Section A-Research paper

Risk assessment and Analytical Method Validation for Determination of Elemental Impurity in Nivolumab by using inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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ABSTRACT

Nivolumab is a PD-1 blocking antibody used to indulgence of renal cell cancer, head and neck cancer ,melanoma, non small-cell lung cancer, and Hodgkin lymphoma.

Nivolumab drug risk assessment and Analytical Method Validation were developed and analyzed for determination of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, , Tl ,V, Mg and Al by ICP MS. operated at standard mode, while pneumatic nebulization was used for introducing the sample solution into the ICP. Mercury was determined using cold vapor generation (CVG) coupled to ICP-MS. Chromium, Mn, Ni and V were detected by means of dynamic reaction cell-inductively coupled plasma mass spectrometry (DRC–ICP-MS).

The operational conditions of each technique were optimized in order to achieve better sensitivity, precision and accuracy. The influence of the sample matrix, mainly carbon, on all investigated elements was evaluated. The use of DRC was effective to reduce interferences on Cr, Mn, Ni and V determination. The other investigated elements (As, Cd, Cu, Pb, Mo and Hg) were determined directly in the samples, which were properly diluted. Results obtained were in good agreement (between 96 and 105%) with certified values (certified reference materials of water were analyzed), at the same time as the relative standard deviation was lower than 5%. Sample throughput was relatively high (up to 30 samples of components used for parenteral nutrition solution could be analyzed per hour). In this way, the proposed method can be recommended for routine analysis.

KEY WORDS: ICPMS, ICH Q3D, Nivolumab and ICH Guidelines.

1. INTRODUCTION

Nivolumab is a type of drug called an immune checkpoint inhibitor which is used to control and treat metastatic melanoma and various other tumours. It is a monoclonal antibody that works by binding to the anti-PD1 receptor, on the surface of certain immune cells called T cells, which keeps cancer cells from suppressing the immune system. This allows the immune system to attack the cancer cells.

This activity outlines the indications, mechanism of action, and administration for Nivolumab to manage advanced cancers.

Based on the previously mentioned analytical techniques, our primary goal is to develop an efficient, rapid, sensitive, selective, linear, and accurate ICPMS approach for Nivolumab determination. The process was evaluated using ICH criteria and USP 26. Linearity, accuracy, precision, specificity, the limit of detection (LOD), and the limit of quantification (LOQ) are performed and utilized to evaluate the drug concentration of Nivolumab in various pharmaceutical products by ICH Q2R1 guidelines. [12, 13]

The classical methods for the determination of elemental impurities is wet chemistry analysis include titrimetry , gravimetry and colorimetry. The process of colorimetry based on changes in color to show qualitative chemical measurements and identify elements. Gravimetric analysis entails the measurement of solids precipitated and weighed from a sample after dissolution. Titration can be used to determine the concentration of a known reactant. Titrations often use visual indicators, such as a color change in the reactant mixture, to indicate the endpoint of a reaction (1)

This technique has several tribulations, it is indistinct, nonspecific, prone to low recuperations recoveries, workup is not optimized and upgraded for volatile elements and is very subjective, the colour change is relatively unpretentious and is very contingent on the operator and is also irksome if a coloured test solution is obtained. Therefore, great effort is required for the development of new advanced methods for the proper risk assessments and multi-element screening to control metals in pharmaceuticals drug product and (API) Active Pharmaceutical Ingredients that rely on modern advance analytical methodologies like ICP-MS.

Various applications of ICP-MS technique are there such as determination of single element, multi element analysis in synthetic drugs, heavy metals in environmental water, determination of toxic and essential elements in different varieties of food samples. It is important and essential that impurities generated in pharmaceuticals at various stages of development, transportation and storage must be detected and quantified. ICP-MS plays an important function in the recognition and revealing of elemental impurities (2).

Nivolumab has a molecular formula of $C_{6362}H_{9862}N_{1712}O_{1995}S_4$ and a molecular weight of 143599.39 g/mol.

Nivolumab is an oncologic class of drugs used to treat different types of cancers as well as tumors. [1] This includes melanoma, lung cancer, renal cell carcinoma, Hodgkin lymphoma,

head and neck cancer, colon cancer, and liver cancer. It is used by slow injection into a vein, they observed side effects including tiredness, rash, liver problems, muscle pains, and cough. [2] Nivolumab is a human IgG4 monoclonal antibody that blocks the PD-1 receptor and shows the mechanism of action. It is a type of cancer treatment drug called immunotherapy and works as a turnpike inhibitor, blocking a wave that prevents activation of T cells from aggressive cancer. [3] The first checkpoint immunotherapeutic drug to receive regulatory authorization for NSCLC is nivolumab. Nivolumab generates a partial or complete tumor response in 15-20% of patients, independent of the number of prior lines of anti-cancer therapy, by permitting host immune-mediated cytotoxic action against tumor cells. [4]

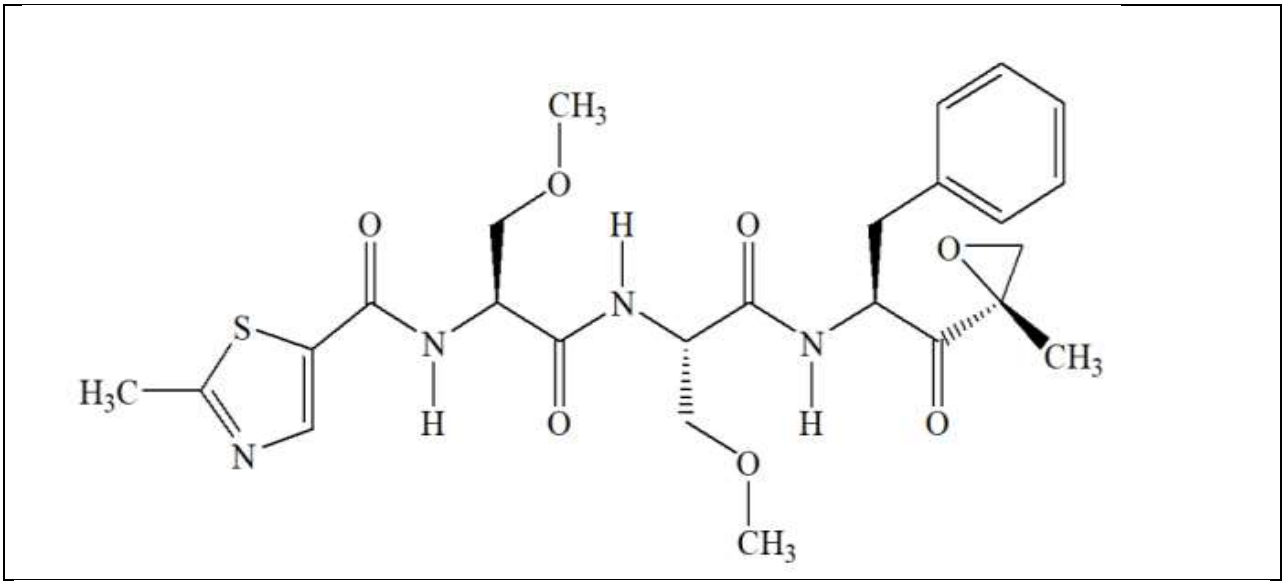

FIGURE 1: Chemical Structure of Nivolumab

The purpose of this enhanced research study is preliminary risk assessment and multi-elemental screening of elemental impurity in Nivolumab by using ICP-MS method for determination of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in terms of specificity, linearity, accuracy and quantitation limits. The method is based on analysis performed with (ICPMS) inductively coupled plasma mass spectrometer Agilent, Model 7700x and Mass hunter workstation software A.01.02.

2. MATERIALS AND METHODS:

Reagents and Chemicals:

The reagents used in this method for preparation of the samples and standard are trace metal grade ultrapure concentrated nitric acid, hydrochloric acid (J.T.Baker), Perchloric acid from (Inorganic Venture) and Ultrapure de-ionized water from a Milli-Q analytical reagent grade. All volumetric flasks used in preperations were made up of polypropylene (PP) and polymethyl pentane (PMP).

Standard Stock solution 1 (SS-1) preparation

Transfer into 50 mL volumetric flask 1.0 mL of concentrated nitric acid and 250 µL of certified reference solution of each Cadmium, Lead and Cobalt, 750µL of certified reference solution of Arsenic, 1500µL of certified reference solution of Mercury, 1000µL of certified reference solution of Nickel and 400µL of certified reference solution of Thallium.

Dilute to volume with de-ionised water.

Standard Stock solution 2 (SS-2) preparation

Transfer into 50 mL volumetric flask 1 mL of concentrated Nitric acid, 2 mL concentrated hydrochloric acid and 1000 µL of Stock solution I(SS-1), 550 µL of certified reference solution of Lithium, 1100 µL of certified reference solution of Chromium , 300 µL of certified reference solution of each copper and molybdenum, 600 µL certified reference solution of Tin, 1200 µL certified reference solution of Antimony, 1400 µL certified reference solution of Barium, 150 µL certified reference solution of each selenium, Silver, 100 µL certified reference solution of each Vanadium, Palladium, Iridium, Platinum, Rhodium, Ruthenium and Gold. Dilute to volume with de-ionised water.

Internal standard stock solution preparation (ISTD)

Transfer 250µL certified reference solution each of Dysprosium and Germanium into 10 mL volumetric flask made of polypropylene and add 100µL concentrated nitric acid dilute to volume with de-ionized water.

Final working standards solution

Take three separate 50 mL volumetric flasks made of polypropylene or polymethylpentene, add 4 mL of concentrated nitric acid, 0.25 mL of concentrated Perchloric acid and 1 mL of concentrated hydrochloric acid and, added Standard Stock solution 2 (SS-2) as per following,

Table 1: Preparation of working standard solutions:

Label	WS-1	WS-2	WS-3
SS -2	150µL	750µL	1000µL
ISTD	100µL	100µL	100µL
Added Volume of Mg	30µL	150µL	200µL
Added Volume of Al	30µL	150µL	200µL
De-ionized water	Dilute to the Volume up-to 50mL		

Table 2: Working standard solutions concentration,

Name of Elemental Impurity	Concentration in c / ng mL^{-1}		
	WS-1	**WS-2**	**WS-3**
Li	33	165	220
V	6	30	40
Cr	660	3300	4400
Co	3	15	20
Ni	12	60	80
Cu	180	900	1200
As	0.9	4.5	6.0
Se	9	45	60
Mo	180	900	1200
Ru	6	30	40
Rh	6	30	40
Ag	9	45	60
Cd	0.3	1.5	2.0
Sn	360	1800	2400
Sb	72	360	480
Ba	84	420	560
Pd	6	30	40
Ir	6	30	40
Pt	6	30	40
Au	6	30	40
Hg	1.8	9.0	12.0
Tl	0.48	2.40	3.20
Pb	0.3	1.5	2.0
Mg	600	3000	4000
Al	600	3000	4000

Quality check solution (QC)

Working standard 2 is used as QC solution.

Blank Solution (Calibration Blank)

Transfer into 50 mL volumetric flask made of polypropylene or polymethylpentene 4 mL of concentrated nitric acid, 0.25 mL of concentrated Perchloric acid, 100 µL of ISTD solution as internal standard and 1 mL of concentrated hydrochloric acid, Dilute to volume with de-ionized water.

Sample Solution preparation (T)

Prepare in duplicate ,Weigh about 100 mg of the substance into digestion tube add 4 mL of concentrated nitric acid, 0.25 mL of concentrated Perchloric acid and 100 µL of ISTD solution as internal standard. Shake each mixture carefully. Wait at least 30 min before the vessels are closed. Heat the sample in the microwave reactor, when the microwave reactor temperature program finishes, allow the digestion vessels to cool down and then transfer sample solutions into a 50 mL volumetric flasks made of polypropylene or polymethylpentene and add 1 mL of concentrated hydrochloric acid. Dilute to volume with de-ionised water.

Calculation formula

Analyte content in ppm is calculated using the following formula:

$$\text{Metal content (ppm) in Sample} = \frac{c\left(\frac{ng}{mL}\right) \times V(mL)}{m(mg)}$$

c (ng/mL) = instrument read-out concentration

V (mL) = final volume of dissolved sample (50 mL)

m (mg) = sample weight (100 mg)

Spiked Sample preparation

Spiked samples were prepared by spiking reference standard materials of the target limit concentration at 50 %(0.5J), 100 %(1J) and 150 %(1.5J) with 250µL, 500µL and 750µL of the standard stock solution 2 (SS-2) alongwith 100µL of internal stock standard solution.

Three preperations at each spiking level were prepared and each solution was analysed in triplicate measurement.

3. MEASUREMENT

Determine Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V, Mg and Al in sample solutions by Inductively Coupled Plasma Mass Spectrometry using the method of direct calibration. Introduce the OISTD solution via a t-piece incorporated into the sample introduction tube before the nebulizer.

3.1 Risk Assessment and Multielemental elemental impurity screening: The purpose of this work to demonstrate Risk Assessment and Multielemental elemental impurity screening for determination of elemental impurity in Nivolumab. Content of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl,V, Mg and Al in with Sc and Dy as internal standard will be determined by Inductively Coupled Plasma Mass Spectrometry.

Method validation: The new analytical development method was validated as per ICH Q2 (R1) guidelines .The following parameters have been tested during this Risk Assessment and Multi elemental impurity screening study:

I. Linearity& Range
II. Accuracy and Recovery
III. Specificity
IV. Quantitation

V. Precision

The Risk Assessment and Multi elemental impurity screening study was performed according to ICH Q3D guidelines[6,7&8.]

I. Linearity & Range:

Linearity is the ability of the analytical method to obtain test results that are directly, or by a well defined mathematical transformation, proportional to the concentration (or amount) of Analyte in samples within a given range.

Working standard solutions for linearity

To demonstrate Linearity, five (5) solutions at different concentrations were prepared, WS-1 Solution, WS-2 Solution, WS-3 Solution, WS-4 Solution and WS-5 Solutions. Transfer into five separate 50 mL volumetric flasks made of polypropylene or polymethylpentene add 4 mL of concentrated nitric acid, 0.25 mL of concentrated perchloric acid and 1 mL of concentrated hydrochloric acid and added as per below table.Each solution was analyzed in triplicate measurements.

Table 3: Working Standard Solution preparation for Linearity test.

Label	WS-1	WS-2	WS-3	WS-4	WS-5
Added Volume of SS-2	150 µL	250 µL	500 µL	750 µL	1000 µL
Added Volume of Mg	30 µL	50 µL	100 µL	150 µL	200 µL
Added Volume of Al	30 µL	50 µL	100 µL	150 µL	200 µL
Added Volume of ISTD	100 µL	100 µL	100 µL	100 µL	100 µL
De-ionized water	Dilute to the volume up-to 50mL				

Procedure:

1. Analyzed the Calibration Blank Solution, Working S-1 Solution, WS-2 Solution, WS-3 Solution, WS-4 Solution and WS-5 Solution.
2. Determined the amount/concentration of Analyte in each solution.
3. Plotted a Linearity curve for linear regression of the sample solutions.
4. Correlation Coefficient (R) was calculated.

Acceptance criteria:

The calculated Correlation Coefficient (R) from Sample Solutions is not less than NLT 0.990.
The results of linearity of detector response for Multielements are presented in the tables and figures below.

Table 1: Linearity summary results:

Sr.No.	Elemental metal	Correlation Coefficient	Acceptance criterion	Sr.No.	Elemental metal	Correlation Coefficient	Acceptance criterion
1	Lithium	0.9997	>0.99	14	Cadmium	1.0000	>0.99
2	Vanadium	0.9999		15	Tin	1.0000	
3	Chromium	1.0000		16	Antimony	0.9998	
4	Cobalt	1.0000		17	Barium	1.0000	
5	Nickel	1.0000		18	Iridium	1.0000	
6	Copper	1.0000		19	Platinum	1.0000	
7	Arsenic	0.9999		20	Gold	1.0000	
8	Selenium	0.9997		21	Mercury	1.0000	
9	Molybdenum	1.0000		22	Thallium	0.9993	
10	Ruthenium	1.0000		23	Lead	1.0000	
11	Rhodium	1.0000		24	Magnesium	0.9999	
12	Palladium	1.0000		25	Aluminum	0.9999	
13	Silver	1.0000					

Hence Linearity result meets the Acceptance Criteria.

Conclusion: Risk assessment and multielemental screening of elemental impurity in Nivolumab for determination of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl,V, Mg and Al in with Sc and Dy as internal standard [10] in Nivolumab substance is linear by using ICP-MS.

II. Accuracy/Recovery

Accuracy is the closeness of test results obtained by the method to the true value. It may often be expressed as percent recovery by the assay of known, added amount of analyte. To demonstrate accuracy, a single unspiked sample and samples spiked with reference material, prepared in triplicate (3x), at concentrations of 0.5J, 1J, and 1.5J were prepared. Each solution was analyzed in triplicate measurements.

Table 5: Concentration levels for Accuracy test

Concentration in c / ng mL^{-1}				
Accuracy level	**Level 1 (0J)**	**Level 2 (0.5J)**	**Level 3 (1J)**	**Level 4 (1.5J)**
Added Standard Stock (µL)	-	100	200	300

A Standard stock solution um was added to nine sample solutions as shown in the table below.

Table 6: Preparation of solutions for Accuracy test:

Flask No.	1-3	4-6	7-9	10-12
Added Volume of SS-2	-	250 µL	500 µL	750 µL
Added Volume of Mg	-	50 µL	100 µL	150 µL
Added Volume of Al	-	50 µL	100 µL	150 µL
Added Volume of ISTD	100 µL	100 µL	100 µL	100 µL
De-ionized water	Dilute to the volume			

Procedure:

1. Analyzed the Unspiked Sample Preparation, 0.5J Spiked Sample Preparations, 1J Spiked Sample Preparations, and 1.5J Spiked Sample Preparations.
2. Determined the amount/concentration of Analyte in each solution.
3. Calculated the Percent Recovery for the average results of triplicate preparations at each concentration level for the Analyte.
4. Calculated the Relative Standard Deviation (RSD) of the triplicate results for each concentration.

Acceptance Criteria:

1. Percent Recovery is 70% - 150% for the average results of triplicate preparations at each concentration of the Analyte.
2. The calculated Relative Standard Deviation (RSD) from the triplicate results of each concentration is NMT 20%.

The calculated contents of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl,V, Mg and Al in with Sc and Dy as internal standard [10] are given in the table below and statistically evaluated.

Table 7: Percentage (%) Recovery for all USP restricted elements analysed in this research study at three different concentration levels are:

Element	**% Recovery at 0.5 J**		**% Recovery at 1.0 J**		**% Recovery at 1.5 J**	
	Mean %	**RSD%**	**Mean %**	**RSD%**	**Mean %**	**RSD%**
Li	104	1.5	107	1.4	105	4.4
V	98	2.6	97	1.3	99	2.4
Cr	98	2.0	99	1.3	100	4.4
Co	101	2.2	100	1.3	99	4.7
Ni	105	7.4	100	1.3	99	4.9
Cu	100	1.7	99	0.7	104	4.4
As	99	2.9	96	1.5	96	4.8

Se	95	2.8	94	2.0	91	3.2
Mo	101	1.7	103	1.3	102	4.4
Ru	103	1.6	103	0.7	101	4.4
Rh	101	1.7	101	0.9	98	4.3
Pd	102	1.3	102	1.2	100	4.3
Ag	99	1.1	101	1.0	98	4.4
Cd	94	2.0	95	1.0	92	4.8
Sn	101	1.4	102	2.3	104	4.9
Sb	94	0.5	99	2.1	99	2.9
Ba	99	1.4	101	1.2	101	3.1
Ir	99	1.5	101	0.9	101	2.8
Pt	101	2.5	102	1.7	99	4.4
Au	96	2.5	98	1.7	95	4.4
Hg	101	1.0	101	0.9	98	4.3
Tl	90	2.2	93	1.9	92	4.3
Pb	92	3.0	97	1.5	96	4.0
Mg	101	3.0	101	1.1	100	4.2
Al	101	4.1	103	4.1	99	4.3

Conclusion: Method for determination of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V, Mg and Al in Nivolumab substance is accurate.

SPECIFICITY

Specificity is the ability of the analytical method to measure accurately and specifically the analyte in the presence of components that may be expected to be presented in the sample matrix, e.g. other metal impurities. Specificity is a measure of the degree of interference (or absence thereof) in the analysis of complex sample mixtures.

To evaluate specificity of the method for the contents of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V, Mg and Al in Nivolumab were compared with contents of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V, Mg and Al contents in Nivolumab in Accuracy test (Level 2).

Conclusion: Method for determination of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V, Mg and Al contents in Nivolumab is specific. All results met Acceptance Criteria for Specificity. Method adequately demonstrated Specificity for elemental Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl,V, Mg and Al contents in Nivolumab.

QUANTITATION LIMIT

Quantitation Limit (QL) is a parameter for low levels of compounds in sample matrices, such as metal impurities. It is the lowest concentration of analytes in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.
To determine QL, the samples were spiked with reference material at concentrations of 0.3J.

Analyte	*Class 1*				*Class 2A*			*Class 2B*									*Class 3*						
	Cd	Pb	As	Hg	Co	V	Ni	Tl	Ag	Se	Au	Ru	Rh	Pd	Ir	Pt	Li	Sb	Ba	Cu	Mo	Sn	Cr
QL (0.3J) ng mL^{-1}	0.3	0.3	0.9	1.8	3	6	12	0.48	9		6						33	72	84	180		360	660

Stock solutions of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl,V, Mg and Al contents in Nivolumab [10] were added to six sample solutions at concentration of 0.3J before microwave digestion as shown in the table below.

Label	QL Sample Solutions (0.3J)
SS	150 µL
ISTD	100 µL

Procedure:

1. Analyzed the 0.3J QL Sample Solution six (6) times each, while appropriately bracketed by the 0.3J Quality Check Solution.
2. Determined the amount/concentration of Analyte in the solutions.

Acceptance Criteria:

1. Percent Recovery is 70% - 150% for the average results of triplicate preparations at each concentration of the Analyte.
2. The Relative Standard Deviation (RSD) of the analyte from the six (6) preparations is NMT 20%.

Table 8: Summarized results of Quantitation Level:

% Recovery at Quantitation Level 0.5 J		
Element	**% Average recovery**	**RSD%**
Li	108	5.2
V	98	2.2
Cr	96	1.1

Co	103		1.4
Ni	104		1.4
Cu	100		1.1
As	99		1.8
Se	94		5.4
Mo	97		0.8
Ru	104		0.9
Rh	101		0.8
Pd	101		0.7
Ag	98		1.1
Cd	95		1.2
Sn	1.3		1.2
Sb	95		0.9
Ba	1.2		1.1
Ir	102		0.9
Pt	102		1.1
Au	90		1.6
Hg	102		0.9
Tl	91		0.9
Pb	89		7.3
Mg	102		2.5
Al	104		2.1
Acceptance Criteria	For Recovery	70-150%	Hence Result meets the Acceptance Criteria.
	For RSD	$\leq$20%	Hence Result meets the Acceptance Criteria.

Application of method: This method is very advance and sophisticated reliable. This method is accurate precise and technically advance and less hazardous method which can be considered for risk assessment and multi elemental screening assessment. It is therefore essential to develop such kind of ICPMS methods which will characterize and determine identified as well as unidentified impurities present in active pharmaceutical ingredients. This method is used to analyze and determine the probability of possible elemental metal degradation impurities.

Precision (including Repeatability) & Intermediate Precision

A second analyst on a different day prepared and analysed six preperations of approximately 100% target limit spiked samples (J) with freshly prepared spiking solutions.The samples were quantified against fresh calibration standards.

The result determine for Intermediate Precision /Ruggedness are summarised in **Table 9.** For each element the second analyst/day mean for all six preparations was within ±20% of the mean result for the first analysis. The % RSD for all preperations (n=12) for each target element was ≤ 25%.All acceptance criteria described in USP Chapters <233/232> for ruggedness were met.

Table 9: Summarized results of Precision & Intermediate Precision

Element	For Precision %RSD (for n=6 Preperations)	For Intermediate Precision %RSD (for n=6 Preperations)	Overall %RSD (for n=12 Preperations)
Li	2.1	1.5	2.2
V	3.1	3.5	3.2
Cr	2.2	2.5	3.5
Co	2.1	2.4	2.2
Ni	1.8	2.5	2.1
Cu	2.1	2.3	2.2
As	3.4	3.4	3.7
Se	3.0	2.8	3.8
Mo	2.1	2.1	5.4
Ru	2.6	2.3	2.4
Rh	2.2	2.3	2.3
Pd	2.1	2.4	2.3
Ag	2.3	2.2	2.2
Cd	3.3	1.6	3.0
Sn	2.2	1.8	3.0
Sb	2.1	1.9	6.5
Ba	2.2	1.6	2.7
Ir	2.9	2.0	2.1
Os	2.3	2.0	2.4
Pt	2.1	1.8	1.8
Au	2.0	1.9	1.9
Hg	2.3	1.9	2.2
Tl	2.2	2.1	2.0
Pb	3.1	3.7	3.3

4. DISCUSSION: This method is developed to be cost effective and less hazardous method which is consider for safety risk assessment, so it is therefore essential to develop such kind of ICPMS methods which will characterize and determine identified as well as unidentified

multielemental impurities present in active pharmaceutical ingredients. This method is used to analyze and determine the probability of possible multielemental impurities.

5. CONCLUSION:

The proposed ICPMS method was developed and validated for the contemporaneous evaluation of Nivolumab within the pharmaceutical dosage form. The proposed method was validated following ICH Q2(R1) guidelines by testing its parameters, including linearity, precision, accuracy, LOD, and LOQ. The newly developed method was precise sensitive, accurate, cost-effective, and scant which can be espoused in regular internal control tests in pharmaceutical industries.

Method adequately demonstrated Specificity,Linearity Quantitation and Accuracy/recovery for risk assessment and multi elemental screening of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V, Mg and Al contents [10] in Montelukast sodium by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).All results met Acceptance Criteria for Specificity. Method adequately demonstrated Specificity for elemental Ca contents in Nivolumab. All system suitability parameters are with in range and satisfactory as per ICH guidelines[8].

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