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## Heavy metals testing in active pharmaceutical ingredients: an alternate approach

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The principle of the pharmacopoeial heavy metals test is detection and estimation of the metallic impurities colored by sulfide ion by comparison against lead standard. The test suffers from a loss of analytes upon ashing and from having varied responses for various metals. An inductively coupled plasma-optical emission spectroscopy (ICP-OES) for estimating 23 metals in active pharmaceutical ingredients is being proposed. The method covers the metals listed in USP, Ph. Eur and EMEA guidance on “Residues of Metal Catalysts or Metal Reagents”.

### 1. Introduction

Metallic impurities in pharmaceutical substances or drug products may originate from several sources like raw materials, metal catalysts or metal reagents used during synthesis, manufacturing equipment and piping, bulk packaging, the environment, cleaning solvents etc. Heavy metals most often implicated in human poisoning are lead, mercury, arsenic, and cadmium. Some heavy metals, such as zinc, copper, chromium, iron, and manganese, are required by the body in small amounts, but these same elements can be toxic in higher quantities.

The content of heavy metals in pharmaceutical substances is tested to control and quantify the levels of metals as a group due to the toxicity of the elements, but also due to the reason that heavy metal residue is a general marker for the quality of the product. The specification for heavy metals in active pharmaceutical ingredients is set taking into account its route of administration, maximum daily dosage, typical duration of intake and risk assessment. The general limit for heavy metals in the United States Pharmacopeia (USP) and European Pharmacopeia (Ph.Eur) is 10 ppm or 20 ppm. EMEA guideline on the “specification limits for residues of metal catalysts or metal reagents” specifies maximum acceptable limits of metal residues in drug substances and excipients.

USP includes a general test for heavy metals by concomitant visual comparison in volume VIII from 1905. The European pharmacopeia was born with a heavy metal test with the known methodology applied for centuries. The aim of the test was to detect the presence of undesirable metallic impurities through sulfide precipitation in both strongly acidic and alkaline medium. These were antimony, arsenic, cadmium, copper, iron, lead, and zinc at that time of inception. Later by the advances in Good Manufacturing Practices, developments in manufacturing of production equipments made of alloys and increase in awareness about metallic toxicities, testing for the target group of metals increased, but the method remained the same. The metallic sulfide precipitation and concomitant visual comparison for

heavy metals testing is not specific and is having recovery issues. The proposed method employs acid digestion of the sample followed by analysis using inductively coupled plasma-optical emission spectroscopy; ICP-OES. Acid digestion of the sample is performed using nitric acid, and hydrogen peroxide in a microwave oven. A few analytical methods have been reported in the literature for analysis of heavy metals in USP and Ph.Eur by various ICP techniques. So far, to our present knowledge no paper is covering ICP-OES determination of all the metals mentioned in USP, Ph.Eur. and EMEA guidance on “Residues of Metal Catalysts or Metal Reagents”. The digested sample is diluted with water to the volume required, further the sample is analyzed for 23 metals. The proposed method exhibits a recovery within 80–120% with a precision below 15% at LoQ level.

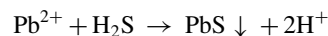
### 2. Investigations and results

#### 2.1. Review of methods in pharmacopeias

##### 2.1.1. United States pharmacopeia [USP] General chapter <231> Heavy metals

USP lists 10 metals under the scope of this chapter i.e. lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum. The principle of the method is detection and estimation of the metallic impurities colored by sulfide ion by comparison against lead standard.

The chemical basis for the determination is the formation of a colloidal precipitate of insoluble heavy metal sulfide salt and comparing color of the test sample with colored lead standard solution



USP specifies three different methods of sample preparation before final color comparison depending on sample solubility. The choice of the method depends upon the sample aqueous solubility and its ability to form colored solution in mild acidic conditions.

**Table 1: USP Monographs list**

Method	Short description	Number of Monographs
Method-I	Aqueous solution	137
Method-II	Ashing	344
Method-III	Wet digestion –Kjeldhal flask	2

*Method I* is for non color forming and/or water soluble substances, which are tested by color comparison after having reacted with sulfide ions. *Method II* is for color forming and/or water in-soluble substances which are tested by charring/ashing followed by color comparison after reacting with sulfide ions. *Method III* is for substances which are not suitable for testing either by Method I or Method II. Sample is digested in a Kjeldhal flask and after a clear solution is attained the sulfide reaction is carried out.

Suitability of the method is verified by checking the recovery of lead. A comparison table is given for the sample preparation type along with number of monographs in USP is listed in Table 1.

#### 2.1.2. European Pharmacopeia method [Ph.Eur] General chapter 2.4.8 Heavy metals

Ph.Eur <2.4.8> lists 15 metals under the scope of the chapter i.e. lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, molybdenum, vanadium, palladium, platinum, gold and ruthenium. This includes all ten metals specified by the USP and five metals in addition.

In addition Ph.Eur states “if the result is difficult to judge, filter the solutions through a membrane filter pore size 3 µm”. Ph.Eur mentions 7 methods of sample preparation before color comparison.

*Method A* is for non color forming and/or water soluble substances, which are tested by color comparison after having reacted with sulfide ion. *Method B* is for non color forming and/or organic solvent soluble substances, which are tested by color comparison after reacting with sulfide ion. For the methods A and B the limit for colour of test sample before adding sulphide ions should be less intense than degree 6 scale as specified in Ph.Eur General chapter 2.2.2: Degree of coloration of liquids. Spiking the test sample with lead standard i.e monitor is recommended if sodium sulfide is used in place of thioacetamide for methods A&B.

*Method C* is Charring method i.e charring the sample at temperatures below 800 °C for less than two hours of time and then MgSO<sub>4</sub> is added after preparing the test sample in water it is tested by color comparison after having reacted with sulfide

**Table 2: Ph.Eur Monographs list**

Method	Short description	Number of Monographs
Method-A	Aqueous solution	299
Method-B	Organic solution	50
Method-C	Ashing MgSO <sub>4</sub>	242
Method-D	Ashing MgO	79
Method-E	Aqueous solution Filtration	16
Method-F	Wet digestion -Kjeldhal flask	48
Method-G	Microwave, pressure	3

ions. *Method D* is Charring method i.e charring the sample at temperatures below 800 °C for less than one hour of time and then MgO is added after preparing the test sample in water it is tested by color comparison after having reacted with sulfide ions. Spiking the test sample with lead standard i.e monitor is recommended for methods C&D.

*Method E* is increased sample concentration followed by color comparison after reacting with sulfide ion. This method is generally for lower limits typically ≤5 ppm. *Method F* digests the sample in a Kjeldhal flask and after a clear solution is attained the color reaction is carried out. *Method G* is the microwave pressure digestion method; the sample is digested in fluoro polymer or quartz glass vessels until after a clear solution is attained and the color reaction is carried out. Spiking the test sample with lead standard i.e monitor is recommended for methods F&G. Table 2 compares the different sample preparation methods (Table 3).

#### 2.1.3. Note on pharmacopeial methods

The pharmacopeial heavy metals methods suffer from a lack in specificity to various metals under scope and the recovery when test samples are ashed. The shortcomings of the USP<231> and Ph.Eur <2.4.8> ashing methods were clearly observed when recoveries were tested for all the metals and found that recoveries are less than 50% (Lewen et al. 2004). An experiment is conducted testing all the ten metals mentioned in USP and palladium at 20 ppm in USP <231> Method I/Ph.Eur <2.4.8> Method A and it was observed that the colors developed varied for different metals. Chances of under-reporting and over-reporting when compared against lead standard was observed. The standard solutions of all the metals is of different solubilities due to the variable levels of reactivities of metals with sulfide ion, which is also creating a problem in color comparison with lead standard. This clearly reveals that there is possibility of erroneous results in reporting heavy metal's presence and content by the pharmacopeial methods.

**Table 3: Metal catalysts as per EMEA guidance**

#	Classification	Oral Exposure		Parenteral Exposure	
		PDE (µg/day)	Conc. ppm	PDE (µg/day)	Conc. ppm
1.	Class-1A: Metal of significant safety concern Platinum (Pt), Palladium (Pd)	100	10	10*	1
2.	Class-1B Iridium (Ir), Rhodium (Rh), Ruthenium (Ru), Osmium (Os)	100	10	10	1
3.	Class-1C Molybdenum (Mo), Nickel (Ni), Chromium (Cr), Vanadium (V)	300	30	30	3
4.	Class-2: Metals with low safety concern Copper (Cu), Manganese (Mn)	2500	250	250	25
5	Class-3: Metals With Minimal Concern Iron (Fe), Zinc (Zn)	13000	1300	1300	130

Table 4: ICP OES Analysis results of the 23 metals

USP <231> SPECIFIED METALS				
S. No	Metal	Wavelength	LoQ	Recovery
1.	Lead as Pb	220.353	0.1	94.4%
2.	Mercury as Hg	194.163	0.4	99.2
3.	Bismuth as Bi	223.061	0.4	97.7
4.	Arsenic as As	228.812	0.4	114.9
5.	Antimony as Sb	259.805	0.4	85.6
6.	Tin as Sn	189.926	0.4	115.6
7.	Cadmium as Cd	228.802	0.4	104.4
8.	Silver as Ag	328.068	0.4	101.2
9.	Molybdenum as Mo	202.03	0.4	109.9
10.	Copper as Cu	324.754	0.4	104.2
EUROPEAN PHARMACOPEIA SPECIFIED METALS <2.4.8> [In addition to above]				
S. No	Metal	Wavelength	LoQ	Recovery
11.	Vanadium as V	292.402	0.4	89.7
12.	Palladium as Pd	340.458	0.4	108.2
13.	Platinum as Pt	214.423	0.4	105.9
14.	Ruthenium as Ru	240.272	0.4	105.0
15.	Gold as Au	242.795	0.4	111.5
EMEA GUIDELINE SPECIFIED METAL CATALYSTS [In addition to above]				
S. No	Metal	Wavelength	LoQ	Recovery
16.	Iridium as Ir	224.268	0.4	110.6
17.	Rhodium as Rh	343.489	0.1	86.0
18.	Osmium as Os	225.585	0.4	85.4
19.	Nickel as Ni	231.604	0.4	102.9
20.	Chromium as Cr	267.716	0.4	103.3
21.	Manganese as Mn	257.61	0.4	95.6
22.	Iron as Fe	259.94	0.4	94.4
23.	Zinc as Zn	213.856	0.4	99.5
RESULTS OF TEST TO DETERMINE REACTIVITY WITH SULFIDE ION FOR USP SPECIFIED METALS AND PALLADIUM				
	Heavy metal	Color forming upon reacting with sulfide ion	Clearness of the solution after adding hydrogen sulfide ion	Remarks
<b>+ve bias ↑</b>	<b>Bismuth (Bi)</b>	<b>YES</b>	<b>Hazy</b>	<b>Over reported</b>
	<b>Copper (Cu)</b>	<b>YES</b>	<b>Clear</b>	<b>Over reported</b>
	<b>Palladium (Pd)</b>	<b>YES</b>	<b>Hazy</b>	<b>Over reported</b>
Working level ≈	Lead (Pb)	Positive	Clear	Standard
	Silver (Ag)	YES	Clear	Under reported
	Tin (Sn)	NO	Clear	Under reported
–ve bias ↓	Arsenic (As)	YES	Clear	Under reported
	Mercury (Hg)	NO	Hazy	Under reported
	Cadmium (Cd)	NO	Hazy	Under reported
Ambiguous ↑↓≈	Molybdenum (Mo)	YES	Clear	Ambiguous
	Antimony (Sb)	YES	Hazy	Ambiguous

## 2.2. EMEA guidance on “Residues of Metal Catalysts or Metal Reagents”

EMA guidance on metal catalysts or metal reagents specifies 14 metals i.e. platinum, palladium, iridium, ruthenium, rhodium, osmium, molybdenum, nickel, chromium, vanadium, copper, manganese, iron, and zinc. Different specifications are proposed based on the classification and toxicity levels (Table 4).

EMA guidance is stating the following about the existing heavy metals method in pharmacopeia “General semi-quantitative metal limit tests based on the precipitation at pH 3.5 of colored metal sulfides are described in several publications (e.g. Ph. Eur). Such tests are not suitable to quantitatively determine

the actual levels of a specific metal residue in a pharmaceutical substance”.

## 2.3. ICP-OES determination of heavy metals in levetiracetam

The active pharmaceutical levetiracetam was tested for 23 metals, 10 metals listed in USP and 15 metals listed in Ph. Eur and 13 metals in the EMA Guidance document. [USP Heavy metals: 1. Lead, 2. Mercury, 3. Bismuth, 4. Arsenic, 5. Antimony, 6. Tin, 7. Cadmium, 8. Silver, 9. Copper, 10. Molybdenum, Ph. Eur heavy metals (in continuation of 10 metals listed in

**Table 5: Experimental conditions**

Plasma Gas Flow (L/min)	15	Plasma Power	1.2 kW
Auxiliary Gas Flow (L/min)	1.5	Torch Type	Fixed
Nebulizer Pressure (kPa)	200	Nebulizer	V-Groove
Viewing Height	5 mm	Fast Pump Rinse	50 rpm
Pump speed	15 rpm	Rinse Time	10s
Sample Uptake Delay	15 s	Replicates	5
Replicate Read Time	1 s	Line Background Correction	Fitted
Instrument stabilization delay	15 s		

USP); 11. Vanadium, 12. Palladium, 13. Platinum, 14. Gold, 15. Ruthenium, EMEA Guidance (Except metals in USP and Ph. Eur heavy metals list); 16. Iridium, 17. Rhodium, 18. Osmium, 19. Nickel 20. Chromium, 21. Manganese, 22. Iron, 23. Zinc). The wavelengths for scan were selected based on the ICP-OES manufacturer recommendation and from available literature. The 23 metals tested were not detected in the active pharmaceutical levetiracetam tested.

The LoQ levels were checked by spiking with sample (acceptance criterion 80–120%, Table 4) and with an acceptable precision (acceptance criterion  $\leq 15\%$  [ $n=3$ ]). The LoQ of all the metals was 0.4 ppm except for lead and ruthenium for which the LoQ was 0.1 ppm. The achieved LoQ are in line with requirements of ICH Q1 impurities guidance & USP draft chapter on metallic impurities i.e less than 30% of the specification. The recovery for all the metals was within 80% to 120% at LoQ level ranging from 85.6% to 115.6%. Precision at LoQ was less than 15% [ $n=3$ ] (Table 4).

### 3. Discussion

Pharmacopieal heavy metals testing procedure of metallic sulfide precipitation and concomitant visual comparison is not specific for all metals and it is having recovery issues. The method is also having drawbacks of non-compatibility for all sample matrices.

The proposed method of acid digestion of the sample followed by analysis using ICP–OES can be used for estimation of the metals specified in USP, Ph.Eur, and the EMEA Guidance document on metal residues for levetiracetam or any other active pharmaceutical ingredients (recovery is to be checked). The comparatively expensive ICP–MS methodology as suggested in some of the research papers may not be necessary for estimating the listed metals.

## 4. Experimental

### 4.1. Chemicals

Levetiracetam sample was received from Hetero Laboratories Ltd, Hyderabad, India. Nitric Acid – Supra pure, Perchloric Acid, Hydrogen peroxide were purchased from Merck LTD which are above 99.5% purity. 1000 ppm metallic standards were purchased from Merck, Darmstadt, Germany. High purity water was prepared using a Millipore water purification system.

### 4.2. Equipment

Inductively Coupled Plasma-Optical Emission Spectrometer (model-Liberty, make-Varian) was used for experimentation. The output signal was monitored and processed using Liberty software on a Pentium computer (Digital equipment Co.).

### 4.3. Experimental conditions

The instrumental parameters used for operating Inductively Coupled Plasma-Optical Emission Spectrometer are given in Table 5.

### 4.4. Preparation of solutions

#### 4.4.1. Sample preparation

Sample preparation is done by acid digestion method. The sample (1 g) is taken into a digestion vessel and 15 mL of nitric acid and 2 mL of hydrogen peroxide are added and the mixture is digested in a microwave oven until a clear solution is attained. Additional hydrogen peroxide is to be added if any precipitate is observed. The volume is reduced to about 5 mL. The temperature of the oven is increased slowly at a gradual ramp of about 10 °C and maintained at 250 °C for about 10 min. The digested solution was mixed, transferred to a 25-mL sample vial and made up with Millipore water for analysis by ICP–OES.

#### 4.4.2. Blank preparation

A blank solution was prepared as described as described above but without sample.

#### 4.4.3. Standard preparation

The intended working standard solutions are prepared by diluting respective 1000 ppm standard solution. Working standards were prepared from LOQ level to 10 ppm concentrations (Table 4).

#### 4.4.4. Spike solutions

Spike solutions were prepared by the sample preparation procedures above by adding a known concentration of intended metallic standard solution to the sample before digestion. After digestion the solution is mixed and transferred to a 25-mL sample vial and made up with Millipore water for analysis by ICP–OES. Aquaregia conc  $\text{HNO}_3 + \text{HCl}$  [1:3] v/v was used instead of hydrogen peroxide, for platinum, iridium and gold.

#### 4.4.5. Study design

The sample was tested for 23 metals, 10 metals listed in USP and 15 metals listed in Ph. Eur and 13 metals in EMEA Guidance document. The LoQ levels were checked by spiking with sample at an acceptable level of 80–120% (Table 4) and with an acceptable precision less than 15% [ $n=3$ ].

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