td>234.7</td>
<td>239.1</td>
<td>235.6</td>
<td>229.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Lead</td>
<td>45.9</td>
<td>45.2</td>
<td>44.6</td>
<td>47</td>
<td>46.6</td>
<td>43</td>
<td>3.2</td>
</tr>
<tr>
<td>Inorganic arsenic</td>
<td>12.1</td>
<td>12.7</td>
<td>12.8</td>
<td>14</td>
<td>12.9</td>
<td>11.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Inorganic mercury</td>
<td>130.7</td>
<td>130.8</td>
<td>132.5</td>
<td>136.5</td>
<td>131.8</td>
<td>127.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Iridium</td>
<td>944.5</td>
<td>941.3</td>
<td>948.2</td>
<td>963.7</td>
<td>950.9</td>
<td>924.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Osmium</td>
<td>954.8</td>
<td>952.7</td>
<td>959</td>
<td>974.9</td>
<td>960.5</td>
<td>940</td>
<td>1.2</td>
</tr>
<tr>
<td>Palladium</td>
<td>918.8</td>
<td>914.7</td>
<td>914.6</td>
<td>928.6</td>
<td>929.4</td>
<td>890.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Platinum</td>
<td>924.4</td>
<td>917.6</td>
<td>931.5</td>
<td>949.9</td>
<td>934.6</td>
<td>910.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Rhodium</td>
<td>921.5</td>
<td>907.2</td>
<td>907.5</td>
<td>917.6</td>
<td>915.8</td>
<td>874.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Ruthenium</td>
<td>955.5</td>
<td>966.5</td>
<td>953.6</td>
<td>972.8</td>
<td>967.5</td>
<td>932.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Molybdenenum</td>
<td>956.8</td>
<td>952</td>
<td>959.6</td>
<td>974</td>
<td>959.5</td>
<td>937.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Nickel</td>
<td>4669</td>
<td>4666</td>
<td>4706</td>
<td>4787</td>
<td>4718</td>
<td>4610</td>
<td>1.3</td>
</tr>
<tr>
<td>Vanadium</td>
<td>962.5</td>
<td>952.9</td>
<td>945.5</td>
<td>960.1</td>
<td>961.7</td>
<td>928.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Copper</td>
<td>9680</td>
<td>9590</td>
<td>9522</td>
<td>9666</td>
<td>9668</td>
<td>9318</td>
<td>1.5</td>
</tr>
</tbody>
</table>
• The key benefits of ICP-MS relative to ICP-OES are:
  • Improved detection limits:
    • Up to 1000x lower for USP regulated elements such as As, Cd, Hg and Pb
  • Able to access a broader elemental package
  • Wider dynamic range, ppt to ppm
  • Straightforward interfacing to speciation techniques (IC etc.)
Microwave Sample Preparation

- Four over-the-counter products were locally sourced
- Two samples of each were weighed into 15 ml disposable glass vials
- 3 ml of conc. HNO₃ was added to each vial
- System was closed, and pressurized with N₂ at 40 bar
- Microwave digestion recipe:

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Power (kW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>200</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>200</td>
<td>1.5</td>
</tr>
</tbody>
</table>

- When <60 °C, the digest was transferred to a polypropylene vial and made up to 50 ml with 1% HCl
- Samples were further diluted before analysis (with high purity 2% HNO₃) to give total dilution factors of between *100 and *1000
Results: Spike Recoveries (0.5 J)
<table>
<thead>
<tr>
<th>Element</th>
<th>Instrumental Detection Limit (ng/mL)</th>
<th>Method Detection Limit (μg/g)</th>
<th>Concentration Limit Max. Daily Dose of ≤10 g/day (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.5</td>
</tr>
<tr>
<td>Lead</td>
<td>0.0005</td>
<td>0.0005</td>
<td>0.5</td>
</tr>
<tr>
<td>Inorganic arsenic</td>
<td>0.0005</td>
<td>0.0005</td>
<td>1.5</td>
</tr>
<tr>
<td>Inorganic mercury</td>
<td>0.003</td>
<td>0.003</td>
<td>1.5</td>
</tr>
<tr>
<td>Iridium</td>
<td>0.002</td>
<td>0.002</td>
<td>10</td>
</tr>
<tr>
<td>Osmium</td>
<td>0.0006</td>
<td>0.0006</td>
<td>10</td>
</tr>
<tr>
<td>Palladium</td>
<td>0.0008</td>
<td>0.0008</td>
<td>10</td>
</tr>
<tr>
<td>Platinum</td>
<td>0.0005</td>
<td>0.0005</td>
<td>10</td>
</tr>
<tr>
<td>Rhodium</td>
<td>0.0007</td>
<td>0.0007</td>
<td>10</td>
</tr>
<tr>
<td>Ruthenium</td>
<td>0.001</td>
<td>0.001</td>
<td>10</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.003</td>
<td>0.003</td>
<td>18</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.003</td>
<td>0.003</td>
<td>60</td>
</tr>
<tr>
<td>Vanadium</td>
<td>0.006</td>
<td>0.006</td>
<td>12</td>
</tr>
<tr>
<td>Copper</td>
<td>0.009</td>
<td>0.009</td>
<td>130</td>
</tr>
</tbody>
</table>
Qtegra ISDS: A Simple Workflow to Quality Results

1. Dashboard
   “Get Ready”

2. LabBook
   “Create LabBook”

3. LabBook
   Results and report

Template
Experiment method and QA/QC protocol

Sample Details
- Manual
- LIMS
- Bar / QR Code

LIMS

Thermo Fisher Scientific
Qtegra ISDS: Get Ready

- Get Ready
  - Performance Check
    - AutoTune
    - Corrective Action (e.g. calibration)
    - Performance Check
- Performance OK
  - User Action
- Process Queued Labbooks
- Export Data
Qtegra ISDS: LabBook

- Method
- QA/QC Protocol
- Sample List
- Results
- Report
- Audit trail

- Prepared in just five clicks
Qtegra ISDS: Reporting

• Flexible tool for on-line generation of printable records
  • Can be printed online with data acquisition
  • Password protected .pdf files can be generated

• Tables are completely customizable
  • Information about Instrument, LabBook, Method Parameters

• Filtering options for reporting of specific samples
  • Sample name, Sample type, Analyst etc.
  • Individual tables for subsamples in a bigger batch
  • Filtering across all acquired data sets is possible to generate e.g. history records of QA/QC samples
Qtegra ISDS Reporting – Example 1

LabBook information:
Created by, Acquired by, Last changed by,…

Customized table:
Direct overview on precision test for Sample XYZ, Average Recovery and RSD are automatically calculated
Qtegra ISDS Reporting – Example 2

Report Intermediate Precision Test:

✓ Results obtained by two operators are summarized in one table

✓ Average value and RSD are calculated

✓ Every individual LabBook as datasource can be indentified and history can be displayed
Qtegra ISDS: IQ/OQ

- Verification of all installed files
- Checksums to verify consistency
- Verification of data evaluation algorithms
- Executable whenever required
## Comparison ICP-OES – ICP-MS

<table>
<thead>
<tr>
<th>Feature</th>
<th>ICP-OES</th>
<th>ICP-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection Power (USP)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Dynamic Range</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Speciation Capabilities</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Lab Requirements</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Operating Cost</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Software</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Investment</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Future Proof (USP)</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>
Implementing ICP-OES or ICP-MS for USP compliance can be pain free

- ICP-OES and ICP-MS provide multi-elemental determination of heavy metal impurities below the limits listed in USP <232>
- Recent developments have simplified user experience, increased throughput speed and reduced maintenance
- Software advances offer intuitive method development and ensure 21CFR part 11 compliance