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## RESEARCH ARTICLE

### Development and Validation of Inductively Coupled Plasma Mass Spectrometry Method for Simultaneous Determination of TIN(SN) content in Excipients and Formulations

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#### ABSTRACT

The pharmaceutical formulation manufacturing process use some of polymers, which contains traces of inorganic elements like Tin (Sn) content. The traces of inorganic elements were determined by using inductively coupled plasma mass spectroscopy (ICP-MS). It is a sensitive method used to determine the inorganic elements by atomic mass value. In the present study Tin (Sn) was extracted by the sample using microwave-assisted digestion with hydrochloric acid. Different validation parameters were employed to evaluate the method in terms of selectivity, specificity, method precision, accuracy, intermediate precision and standard measurement uncertainty. The mass value of tin element is 118 as per the perkin Elmer instrument recommendations. The results indicate that this method could be used for the routine measurement of tin (Sn) in the pharmaceutical laboratories.

**Keywords:** inductively coupled plasma mass spectrometry, Analytical Method development, Analytical Method validation and Tin (Sn) content

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## 1. Introduction

The pharmaceutical formulation such as injections, lyophilized powder and some solid dosage forms, use polymers for the homogenization in the manufacturing process [1], The pharmaceutical formulation contain mixture of ingredients that have been proven suitable for humans [2]. The copolymer poly (D,L-lactide-co-glycolide) is one of the best biodegradable polymer. Used for medical applications. It is very favorable polymer to easily bioreabsorbable, biocompatible and non-toxic, while its degradation kinetics can be modified by the copolymerization ratio of the monomers. By opening the rings of the cyclic dimers of the D,L-lactide and glycolide monomers in the presence of stannous octoate initiator and lauryl alcohol co-initiator. The synthesized polymers contains some trace elements [3] such as arsenic (As), copper (Cu), chromium (Cr), selenium (Se), tin (Sn), cadmium Cd, Antimony (Sb) and Lead (Pb) [4]. Out of these elements tin (Sn) is the major trace element. The analytical techniques were used for identification and characterization are differential Scanning Calorimetry (DSC), Thermogravimetry (TG), Nuclear Magnetic Resonance Spectroscopy (NMR) and Fourier Transform Infrared Spectroscopy (FTIR).

Laser ablation (LA) [5] in combination with inductively coupled plasma–mass spectrometry (ICP-MS) is a powerful technique for the direct elemental analysis of solid samples. The application of vacuum to the reaction medium, coupled with adequate stirring, is essential for obtaining good results. The input monomers and the reaction products were analyzed. Important elemental characteristics, such as melting temperature, glass transition temperature, thermal stability, chemical composition and the ratio of the monomers in the synthesized copolymer, were obtained from these analyses. These results helped to infer the absence of residual monomers in the synthesized copolymers. In pharmaceutical organization, drug product manufacturing process uses some of the polymers [6-9].

As per the International Conference On Harmonization the tin content was allowable for oral dosage form 6400 PDE ( $\mu\text{m}/\text{day}$ ), parental 640 PDE ( $\mu\text{m}/\text{day}$ ) and inhalation 64 PDE ( $\mu\text{m}/\text{day}$ ) [10-13]. Tin (Sn) is a silvery-white metal that exists in valence states of 2 and 4. The most important inorganic compounds of tin are its oxides, chlorides, fluorides and halogenated sodium stannates and stannites. Tin is present in some multi-vitamin and mineral food supplements (levels up to 10  $\mu\text{g}$  Sn/tablet). Tin is possibly nutritionally essential for some animals, it has not been shown to be essential for humans. Tin (II) chloride is being used as a reducing agent, and as a stabilizer of polyvinylchloride (PVC). This safety assessment focuses on inorganic tin considering that the more frequent occurrence of inorganic tin is more relevant with respect to metal impurities in drug products than organic tin compounds. By hydride generation inductively coupled plasma mass spectrometry using there for it is essential to develop a method and validate as per the ICH and FDA regulation [14-18].

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## 2. Materials and Methods

### Chemicals

The Tin reference standard material (SRM316) a certified by National Institute of Standards and Technology used as a primary calibration standard for the quantitative determination of tin (Sigma Aldrich, USA). The solution is prepared gravimetrically and contains 10.0 mg/g of nitric acid at a volume fraction of approximately (5%) and hydrofluoric acid at (1 %), equivalent to 0.86 mol/L and 0.28 mol/L, respectively. HPLC grade water was obtained from Merck (Darmstadt, Germany). All other chemicals used in the present study were analytical grade and obtained from commercial source.

### Instrumentation:

The ICP MS system was used for the method development and method validation consists of sample injecting system, inductively coupled plasma, plasma sampling interface and mass spectrophotometer. The output signal was monitored and processed using dellwork station computer with Empower software (Version 3). Microwave digester (perkin elmer) and photo stability studies were carried out using photo stability chamber (Newtronic life care, India). Thermal stability studies were performed using dry hot air oven (Newtronic life care, India).

### Preparation of standard solutions and sample solutions:

The standard solution was prepared by dissolving reference standard material in diluent (consist of 125 mL of concentrated nitric acid and 125 mL of concentrated hydrochloric acid and subsequently diluted to 30 ppb to 600 ppb. The test sample solution (1 mg/mL) was prepared as per the specification limit with 25 ppm of tin.

### Method development

#### Method development and optimization

The method development study was initiated with as per the user Manuel conditions 1, 2 and 3 and microwave digestion conditions. In experiment 1: The polymer was mixed with 1 mL of nitric acid, 1 mL of concentrated hydrochloric acid and 2 mL of water in the Teflon tube with cap and digested as per the Microwave digestion conditions using microwave digestion cell. Then the Sample was allowed to room temperature for 30 min and then diluted to 50 mL with water (1 mg/mL). In experiment 2: Same the polymer sample containing 20 mg, of under similar experimental conditions mentioned experiment 1. The polymer sample was taken and digested (2 mg/mL) with water. In experiment 3: Same experiment conditions, 50 mg of polymer sample was conducting experiment 1 conditions digested as mentioned earlier and initially diluted to 5 mg/mL and further diluted to 1 mg/mL.

### Method validation parameters as per current ICH guidelines Q2R1

**Specificity:** The specificity studies were performed to evaluate the capability to read the absorbance at 118 mass value for standard blank solution, sample blank solution and standard solution and sample solution. The tin (Sn) standards were spiked to the sample at a 250 ppb. Table 1.

### System suitability and System precision:

The system suitability parameters were tested and measured the system performance. The linearity curve was constructed by the absorbance of eight level of different

concentration of tin standard reference solution and evaluated correlation coefficient of tin absorbance (MS Excel, 2009).

**Method precision:** The method precision was explored by preparing six individual preparations of polymer sample solutions. The % RSD of the tin content was calculated.

**Intermediate precision:**

The intermediate precision of polymer sample was performed by using different instruments, different analysts, and different locations and on different days. The intermediate precision was explored by preparing six more individual preparations of polymer sample solution. The % RSD of the tin content were calculated.

**Accuracy:**

The accuracy of the method was evaluated in triplicate at each concentration of 50, 100 and 150% and the percent recovery was calculated for tin content. The tin was spiked into polymer sample and recovery experiments were performed to determine the accuracy of the tin quantification. The study was carried out in triplicate of the analyte (5mg/ml). The tin content was spiked at 250 ppb and percent of recovery for tin was calculated.

**Linearity:**

Linearity of tin reference standard solutions was prepared from 30 to 600 ppb for the ICPMS method. The absorbance versus concentration was analyzed with least-squares linear regression. The Linearity standard solutions were prepared by diluting the tin reference standard stock solution to the required concentrations for the method. The solutions were diluted to eight different concentration levels ranging from the 30 to 600 ppb.

**Limit of detection (LOD) and limit of quantification (LOQ):** The LOD and LOQ for tin reference standard at a of 3:1 and 10:1, signal-to noise ratio by injecting a series of dilute solutions with known concentrations. The precision study was also performed at the LOQ level by readings of six individual samples and calculated the % RSD of the tin content.

### 3. Results and Discussion

**Method development and optimization for tin content:**

The existing methodology for determination of content in polymers preparation is not official in USP, BP and EP

pharmacopeia. According to the USP general chapter <232> and <233> and ICH guideline Q3D. a new in house method was developed and validated. As per the polymer supplier list the tin content showed not be more than 20 ppm or 200 ppb. The target of the current study is to determine the content of tin (Sn) using an inductive coupled plasma mass spectroscopy (ICP-MS) method. The sequence of tin (Sn) was depicted in the table2. The results on experimental setup 1 of method development study reveals that the tin (Sn) content was not recovered at both at 118 mass and 120 mass and recovery also found at lower side. Screening experimental setup-2 with increasing sample concentration, the recovery was observed both at 118 mass and 120 mass with 85%.respectively, Hence experimental setup-3 was done by changing the diluents for sample preparation and recovery were observed both at 118 mass and 120 mass with about 100%.

**Specificity:**

The polymer samples and spiked samples were recorded the absorbance and calculated the tin (Sn) content. The results show that no interference was observed between the sample blank solution and standard blank solution (table 3).

**System suitability and System precision:**

System suitability parameters were tested and measured the system performance. The linearity curve was determined on to take the absorbance of eight level of different concentration of tin standard reference solution and evaluated correlation coefficient of tin absorbance was found to be 0.999866. The results are listed in the table 4 and 5 also figure 2demonstrated linearity plot.

**Method precision:**

The method precision study was carried out within 10.0 % RSD. The % RSD of six individual polymer sample solutions. The results show that %RSD of tin (Sn) content. Results listed Table 6.

**Intermediate precision:**

The method precision study was carried out within 10.0 % RSD. The % RSD of six individual polymer sample solutions. The results show that % RSD of tin (Sn) content. Results listed Table 7.

**Accuracy:** The percent recovery of tin content from 101.2, 102.8and 108.These results indicate the accuracy of method (Table 8).

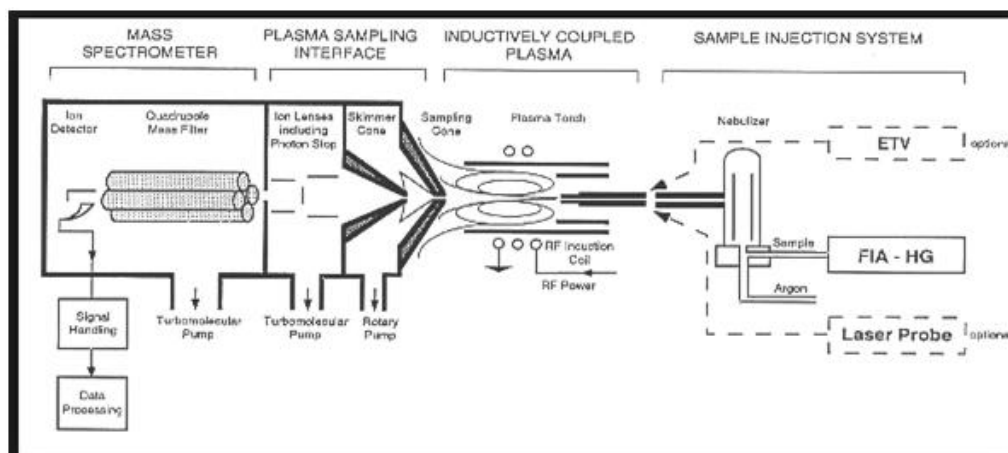


Figure. 1: A: Diagram of inductively coupled plasma mass spectrometry

**Optimization of instrument parameters:**

a) The instrument condition was used on KED mode and at mass of 117.902

<b>Condition 1</b>		
<b>Description</b>	<b>KED mode</b>	
Nebulizer Gas flow	0.81	
Auxiliary Gas flow	1.2	
Plasma Gas flow	18	
ICP RF Power	1600	
<b>Condition 2</b>		
<b>Description</b>	<b>KED mode</b>	
Sweeps	20	
Readings	1	
Replicates	3	
<b>Condition 3</b>		
<b>Parameter</b>	<b>Time (Sec)</b>	<b>Speed (+/- rpm)</b>
Sample Flush	35	-24.0
Ready delay	15	-20.0
Analysis	-	-20.0
Wash	45	-24.0

b) Microwave digestion condition:

<b>Temperature (°C)</b>	<b>Pressure (in bar)</b>	<b>Ramp</b>	<b>Hold</b>	<b>P (%)</b>
100	30	5	5	70
150	35	5	5	75
200	35	10	15	80
50	20	1	2	0
50	20	1	1	0

**Table No.1.** Sequence of specificity parameter

<b>S.No.</b>	<b>Name of the injection</b>	<b>No. of injections</b>
1	Standard blank	1
2	Sample blank	1
3	Standard solution level 1 (30 ppb)	1
4	Standard solution level 2 (100 ppb)	1
5	Standard solution level 3 (150 ppb)	1
6	Standard solution level 4 (200 ppb)	1
7	Standard solution level 5 (250 ppb)	1
8	Standard solution level 6 (300 ppb)	1
9	Standard solution level 7 (400 ppb)	1
10	Standard solution level 8 (600 ppb)	1
11	Standard blank	1
12	Sample blank	1
13	Sample solution	1
14	Spiked sample	1
15	Standard blank	1
16	Sample blank	1
17	Standard at 100% level solution (BKT)	1

**Table 2.** Sequence of tin (Sn) content sample analysis

S.No.	Name of the injection	No. of injections
1	Standard blank	1
2	Standard solution level 1 (30 ppb)	1
3	Standard solution level 2 (100 ppb)	1
4	Standard solution level 3 (150 ppb)	1
5	Standard solution level 4 (200 ppb)	1
6	Standard solution level 5 (250 ppb)	1
7	Standard solution level 6 (300 ppb)	1
8	Standard solution level 7 (400 ppb)	1
9	Standard solution level 8 (600 ppb)	1
10	Standard blank	1
11	Sample blank	1
12	Sample solution	1
13	Sample blank	1
14	Standard at 100% level solution (BKT)	1

**Table 3:** Results of tin (Sn) content specificity

S.No.	Name of the sample	Tin (Sn) intensity at 118 mass
1	Standard Blank	Intensity of Standard blank should be subtracting in the standard solution.
2	Standard solution level 1 (30 ppb)	
3	Standard solution level 2 (100 ppb)	
4	Standard solution level 3 (150 ppb)	
5	Standard solution level 4 (200 ppb)	
6	Standard solution level 5 (250 ppb)	
7	Standard solution level 6 (300 ppb)	
8	Standard solution level 7 (400 ppb)	
9	Standard solution level 8 (600 ppb)	
10	Standard Blank	
11	Sample solution	
12	Sample blank	
13	Standard at 100% level solution (BKT)	

**Table 4:** Results of tin (Sn) content system suitability

S.No.	Name of the sample	Concentration in ppb	Intensity
1	Standard Blank	0.0	0.0
2	Standard solution level 1 (30 ppb)	30	53525
3	Standard solution level 2 (100 ppb)	100	178417
4	Standard solution level 3 (150 ppb)	150	267625
5	Standard solution level 4 (200 ppb)	200	356834
6	Standard solution level 5 (250 ppb)	250	446042
7	Standard solution level 6 (300 ppb)	300	535250
8	Standard solution level 7 (400 ppb)	400	713667
9	Standard solution level 8 (600 ppb)	600	1070501
correlation coefficient		1.00000	

**Table 5:** Results of tin (Sn) content system precision

S.No.	Level	Tin (Sn) Intensity at 118 mass
1	Standard solution 250 ppb	446042
2	Standard solution 250 ppb	445021
3	Standard solution 250 ppb	431502
4	Standard solution 250 ppb	445612
5	Standard solution 250 ppb	454126
6	Standard solution 250 ppb	445263
Correlation coefficient		1.00000
Average		444594
STDEV		7294.1
%RSD		1.64

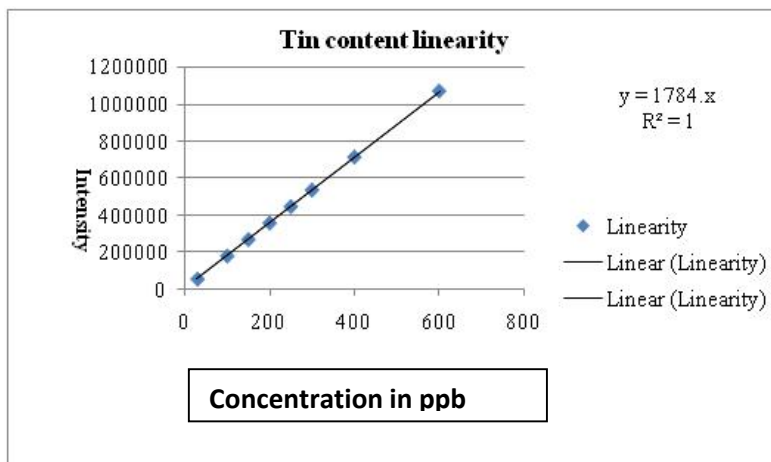


Figure. 2: System suitability linearity plot

Table 6: Method precision results of tin content

S.No.	Tin content in ppb
1	4.8
2	4.6
3	4.8
4	4.9
5	4.7
6	4.8
<b>Average</b>	<b>4.8</b>
<b>STDEV</b>	<b>0.1</b>
<b>%RSD</b>	<b>2.17</b>

Table 7: Intermediate precision for separation of tin

S.No.	Method precision	Intermediate precision
	Tin content in ppb	
1	4.1	4.3
2	4.2	4.0
3	4.3	4.5
4	4.2	4.1
5	4.4	4.4
6	4.2	4.3
Average	<b>4.8</b>	<b>4.6</b>
STDEV	<b>0.1</b>	<b>0.3</b>
%RSD	<b>2.17</b>	<b>6.14</b>
Average		<b>4.7</b>
STDEV		<b>0.2</b>
%RSD		<b>4.86</b>

Table No. 8. Accuracy results

S.No.	Level	% recovery	Mean recovery	STDEV	%RSD
1	50%	101.2	102.6	0.48	0.50
2		102.1			
3		101.5			
1	100%	102.7	104.2	0.63	0.60
2		102.5			
3		101.5			
1	150%	108.8	105.7	1.00	0.95
2		107.5			
3		106.7			

<b>Average</b>	<b>103.8</b>
<b>STDEV</b>	<b>3.335</b>
<b>%RSD</b>	<b>4.5</b>

#### 4. Conclusion

The method for determination of tin (Sn) was established as per ICH recommendations. The Inductively Coupled Plasma Mass Spectrometry method was developed and used for estimation of content of inorganic of tin. This method is suitable for plasma samples for quantification of tin content in polymers. The results of this study demonstrate the stability-indicating power of the method. This method is sensitive and can detect up to 5 PPB of tin content. This method precise, accurate and stability indicative as per ICH recommendations. The developed method can be used to determine the Tin content of polymers and injection in the routine as well as and stability sample analysis.

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