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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR MULTI ELEMENTAL IMPURITIES IN DOXORUBICIN DRUG PRODUCT AND RELATED MATRICES BY ICP-MS IN COMPLIANCE WITH USP REQUIREMENTS

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Abstract:

In efforts to control the potential presence of heavy metals in pharmaceuticals, the United States Phar-macopeia (USP) general chapter (<232>, <233>) and International Conference on Harmonization (ICH) guideline ICH Q3D have put forth new requirements and guidelines for their control. The new requirements and guidelines establish permitted daily exposures (PDE) for 24 heavy metals/elemental impurities (EI) based upon their toxicological properties. USP General Chapter <233> provides a general reference procedure for preparing pharmaceutical samples for analysis employing microwave assisted digestion (MWAD). It also provides two Compendial Procedures, Procedure 1 employing ICP-AES, and Procedure 2 employing ICP-MS. High sensitivity detection limits given by ICP-MS, much work has been done in developing and evaluating analytical methods to support the analysis of elemental impurities in finished pharmaceutical products, active pharmaceutical ingredients, and excipients by this analytical technique. In this study, we have evaluated and validated the method for the determination of Class 1 and 2 elemental impurities using pneumatic nebulization. The study also employed closed vessel MWAD to prepare samples for analysis. Limits of quantitation were element specific and significantly lower than the PDEs for parenteral drugs. Spike recoveries for the elements studied ranged between 94.9% and 115.7%, having correlation of more than 0.99 for all elements; the developed method is observed to be specific, linear and precise. The use of ICP-MS provides an alternative to heavy metals in the analysis of elemental analysis requiring low detection limits.

Keywords: Inductively-coupled plasma mass spectrometry, Elemental impurities, Doxorubicin, USP, Development, Validation.

Introduction: The determination of elemental impurities in pharmaceuticals, excipients or drug products has been performed as recommended by pharmacopoeias, generally based on limit tests [1, 2]. The main disadvantage of this method is the lack of specificity and sensitivity. Heavy metals cause chronic toxicological risks and their effects can be very difficult to detect. Growing concern over controlling potential heavy metals exposure has resulted in establishment of element specific daily exposure (PDEs) limits for finished drug products by both the United States Pharmacopeia (USP), International Conference on Harmonization (ICH), and other pharmacopeial and regulatory bodies. These PDEs are based on current toxicological assessments of the elements rather than the capability of the testing methodology. There are 24 elemental impurities of potential concern identified by both USP <232> and ICH Q3D. However, based upon the route of administration, oral, parenteral, inhalation, etc., not all of these 24 elemental impurities need to be monitored.

Table 1 lists their PDEs by route of administration and classification, according to risk based upon toxicity and likelihood of occurrence. USP <232> and ICH Q3D also provide guidance as to which of these 24 elemental impurities must be tested for. For example, an oral drug product need only be tested for Class 1 and Class 2A elemental impurities which total 7, assuming that through a paper risk assessment, no other elemental impurities are identified as contributory from the manufacturing process. If the use of a catalyst, such as silver, palladium or platinum is used in the manufacturing process, including raw materials such as excipients, then the additional 10 Class 2 B elemental impurities must also be tested for.

Thus, if no further sources are identified, for an oral drug where catalysts are used in the manufacturing process a total of 17 specific elemental impurities out of the 24 must be tested for. The compendial methods that have historically been used to determine heavy metals have relied on the precipitation of metal sulfides from an aqueous solution, and visual comparison to a lead standard similarly treated. In 2008, the USP proposed replacing the historical compendial method for heavy metals <231> with two new General Chapters. These two chapters, <232> Elemental Impurities- Limits and <233> Elemental Impurities-Procedures, respectively, establish safety based limits on elemental impurities and modern methods for their analysis. Following several

years of revisions with input from industry and regulatory stakeholders, these two chapters were issued in final form, being subsequently harmonized with ICH Q3D, and industry compliance beginning in January 1, 2018. Prior to the development of USP <233>, Wang [3] and Lewen [4] had proposed and demonstrated that ICP-MS could be used as a rapid screening technique for heavy metals in pharmaceutical compounds and materials. This, along with Lewen's involvement on the USP Expert Committee developing <232> and <233> led to the inclusion of both ICP-AES and ICP-MS as the referee methods.

More recently, the use of ICP-MS for the determination of the original 15 elemental impurities covered by <232>, but also the additional 9 included in ICH Q3D guidelines, and subsequently added by USP to <232> and <233>, was demonstrated by Li [5] for the analysis of these 24 elemental impurities in pharmaceutical excipients. The use of atomic spectrometry as an analytical technique has found widespread use for the analysis of trace metals in the environment since the mid-1970s.

The USEPA has developed numerous methods for using flame atomic absorption spectrophotometry (AAS), graphite furnace atomic absorption spectrophotometry (GFAAS), ICP-AES, and ICP-MS. In 1974, inductively coupled plasma mass spectrometry is one of the most sensitive analytical technique and, therefore, very appropriate for multielemental impurities determination at trace and ultra-trace levels in different sample matrices [6].

In the last years, this technique has gained a good acceptance, being used as an alternative over the classical methods for elemental impurities determination in pharmaceutical and nutritional supplements [6].

The aim of the present research work was to study the analysis of elemental impurities, Class 1, 2A and 2B, in parenteral drug requiring low detection limits employing ICP-MS.

ICP-MS is proposed for Arsenic (As), Cadmium (Cd), Cobalt (Co), Copper (Cu), Mercury (Hg), Lithium(Li), Antimony(Sb), Nickel (Ni), Lead (Pb) and Vanadium (V) determination in PEGylated doxorubicin, lipids and cholesterol used as component of parenteral drug solutions. Analytes determination is carried out with sample digestion, whereas sample matrix effect is critically evaluated. The ICP-MS instrument is operated in standard mode or using DRC.

Table 1: Permitted Daily Exposures for Elemental Impurities.

Element	Class	Oral PDE µg/day	Parenteral PDE, µg/day	Inhalation PDE, µg/day
Cd	1	0.5	0.2	0.2
Pb	1	0.5	0.5	0.5
As	1	1.5	1.5	0.2
Hg	1	3	0.3	0.1
Co	2A	5	0.5	0.3
V	2A	10	1	0.1
Ni	2A	20	2	0.5
T1	2B	0.8	0.8	0.8
Au	2B	10	10	0.1
Pd	2B	10	1	0.1
Ir	2B	10	1	0.1
Os	2B	10	1	0.1
Rh	2B	10	1	0.1
Ru	2B	10	1	0.1
Se	2B	15	8	13
Ag	2B	15	1	0.7
Pt	2B	10	1	0.1
Li	3	55	25	2.5
Sb	3	120	9	2
Ba	3	140	70	30
Mo	3	300	150	1
Cu	3	300	30	3
Sn	3	600	60	6
Cr	3	1100	110	0.3

Method development and validation:

In order to minimize interferences a high pure grade solvents used. Concentrations were optimized in order to obtain the lowest LOD as possible [6]. All investigated elements, were determined using the same instrumental conditions. The equipment used changes automatically from standard mode to DRC mode during each

measurement cycle. Doxorubicin injection was diluted considering the maximum matrix concentration that could be present without causing interferences on the investigated element. Due to the viscosity of the original solutions, dilutions were performed by weighing the original solution. A given amount of each sample was weighed and diluted in 2.5% (v/v) nitric acid and hydrochloric acid. In

order to achieve the desired concentration. The method developed for impurities determination in components of parenteral drug product and solutions is considered specific for the investigated elements. The accuracy of the proposed method was evaluated by analyte recovery tests and analysis of certified reference materials. Spiked solutions of the analyzed samples were prepared by adding a given amount of the analytes.

Materials and Methods:

Concentrated nitric acid (70%, v/v, trace metal grade) and concentrated hydrochloric acid (36%, v/v, and trace metal grade) were purchased from Fisher Scientific, and used throughout this work to prepare standards and samples. Ultrapure water with a resistivity of 18.2M used in the experiments was prepared by passing water through deionizer polishing filters, and then through a Milli-Q Type 1 Ultra-pure water system (EMD Millipore, Billerica, MA, USA). Standard solutions for elemental analysis were prepared by diluting commercially available, NIST traceable, single element 1000 mg per liter stock solutions (Reagecon and spec pure). Spike solutions for recovery assessment were also prepared from these stock solutions. Test samples employed for this study consisted of PEGylated doxorubicin, lipid base placebo consisting of formulation without drug and available market sample Lipodox.

Sample preparation:

Liposomal injectable sample preparations were prepared by as per USP <233>. Briefly,0.5 g of the sample was weighed into a microwave digestion vessel. Pre-digestion was initiated by adding 0.6 mL of nitric acid with the careful addition of 0.6 mL of hydrochloric acid to each vessel, and waiting 5 min for the reaction to subside. After 5 min an additional 1.0 mL of nitric acid was added, along with 1.0 mL of hydrochloric acid. Each vessel was then assembled and carefully sealed tight. Samples were then subjected to closed vessel microwave digestion allowing sample decomposition under high temperature and pressure. A modified microwave digestion program was used employing a two-step microwave program consisting of a 5 min ramp to 210°C,

with a hold for 5 min; then a 3 min ramp to 250°C, with a final hold of 6 min at 250°C. Samples were allowed to cool for 20 min prior to venting and opening the digestion vessels. Digested samples were quantitatively transferred into 25 mL volumetric flasks.

Preparation of standards:

Standards were prepared from the stock 1000 ug per mL stock solutions by serial dilution with 2.5% nitric acid and hydrochloric acid. These standards encompassed the range of 0.5–2.0 of the Target Limit (J-value) called for in USP <233>.The standard preparation are listed in Table 2 A, 2 B and 2C.

Table-2 A: Preparation of Standard stock solutions:

Name of Standard 1000 ppm	**Volume of standard (mL)**	**Dilution With diluent (mL)**	**Concentration (ppm)**	**Label**
Cadmium	0.100	10	10	Stock-A
Lead & Cobalt	0.100	10	10	Stock-B
Arsenic	0.100	10	10	Stock-C
Mercury	0.100	10	10	Stock-D
Nickel	0.100	10	10	Stock-E
Vanadium	0.100	10	10	Stock-F

Table-2 B: Preparation Stock – G solution (Mixed stock solution):

Name of standard stock and Standard 1000 ppm	**Volume of standard (mL)**	**Dilution With diluent (mL)**	**Concentration (ppm)**	**Label**
Stock-A	0.2	50	0.04	Stock-G solution (Mixed stock solution)
Stock-B	0.5		0.1	
Stock-C	1.5		0.3	
Stock-D	0.3		0.06	
Stock-E	2.0		0.4	
Stock-F	1.0		0.2	
Lithium 1000 ppm	0.25		5	
Antimony 1000 ppm	0.09		1.8	
Copper 1000 ppm	0.3		6	

Table-2 C: Preparation of Calibration standard solutions:

Name of stock Solution	Volume of standard (mL)	Dilution With diluent (mL)	Concentration (ppb)									Label
			Cd	Pb & Co	As	Hg	Ni	V	Sb	Li	Cu	
G	0.250	10	1	2.5	7.5	1.5	10	5	45	125	150	Cal.Std -1
	0.500	10	2	5	15	3	20	10	90	250	300	Cal.Std -2
	1.000	10	4	10	30	6	40	20	180	500	600	Cal.Std -3
	1.500	10	6	15	45	9	60	30	270	750	900	Cal.Std -4
	2.000	10	8	20	60	12	80	40	360	1000	1200	Cal.Std -5
	2.500	10	10	25	75	15	100	50	450	1250	1500	Cal.Std -6
	1.000	10	4	10	30	6	40	20	180	500	600	Standard check/Bracketing standard
	2.500	25	4	10	30	6	40	20	180	500	600	Standard solution

Instrumentation:

Samples were digested using microwave digestion system. Employing this system is equipped with temperature and pressure sensors. However, the method parameters are based on temperature control, so pressure was not controlled during sample digestion. The digestion system have vessels with a volume of 100 mL, a maximum pressure of 100 bar and a maximum temperature of 300°C, and, feature a "vent-and-reseal" design that prevents over-pressure without losing samples. Thermo Fischer (model i-CAP Q series sequential ICP-MS with pneumatic nebulizer. Standards and samples were introduced using Thermo Fischer auto sampler. The instrumental parameters are listed in Table 3. Instrument control and data analysis were carried by Qtegra software.

Table-3: ICPMS Instrument and Method Conditions:

Measurement Mode	KED
Plasma Power	1550 Watts
Nebulizer flow	1.05 L/min
Auxilliary flow	0.8L/min
Helium gas flow	4.2 mL/min
No. of Replicates	3
Mass of Lithium	7 amu
Mass of Vanadium	51 amu
Mass of Cobalt	59 amu
Mass of Nickel	60 amu
Mass of Copper	63 amu
Mass of Arsenic	75 amu
Mass of Cadmium	111 amu
Mass of Antimony	121 amu
Mass of Mercury	202 amu
Mass of Lead	208 amu
Wash time (s)	80 seconds
Up take time (s)	70 seconds
Dwell time (s)	0.01
Spacing (u)	0.1

Result and discussion:

Method linearity:

Method linearity was evaluated by analyzing a series of multi-element standards prepared at concentrations of 0.0, 0.5J, 1.0J, 1.5J,2.0J and 2.5J in 2.5% nitric acid and hydrochloric acid. Linear regression using the peak emission intensity counts of the standards as the y-axis and the concentration of the standards as the x-axis

yielded correlation coefficients (r2)>0.99 for all 10 elemental impurities evaluated. The results clearly demonstrate the linearity of the instrument response as a function of the concentration over the entire concentration range studied.

Precision:

Method precision was determined by repeated analysis of both PEGylated doxorubicin, evaluating the degrees of reproducibility among the replicate results from the same sample. To take the sample matrix into account, samples were spiked with each elemental impurity at 1J and carried through sample preparation. Replicate analysis of each sample gave the values listed in Table 4, The results showed <5.0% RSD for samples, indicating good precision of the method, significantly less than the 20% RSD stated in USP <233>.

Table-4: Results for Method Precision

Aspiration	**Amount of analyte in ppm**									
	Antimony	**Arsenic**	**Cadmium**	**Cobalt**	**Copper**	**Lead**	**Lithium**	**Mercury**	**Nickel**	**Vanadium**
Spiked Sample-1	11.149	1.863	0.237	0.563	34.301	0.495	29.627	0.333	2.293	1.153
Spiked Sample-2	11.200	1.849	0.237	0.567	34.396	0.491	29.765	0.336	2.289	1.157
Spiked Sample-3	11.181	1.857	0.232	0.565	33.438	0.515	29.850	0.324	2.197	1.149
Spiked Sample-4	10.107	1.707	0.215	0.523	30.569	0.464	28.146	0.308	2.043	1.067
Spiked Sample-5	11.517	1.811	0.235	0.573	33.677	0.505	30.721	0.335	2.243	1.156
Spiked Sample-6	11.115	1.878	0.231	0.563	34.510	0.500	30.374	0.328	2.288	1.179
Mean	**11.045**	**1.828**	**0.231**	**0.559**	**33.482**	**0.495**	**29.747**	**0.327**	**2.226**	**1.144**
% RSD	**4.4**	**3.5**	**3.5**	**3.2**	**4.6**	**3.4**	**3.0**	**3.4**	**4.4**	**3.4**

Accuracy:

Method accuracy was demonstrated by the recovery of a known amount of an elemental impurity spiked into each drug product. To demonstrate the accuracy of the method, replicate samples were spiked with each elemental impurity at 1J and carried through sample preparation. Table 5 summarizes the recovery results for the various samples. The results showed excellent recovery of the elemental impurities spiked into the samples. Spike recoveries for the elements studied ranged between 94.9% and 115.7%.

Table-5: Results for accuracy.

Element	**Concentration with respect to J value**			**Mean recovery (%)**
	50% level	**100% level**	**150% level**	
Cadmium	0.1 ppm	0.2 ppm	0.3 ppm	109.6
Arsenic	0.75 ppm	1.5 ppm	2.25 ppm	115.1
Lead	0.25 ppm	0.5 ppm	0.75 ppm	94.9
Cobalt	0.25 ppm	0.5 ppm	0.75 ppm	113.1
Mercury	0.15 ppm	0.3 ppm	0.45 ppm	100.0
Nickel	1.0 ppm	2.0 ppm	3.0 ppm	111.7
Vanadium	0.5 ppm	1.0 ppm	1.5 ppm	115.7
Lithium	12.5 ppm	25.0 ppm	37.5 ppm	110.5
Antimony	4.5 ppm	9.0 ppm	13.5 ppm	107.2
Copper	15.0 ppm	30.0 ppm	45.0 ppm	111.9

Limit of detection:

The determination of the Limit of Detection (LOD) in atomic emission spectroscopy, has been derived by Bauman's [7], using the signal-to-background ratio and the relative standard deviation of the signal background fluctuations. The LOD can thus be determined as the analytic concentration that yields a signal equal to three times the standard deviation (σ) of the background of instrument response for the blank. A blank was carried throughout the entire sample preparation process, with 12 replicate aliquots analyzed for all 10 elements. The LOD is defined as three times the standard deviation of the 12 measurements, while the Limit of Quantitation (LOQ) is defined as ten times the LOD. The results are given in Table 6. It can be seen that the LODs and

LOQs for the ten elemental impurities are all significantly below 0.5 J value level, in ug/L relative to these values, for parenteral drug doxorubicin injection.

Table-6: Results for LOD & LOQ Establishment.

Aspiration	**Sample Blank Intensity/Response (cps)**									
	Antimony	**Arsenic**	**Cadmium**	**Cobalt**	**Copper**	**Lead**	**Lithium**	**Mercury**	**Nickel**	**Vanadium**
1	5905	13	7	10	2180	447	120	240	243	12640
2	4244	50	7	7	2194	417	127	163	250	12323
3	3504	30	0	17	2037	390	160	227	230	12473
4	3120	30	7	10	2100	390	133	207	330	12186
5	2794	27	0	10	2030	450	93	233	230	12410
6	2754	33	3	40	4591	923	167	237	313	9143
7	2277	20	3	47	1970	423	127	220	403	13097
8	2040	33	7	33	1970	410	133	177	313	12743
9	2007	37	3	30	1863	380	110	187	430	12633
10	1880	40	10	37	1843	423	117	173	377	13140
Mean	3053	31	5	24	2278	465	129	206	312	12279
Standard deviation	1247.9	10.3	3.4	14.9	821.2	162.5	21.9	29.1	73.6	1144.1
Slope (Calibration standard curve)	17020.2	1610.6	8182.9	31752.5	25659.8	108860.5	129.4	23833.7	8595.0	11231.7
LOD (ppb)	22.5	3.75	0.5	1.25	75.0	1.25	62.5	0.75	5.0	2.5
LOD wrt sample (ppm)	1.125	0.1875	0.025	0.0625	3.75	0.0625	3.125	0.0375	0.25	0.0125
LOQ (ppb)	45.0	7.5	1.0	2.5	150.0	2.5	125	1.5	10.0	5.0
LOQ wrt sample (ppm)	2.25	0.375	0.05	0.125	7.5	0.125	6.25	0.075	0.5	0.25

Conclusion:

The low detection limits needed to determine a number of elemental impurities in pharmaceutical products and excipients can present challenges in using conventional ICP-MS with pneumatic nebulization. While ICP-MS has the ability to quantitatively measure extremely low levels of elemental impurities, relative to AAS. In this study we have seen that use of MWAD and pneumatic nebulizer gives significant improvement in detection limits for the determination of elemental impurities in parenteral drug products. Such a method for the analysis

of elemental impurities according to USP <233>, which employs ICP-MS, has been developed for 10 of the elemental impurities. The method has been shown to be linear, precise, and accurate over the range of 50% – 200% of the target value. The use of ICP-MS was considered adequate for the determination of elemental impurities in components (phospholipids and cholesterol) for parenteral drug product like doxorubicin, allowing accuracy better than 96%. By using pure grade solvents interference were reduced and relative standard deviations lower than 5% were obtained. By analyzing samples properly diluted, no problems related to loss of sensitivity were observed and the LODs obtained were in agreement with the limits proposed in USP. The proposed method can be applied and useful in routine analysis of components for parenteral injections, nutritional solutions with respect to Arsenic (As), Cadmium (Cd), Cobalt (Co), Copper (Cu), Mercury (Hg), Lithium(Li), Antimony(Sb), Nickel (Ni), Lead (Pb) and Vanadium (V) determination.

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