Analytical Method Development and Validation of an ICP-MS Method for the Determination of Elemental Impurities in Darunavir Tablets

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Abstract

The new guidelines of the United States pharmacopeia (USP), European pharmacopeia (EP) and international conference on harmonization (ICH) regulating elemental impurities in pharmaceutical dosage form. The new guidelines specify both daily doses and concentration/limits of elemental impurities in pharmaceutical final products, active pharmaceutical ingredients (API) and excipients. In chapter USP <233> method implementation, validation and quality control during the analytical process are described. A sensitive and selective method has been developed and validated for the simultaneous quantitative (As, Cd, Hg, Pb, V, Co, Ni, Mo, Mg, Cr) in Darunavir Tablets by inductively coupled plasma mass spectrometry. Inductively coupled plasma mass spectrometry is an advanced technique which is capable of analyzing multiple elements simultaneously with high selectivity, sensitivity and much lower detection limit. The test sample was prepared with microwave digestion and introduced into optimized instrumental parameters for use of a quadrupole based inductively coupled plasma mass spectrometry instrument. Gold, Scandium, Yttrium, Bismuth and Terbium is used as an internal standard in the study. The developed method was validated in terms of specificity, linearity, accuracy, precision, range, limit of detection, limit of quantitation and ruggedness. The results for recoveries of all elements were found between 90.5 to 110.2 %. The relative standard deviation for precision was within 15 %. Calibration plots were linear. The low relative standard deviation values and high recoveries of the method confirm the suitability of the method.

Key words: Darunavir, inductively coupled plasma mass spectrometry (ICP-MS), microwave digestion, method validation, Vanadium, Internal Standard

1. Introduction [1-8]

Presently in USP there is a Heavy Metal test <231> in General chapter for Metallic impurities. There are three different procedures for Metallic impurities in <231>.All three procedures have limitations viz. Qualitative, Visual comparison, no discrimination amongst different elements, Specificity, Recovery, safety concern, Toxicity concern and limits is based on visual comparison. All above limitations triggered the new standards to be establish for Metallic impurities. In USP there are three different General chapters related to Elemental Impurities. (1) <232> Elemental Impurities - limits (2) <233> Elemental Impurities-Procedures (3) <2232> Elemental contaminants in dietary supplements.

In ICH - Q3 D Elemental Impurities Guidance for Industry

1. Elemental impurities in Drug products (DP) may arise from several sources, they may be residual catalysts that were added intentionally in synthesis or may be present as impurities (e.g., through interactions with processing equipment or container/closure systems or by being present in components of the drug product) or Environmental contaminants.
2. When Elemental Impurities are known to be present, have been added or have the potential for introduction, assurance of compliance to the specified level is required.
3. A risk based control strategy may be appropriate to assure compliance with standard.
4. Due to ubiquitous nature of Arsenic, Cadmium, Lead & Mercury they must be considered in Risk assessment.

5. Regardless of the approach used, compliance with the limits specified is required for all drug products unless otherwise specified in an individual monograph or excluded as given in Scope.

The elements included in ICH Q3 D Placed into three classes based on their toxicity(PDE) likelihood of occurrence in the drug product. The likelihood of occurrence is derived from several factors including

- Probability of user in pharmaceutical process
- Probability of being a co-isolated impurity with other elemental impurities in materials used in pharmaceutical processes
- The observed natural abundance and environmental distribution of elements.

Elemental Impurity Classes are

- ❖ Class 1: Arsenic (As), Cadmium (Cd), Mercury (Hg), Lead (Pb).
- ❖ Class 2A: Cobalt(Co), Nickel(Ni), Vanadium (V).
- ❖ Class 2B: Silver (Ag), Gold (Au), Iridium (Ir), Osmium (Os), Palladium (Pd), Platinum (Pt), Rhodium (Rh), Ruthenium (Ru), Selenium (Se) and Thallium (Tl).
- ❖ Class 3: Barium (Ba), Chromium (Cr), Copper (Cu), Lithium (Li), Molybdenum (Mo), Antimony (Sb), and Tin (Sn).

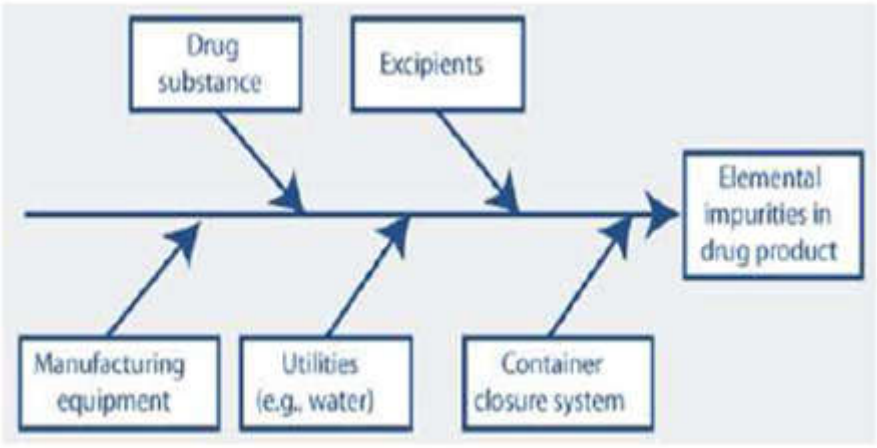

Figure 1 Potential Source of Elemental Impurities

2. Experimental

2.1 Chemical and Laboratory reagents

Trace metal grade concentrated Nitric acid (Fisher Chemical), Trace metal grade concentrated hydrochloric acid (Fisher Chemical), Trace metal grade hydrogen peroxide (30%) (Fisher Chemical) multi-element standard Sc, In, Y, In, Tb and Bi (Inorganic Ventures),V Standard (Inorganic Ventures), Co standard (Inorganic Ventures),Ni standard (Inorganic Ventures), Mo standard (Inorganic Ventures),Hg standard (Inorganic Ventures),Cr standard (Inorganic Ventures), Pb standard (Inorganic Ventures), Cd standard (Inorganic Ventures),As standard (Inorganic Ventures), Test materials were stored at the recommended storage conditions provided on the supplier's certificate of analysis. All solutions prepared from the test materials were stored at room temperature. All volumetric flasks used in preparations were polymethylpentene (PMP), polypropylene (PP) or equivalent quality polymer. All sample preparation were analysed on Mass Spectrometry and carried out under GMP.

2.2 Methodology

Diluent: Dilute 20.0 mL of Concentrated Nitric acid (Trace metal grade), 10.0 mL of hydrogen peroxide (30%) (Trace metal grade) and 10.0 mL of Concentrated Hydrochloric acid (Trace metal grade) to 500mL with purified water and mix.

Gold standard preparation: Transfer accurately 500 µL of Gold standard (1000 µg/mL) into 10 mL volumetric flask and dilute up to the mark with diluent and mix well.

Internal standard stock solution: Transfer accurately 1000 µL of (500 µg/mL Scandium, 125 µg/mL Yttrium, 12.5 µg/mL Bismuth and 5 µg/mL Terbium) and 500 µL of Germanium standard (1000 µg/mL) into 25 mL volumetric flask and dilute up to the mark with diluent and mix well.

Calibration blank preparation: Transfer 50 µL of gold standard solution and 250 µL of internal standard stock solution in to 50 mL volumetric flask and make volume up to the mark with diluent and mix well.

Standard solution: Transfer accurately 2000 µL of (5500 µg/mL Chromium, 1500 µg/mL Molybdenum, 125 µg/mL Manganese, 100 µg/mL Nickel, 50 µg/mL Vanadium, 25 µg/mL Cobalt, 15 µg/mL Mercury, 7.5 µg/mL Arsenic, 2.5 µg/mL Cadmium and 2.5 µg/mL Lead) into 25 mL volumetric flask and dilute up to the mark with diluent and mix well. And prepared calibration standard of LOQ/20% level (100 µL), 50% level (250 µL), 80% level (400 µL), 100% level (500 µL), 120% level (600 µL) ,150% level (750 µL) by diluting upto 50.0 ml.

2.3 Instrumentation

The method was developed and validated on Thermo Fisher Triple Quadrupole ICP-MS (TQ-ICP-MS), equipped with data acquisition and processing software Qtegra. The samples were digested using an Anton paar microwave digester. The ICP-MS parameters were optimized and optimized conditions are as below.

Method parameters

Measurement Mode	: KED
Dwell time	: 0.05 seconds
No. of sweep	: 25
No. of replicate	: 3
Auto sampler	: Wash time: 50 seconds, Uptake time: 80 seconds

Microwave digester parameter	
Application type	: Digestion
Vessel mode	: Multi vessel
Program type	: Temperature control
Rotor	: Rotor 24HVT50
Temperature control mode	: Average
Internal Temperature limit	: 180°C
Temperature	: 100°C
Ramp	: 10 minutes
Hold time	: 10 minutes
Temperature	: 170°C
Ramp	: 15 minutes
Hold time	: 35 minutes
Cooling temperature	: 60°C

3. Result and Discussion

Method Validation

The developed method was validated as per International Conference on Harmonization (ICH) Q2 (R1) United States Pharmacopoeia general chapter <233> and United States Pharmacopoeia general chapter <1225>.

3.1 System suitability and Linearity

System suitability and Linearity was demonstrated by injecting linearity solution [LOQ (20%) to 150 %] prepared as per the test method. The correlation co-efficient between concentration and response was evaluated.

Table 1 Correlation co-efficient of Elements

Name of Elements	Correlation co-efficient
Vanadium	1.00000
Chromium	0.99999
Manganese	0.99806
Cobalt	0.99998
Nickel	0.99997
Arsenic	0.99992
Molybdenum	1.00000
Cadmium	0.99998
Mercury	0.99998
Lead	0.99990

Acceptance criteria

The correlation coefficient should be not less than 0.990.

Conclusion

The results obtained meet the system suitability and linearity requirement, which indicates that the system is suitable and method is linear over the range of LOQ (20%) to 150 % of specification limit.

3.2 Specificity

Interference from calibration blank with each of the elements were studied by preparing the following specificity samples.

- By injecting calibration blank preparation.
- By preparing LOQ solution.

Following were the observations:
No significant interference was observed from calibration blank solution.

Acceptance criteria

The response of the elements from Calibration blank should be less than the response of elements of the standard preparation at LOQ level concentration.

Conclusion

The study meets the acceptance criteria.

3.3 LOD-LOQ

The limit of quantitation was established for each of the elements with respect to sample concentration. The precision at this concentration level has been established and limit of detection was considered as a concentration three times lesser than LOQ.

The responses obtained for Vanadium, Chromium, Manganese, Cobalt, Nickel, Arsenic, Molybdenum, Cadmium, Mercury and Lead content for the duplicate injections at LOD concentration and six replicate injections at LOQ concentration are tabulated.

Table 2 Established LOD-LOQ of Elements in (µg/g)

Sr. No.	Name of Elements	LOD(µg/g)	LOQ (µg/g)
1	Vanadium	1.334	4.002
2	Chromium	146.667	440.000
3	Manganese	3.333	10.000
4	Cobalt	0.667	2.000
5	Nickel	2.667	8.000
6	Arsenic	0.200	0.400
7	Molybdenum	40.000	120.000
8	Cadmium	0.067	0.200
9	Mercury	0.400	1.200
10	Lead	0.067	0.200

Table 3 Response of Elements in LOD Solution

Injection No.	Response				
	Vanadium	**Chromium**	**Manganese**	**Cobalt**	**Nickel**
1	34078	608132	76346	39798	42099
2	33784	601193	75669	39002	41245
Average	**33931**	**604662**	**76008**	**39400**	**41672**

Injection No.	Response				
	Arsenic	**Molybdenum**	**Cadmium**	**Mercury**	**Lead**
1	664	988876	1425	12788	23404
2	652	982912	1403	12666	23276
Average	**658**	**985894**	**1414**	**12727**	**23340**

Table 4 Response of Elements in LOQ Solution

Sample Set No.	Content (µg/g)				
	Vanadium	**Chromium**	**Manganese**	**Cobalt**	**Nickel**
1	35.236	2373.230	53.460	10.785	38.726
2	35.040	2350.466	52.888	10.660	38.430
3	35.134	2376.794	53.522	10.742	38.594
4	35.683	2394.507	53.775	10.782	38.873
5	35.546	2389.063	53.911	10.731	38.617
6	35.348	2390.893	56.508	10.674	38.388
Average	**35.331**	**2379.159**	**53.511**	**10.729**	**38.605**
% RSD	**0.7**	**0.7**	**0.7**	**0.5**	**0.5**

Sample Set No.	Content (µg/g)				
	Arsenic	Molybdenum	Cadmium	Mercury	Lead
1	3.296	757.344	1.275	6.839	1.113
2	3.214	753.030	1.262	6.814	1.093
3	3.231	749.385	1.259	6.826	1.106
4	3.274	751.575	1.260	6.852	1.105
5	3.213	751.758	1.255	6.792	1.103
6	3.223	745.248	1.245	6.759	1.150
Average	**3.242**	**751.390**	**1.259**	**6.814**	**1.111**
% RSD	**1.1**	**0.5**	**0.8**	**0.5**	**1.8**

Acceptance criteria

The detector response should be positive for LOD solution and the RSD at LOQ level for each element shall not be more than 20.0 %.

Conclusion

From the established data it can be concluded that the test method is capable of detecting and quantifying the Vanadium, Chromium, Manganese, Cobalt, Nickel, Arsenic, Molybdenum, Cadmium, Mercury and Lead content, if present in the sample, to the extent that mentioned in Table

3.4 Method Precision

Method precision was demonstrated by preparing sample as such as per the test method and six samples, in which Vanadium, Chromium, Manganese, Cobalt, Nickel, Arsenic, Molybdenum, Cadmium, Mercury and Lead content were spiked at respective specification level, representing a single batch. The elements were quantified for each of these samples. The precision of the method was evaluated by computing the percentage relative standard deviation for above mention elements.

Table 5 Results of Elements present in As such sample

Name	Content (µg/g)				
	Vanadium	Chromium	Manganese	Cobalt	Nickel
Sample as such	11.892	BDL	BDL	BDL	BDL
Name	**Content (µg/g)**				
	Arsenic	**Molybdenum**	**Cadmium**	**Mercury**	**Lead**
Sample as such	BDL	BDL	BDL	BDL	BDL

Table 6 Results of Spiked sample

Sample Set No.	Content (µg/g)				
	Vanadium	Chromium	Manganese	Cobalt	Nickel
1	35.236	2373.230	53.460	10.785	38.726
2	35.040	2350.466	52.888	10.660	38.430
3	35.134	2376.794	53.522	10.742	38.594
4	35.683	2394.507	53.775	10.782	38.873
5	35.546	2389.063	53.911	10.731	38.617
6	35.348	2390.893	56.508	10.674	38.388
Average	**35.331**	**2379.159**	**53.511**	**10.729**	**38.605**
% RSD	**0.7**	**0.7**	**0.7**	**0.5**	**0.5**

Sample Set No.	Content (µg/g)				
	Arsenic	Molybdenum	Cadmium	Mercury	Lead
1	3.296	757.344	1.275	6.839	1.113
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3	3.231	749.385	1.259	6.826	1.106
4	3.274	751.575	1.260	6.852	1.105
5	3.213	751.758	1.255	6.792	1.103
6	3.223	745.248	1.245	6.759	1.150
Average	**3.242**	**751.390**	**1.259**	**6.814**	**1.111**
% RSD	**1.1**	**0.5**	**0.8**	**0.5**	**1.8**

Acceptance criteria

The % RSD for elements of six preparations (N=6) should not be more than 20.0 %

Conclusion

As the precision results obtained are found within the acceptance criteria, this implies that the method is precise for quantification of elements.

3.5 Stability of Analytical Solution

For sample solution spiked with elements at respective specification level, the study was conducted at room temperature (about 25°C) up to 29 hours. The changes in responses of each element were evaluated.

Table 7 For sample solution at room temperature (about 25°C)

Time	Content (µg/g)				
	Vanadium	Chromium	Manganese	Cobalt	Nickel
Initial	35.236	2373.230	53.460	10.785	38.726
8 hours	36.183	2441.305	54.754	10.864	39.128
29 hours	40.207	2718.731	61.294	12.084	37.421
Time	**Content (µg/g)**				
	Arsenic	**Molybdenum**	**Cadmium**	**Mercury**	**Lead**
Initial	3.296	757.344	1.275	6.839	1.113
8 hours	3.268	748.812	1.253	6.757	1.106
29 hours	3.178	739.968	1.240	6.630	1.094

Table 8 % Deviation From Initial Results (From Initial Results)

Time	% Deviation from initial result				
	Vanadium	**Chromium**	**Manganese**	**Cobalt**	**Nickel**
8 hours	2.7	2.9	2.4	0.7	1.0
29 hours	14.1	14.6	14.7	12.0	-3.4
Time	**% Deviation from initial result**				
	Arsenic	**Molybdenum**	**Cadmium**	**Mercury**	**Lead**
8 hours	-0.8	-1.1	-1.7	-1.2	-0.6
29 hours	-3.6	-2.3	-2.7	-3.1	-2.1

Acceptance Criteria

For sample preparation

The elements in sample solution should not differ by more than 20.0 % from initial elements value.

Conclusion

Sample solution is stable at room temperature (about 25°C) up to 29 hours.

3.6 Accuracy

The accuracy of the method was demonstrated by preparing recovery samples (i.e. spiking sample with known quantities of elements) at the level of LOQ (20%), 50%, 100 % and 150 % of specification limit. The recovery samples were prepared in triplicate at each level. The above samples were analyzed in ICP-MS system and the percentage recovery for the amount added was estimated.

Table 9 Recovery Results of Vanadium (LOQ to 150%)

Sample No.	Recovery of Vanadium			
	LOQ (20%)	**50 %**	**100 %**	**150 %**
1	95.8	98.2	101.2	98.5
2	96.3	94.1	99.2	97.4
3	97.2	93.5	98.5	92.5
Average	**96.4**	**95.3**	**99.6**	**96.1**

Table 10 Recovery Results of Chromium (LOQ to 150%)

Sample No.	Recovery of Chromium			
	LOQ (20%)	**50 %**	**100 %**	**150 %**
1	110.2	105.2	101.2	100.2
2	105.2	110.2	99.5	96.5
3	101.2	106.5	98.5	98.5
Average	**105.5**	**107.3**	**99.7**	**98.4**

Table 11 Recovery Results of Manganese (LOQ to 150%)

Sample No.	Recovery of Manganese			
	LOQ (20%)	50 %	100 %	150 %
1	105.2	98.5	92.5	99.6
2	96.5	99.5	94.6	92.4
3	97.9	92.5	98.7	96.2
Average	**99.9**	**96.8**	**95.3**	**96.1**

Table 12 Recovery Results of Cobalt (LOQ to 150%)

Sample No.	Recovery of Cobalt			
	LOQ (20%)	50 %	100 %	150 %
1	95.8	96.4	91.5	95.4
2	98.5	98.6	99.6	96.8
3	99.9	99.2	98.5	94.6
Average	**98.1**	**98.1**	**96.5**	**95.6**

Table 13 Recovery Results of Nickel (LOQ to 150%)

Sample No.	Recovery of Nickel			
	LOQ (20%)	50 %	100 %	150 %
1	93.0	92.3	93.1	91.4
2	94.5	92.4	93.3	90.7
3	94.4	92.9	92.1	90.9
Average	**94.0**	**92.6**	**92.9**	**91.0**

Table 14 Recovery Results of Arsenic (LOQ to 150%)

Sample No.	Recovery of Arsenic			
	LOQ (20%)	50 %	100 %	150 %
1	104.5	105.1	106.4	106.2
2	107.8	104.5	107.6	106.5
3	105.3	104.8	107.8	107.8
Average	**105.9**	**104.8**	**107.2**	**106.8**

Table 15 Recovery Results of Molybdenum (LOQ to 150%)

Sample No.	Recovery of Molybdenum			
	LOQ (20%)	50 %	100 %	150 %
1	99.8	98.6	102.5	102.5
2	98.5	97.4	99.0	98.5
3	105.6	99.1	98.4	99.4
Average	**101.3**	**98.4**	**100.0**	**100.1**

Table 16 Recovery Results of Cadmium (LOQ to 150%)

Sample No.	Recovery of Cadmium			
	LOQ (20%)	50 %	100 %	150 %
1	98.5	99.7	105.6	102.5
2	94.5	95.1	102.0	100.8
3	98.9	102.5	99.8	105.6
Average	**97.3**	**99.1**	**102.5**	**103.0**

Table 17 Recovery Results of Mercury (LOQ to 150%)

Sample No.	Recovery of Mercury			
	LOQ (20%)	50 %	100 %	150 %
1	102.5	104.3	100.5	99.8
2	110.2	101.2	102.3	102.5
3	106.5	105.6	106.5	103.2
Average	**106.4**	**103.7**	**103.1**	**101.8**

Table 18 Recovery Results of Lead (LOQ to 150%)

Sample No.	Recovery of Lead			
	LOQ (20%)	50 %	100 %	150 %
1	99.8	92.5	102.5	95.2
2	94.5	96.8	99.8	96.8
3	96.8	99.7	96.5	98.5
Average	**97.0**	**96.3**	**99.6**	**96.8**

Acceptance criteria

The recovery should be between 70.0 – 130.0 % for each of the replicate preparations at each concentration.

Conclusion

As the recovery results obtained for elements were within the acceptable limits of recovery, the study proves that the method is accurate for quantification of each element at LOQ to 150 % of specification limit.

3.7 Robustness

Concentration of acid and hydrogen peroxide in sample solution during sample preparation was changed by ± 10% [1800 µL of concentrated nitric acid, 900 µL of hydrogen peroxide (30%) and 900 µL of concentrated hydrochloric acid for -10 % and 2200 µL of concentrated nitric acid, 1100 µL of hydrogen peroxide (30%) and 1100 µL of concentrated hydrochloric acid for +10 %].

Table 19 Results of Robustness for Change in Concentration of Diluent

Condition	Content (µg/g)				
	Vanadium	**Chromium**	**Manganese**	**Cobalt**	**Nickel**
Normal	35.236	2373.230	53.460	10.785	38.726
– 10 %*	35.610	2389.689	53.742	10.685	38.482
+ 10%#	35.881	2408.014	54.172	10.721	38.712
Condition	**Content (µg/g)**				
	Arsenic	**Molybdenum**	**Cadmium**	**Mercury**	**Lead**
Normal	3.296	757.344	1.275	6.839	1.113
– 10 %*	3.207	740.529	1.236	6.748	1.095
+ 10%#	3.191	740.605	1.242	6.745	1.099

Table 20 % Deviation From Initial Results for Change in Concentration of Diluent

Condition	% Deviation from initial result				
	Vanadium	**Chromium**	**Manganese**	**Cobalt**	**Nickel**
– 10 %*	1.1	0.7	0.5	-0.9	-0.6
+ 10%#	1.8	1.5	1.3	-0.6	0.0
Condition	**% Deviation from initial result**				
	Arsenic	**Molybdenum**	**Cadmium**	**Mercury**	**Lead**
– 10 %*	-2.7	-2.2	-3.1	-1.3	-1.6
+ 10%#	-3.2	-2.2	-2.6	-1.4	-1.3

Assessment of robustness study

The elements in changed condition of sample solution should not differ by more than 20.0 % from normal condition of elements value.

Conclusion

The % difference of each element from the normal condition to changed condition does not exceed 20 %, it proves that the method is robust.

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