Development and validation of inductively coupled plasma mass spectrometry method for simultaneous determination of tin (SN) levels in excipients and formulations

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Abstract

The pharmaceutical dosage form 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: Analytical Method development, Analytical Method validation, Inductively coupled plasma mass spectrometry and Tin

Introduction

The pharmaceutical dosage forms such as injections, lyophilized powder and some solid dosage forms, use polymers for the homogenization in the manufacturing process.(1) The pharmaceutical dosage forms 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 bio-reabsorbable, biocompatible and non-toxic. Its degradation kinetics can be modified by the copolymerization ratio of the monomers and 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 such as arsenic (As), copper (Cu), chromium (Cr), selenium (Se), tin (Sn), cadmium Cd, Antimony (Sb) and Lead (Pb).(3,4) Out of these elements, tin (Sn) is the major trace element. The routine analytical techniques were used for identification and characterization of Sn 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 (μm/day), parental 640 PDE (μm/day) and inhalation 64 PDE (μm/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 μg Sn/tablet). Tin is possibly nutritionally essential for some animals but not for humans. Tin (II) chloride is being used as a reducing agent, and as a stabilizer of polyvinylchloride (PVC). The present study is mainly focusing on assessment of inorganic tin in drug products by hydride generation inductively coupled plasma mass spectrometry, which is developed and validated as per the ICH and FDA regulations.(14-18)

Experimental

Chemicals: The Tin reference standard material (SRM316) a certified by National Institute of Standards and Technology was used as a primary calibration standard for the quantitative determination of tin (Sigma Aldrich, USA). The solution was 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 is consists of sample injecting system, inductively coupled plasma, plasma sampling interface and mass spectrophotometer. The output signal was monitored and processed using dell work 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).

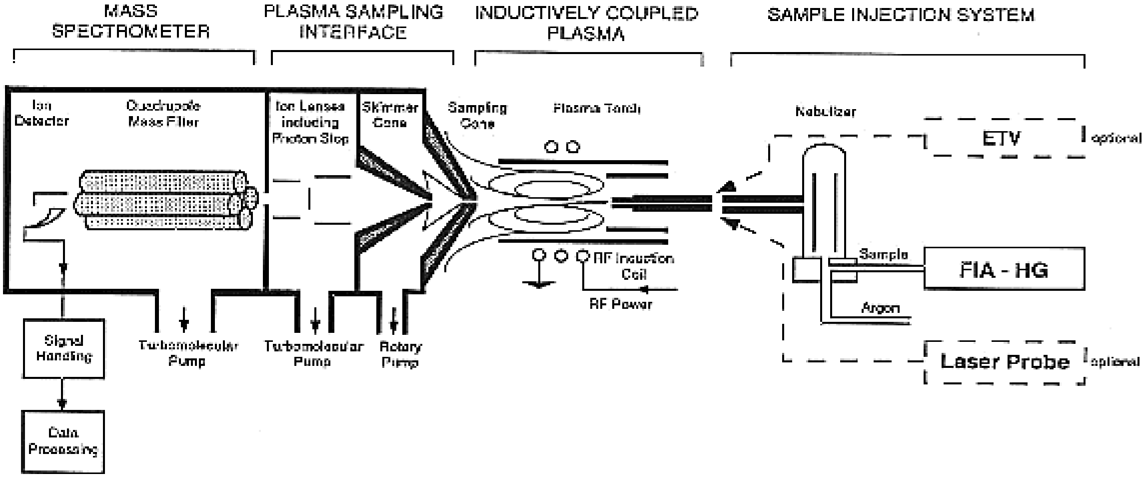

Fig. 1: A: Diagram of inductively coupled plasma mass spectrometry

Optimization of instrument parameters

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

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

b. Microwave digestion condition

Temperature (°C)	**Pressure (in bar)**	**Ramp**	**Hold**	**P (%)**
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

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 manual 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 of experiment 1.50 mg of polymer sample was 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

Table 1: Sequence of specificity parameter

S. No.	Name of the injection	No. of injections
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

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 were calculated.

Intermediate precision: The intermediate precision of polymer sample was performed by using different instruments, different analysts, 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.

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 table 2. 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%.

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

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).

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)	

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 Fig. 2 demonstrated linearity plot.

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	

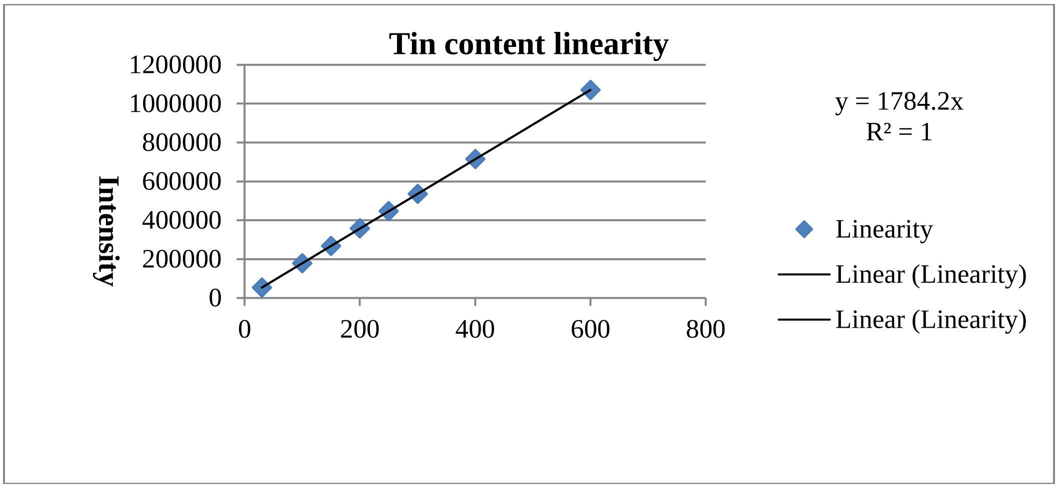

Fig. 2: System suitability linearity plot

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

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.

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

Average	4.8
STDEV	0.1
%RSD	2.17

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.

Table 7: Intermediate precision for separation of tin

S. No.	Method precision	Intermediate precision
	Tin content in ppb	
1	4.8	4.5
2	4.6	4.7
3	4.8	4.9
4	4.9	4.8
5	4.7	4.2
6	4.8	4.3
Average	4.8	4.6
STDEV	0.1	0.3
%RSD	2.17	6.14
Average		4.7
STDEV		0.2
%RSD		4.86

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

Table 8: Accuracy results

S. No.	Level	% recovery	Mean recovery	STDEV	%RSD
1	50%	101.2	101.6	0.458	0.45
2		102.1			
3		101.5			
1	100%	102.7	102.2	0.643	0.63
2		102.5			
3		101.5			
1	150%	108.8	107.7	1.060	0.98
2		107.5			
3		106.7			
Average		103.8			
STDEV		3.335			
%RSD		3.21			

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 in 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 was precise, accurate and stability indicative as per ICH recommendations. The developed method can be used to determine the in Tin content of polymers and injection in the routine as well as and stability sample analysis.

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Fig. 3: Typical inductively coupled plasma mass spectroscopy blank, standard and sample readings

Fig. 3 A. Sample Blank:

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode
Sn-1	118	452	4.4	-2.13	0.011	0.5	ppb	4238	KED
Sn	120	651.6	5.3	-2.19	0.013	0.6	ppb	6389	KED

Fig. 3 B. Standard Blank:

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode
Sn-1	118	4552.4	0.9	0.18	0.024	13.5	ppb	4238	KED
Sn	120	6756.9	3.5	0.14	0.091	64.9	ppb	6389	KED

Fig. 3 C. Standard solution level 1 (30 ppb)

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode
Sn-1	118	60278.5	0.3	30	0.097	0.3	ppb	4238	KED
Sn	120	87475.3	1.4	30	0.442	1.5	ppb	6389	KED

Fig. 3 D. Standard solution level 2 (100 ppb)

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode
Sn-1	118	193608.5	1	100.11	1.001	1.0	ppb	4238	KED
Sn	120	284792.8	0.7	100.24	0.706	0.7	ppb	6389	KED

Fig. 3 E. Standard solution level 3 (150 ppb)

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode
Sn-1	118	281885.7	0.8	148.93	1.179	0.8	ppb	4238	KED
Sn	120	413038	0.6	148.81	0.904	0.6	ppb	6389	KED

Fig. 3 F. Standard solution level 4 (200 ppb)

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode
Sn-1	118	366063	1.0	197.27	1.957	1	ppb	4238	KED
Sn	120	527653.6	3.0	195.68	5.882	3	ppb	6389	KED

Fig. 3 G. Standard solution level 5 (250 ppb)

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode
Sn-1	118	446042.2	0.6	244.98	1.524	0.6	ppb	4238	KED
Sn	120	650614.6	1.6	245.53	3.934	1.6	ppb	6389	KED

Fig. 3 H. Standard solution level 6 (300 ppb)

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode
Sn-1	118	531022.8	1.6	295.2	4.904	1.7	ppb	4238	KED
Sn	120	776647.6	0.9	296.09	2.59	0.9	ppb	6389	KED

Fig. 3 I. Standard solution level 7 (400 ppb)

Results (Mean Data)

Analyte		Intensity	RSD	Conc.	SD	RSD	Units	Blank Intens.	Mode

Sn-1	118	712121.2	1.9	398.05	7.693	1.9	ppb	4238	KED
Sn	120	1047818	1.4	400.2	5.591	1.4	ppb	6389	KED
Fig. 3 J. Standard solution level 8 (600 ppb)									
Results (Mean Data)									
Analyte		**Intensity**	**RSD**	**Conc.**	**SD**	**RSD**	**Units**	**Blank Intens.**	**Mode**
Sn-1	118	1067077	0.7	598.78	4.336	0.7	ppb	4238	KED
Sn	120	1583875	1.8	603.19	10.968	1.8	ppb	6389	KED
Fig. 3 K. Sample preparations:									
Results (Mean Data)									
Analyte		**Intensity**	**RSD**	**Conc.**	**SD**	**RSD**	**Units**	**Blank Intens.**	**Mode**
Sn-1	118	20177.6	1.9	8.98	0.219	2.4	ppb	4238	KED
Sn	120	30081.6	0.5	9.06	0.053	0.6	ppb	6389	KED
Fig. 3 L. Accuracy sample preparation 50%									
Results (Mean Data)									
Analyte		**Intensity**	**RSD**	**Conc.**	**SD**	**RSD**	**Units**	**Blank Intens.**	**Mode**
Sn-1	118	317636.1	1.3	176.56	2.295	1.3	ppb	4238	KED
Sn	120	466317.6	1.9	175.86	3.326	1.9	ppb	6389	KED
Fig. 3 M. Accuracy sample preparation 100%									
Results (Mean Data)									
Analyte		**Intensity**	**RSD**	**Conc.**	**SD**	**RSD**	**Units**	**Blank Intens.**	**Mode**
Sn-1	118	633689.8	2.0	354.62	7.055	2.0	ppb	4238	KED
Sn	120	917818.2	0.8	348.5	2.944	0.8	ppb	6389	KED
Fig. 3 N. Accuracy sample preparation 150%									
Results (Mean Data)									
Analyte		**Intensity**	**RSD**	**Conc.**	**SD**	**RSD**	**Units**	**Blank Intens.**	**Mode**
Sn-1	118	856919.8	3.0	480.38	14.58	3.0	ppb	4238	KED
Sn	120	1261552	2.0	479.94	9.418	2.0	ppb	6389	KED

References

1. Gianluigi Maria Lo Dico, Gaetano Cammilleri, Andrea Macaluso, Antonio Vella, Giuseppe Giangrosso, Mirella Vazzana and Vincenzo Ferrantelli, Simultaneous Determination of As, Cu, Cr, Se, Sn, Cd, Sb and Pb Levels in Infant Formulas by ICP-MS after Microwave-Assisted Digestion: Method Validation, from J Environ anal toxical 2015, 5-6.
2. Centre for drug evaluation and research, application number 21-673, http://www.accessdata.fda.gov.
3. Andreas Limbeck, Patrick Galler, Maximilian Bonta, Gerald Bauer, Winfried Nischkauer, Frank Vanhaecke, Recent advances in quantitative LA-ICP-MS analysis: challenges and solution in the life sciences and environmental from Anal Bioanal Chem, 2015, 407;6593-6617.
4. Jens Sondergaard, Gert Asmund, Martin M. Larsen, Trace elements determination in seawater by ICP-MS with online pre-concentration on a Chelex-100 column using standard instrument setup, from Elsevier methods x 2, 2015, 323-330.
5. Yiannis Fiamegos, Mitja Vahcic, Hakan Emteborg, James Snell, George Raber, Fernando Corderiro, Piotr Robouch, Beatriz De la Calle, Determination of toxic trace elements in canned vegetables, the importance of sample preparation, from Elsevier trends in analytical chemistry 85, 2016, 57-66.
6. Sanda Roncevic, Anica Benutic, Ivan Nemet, Buga Gabelica, Tin Content determination in canned fruits and vegetables by hydride generation inductively coupled plasma optical emission spectrometry, from Hindawi publishing corporation, volume 2012,article ID 376381,7.
7. Centre for drug evaluation and research, application number 21-673, http://www.accessdata.fda.gov.
8. Description of leprolide depot injection, http://www.drugbank.ca/drugs/ DB00631.
9. Description of leprolide depot injection, https://dailymed.nlm.nih.gov/.
10. Chemical and Physical properties of Tin content(2004) Chem Spider.
11. ICH Q2 (R1), Validation of analytical procedures: Text and methodology, 2005.
12. ICH Q1 (R2), Stability testing of New Drug Substances and Products, 2000.
13. ICH, Photo stability testing of new drug substances and products Q1B.
14. ICH Guidelines on validation of analytical Procedures definitions and terminology.

15. Drug stability principles and practices third edition, edited by Jens T, Carstensen Rhodes CT. 2000.
16. Bakshi M, Singh S, Development of validated stability indicating assay methods critical review. J Pharm Biomed. 2002: 28:1011-1040.
17. Validation of compendial methods The United States Pharmacopeia.2016:42.
18. Jens T, Carstensen, Rhodes CT, Drug stability principles and practices, Marcel Dekker, New York, 2000.
19. ICH Stability Testing of New Drug Substances and Products Q1A (R2), International Conference on Harmonization, IFPMA, Geneva, 2003.