RESEARCH ARTICLE

ANALYTICAL METHOD VALIDATION FOR DETERMINATION OF HEAVY METAL IN CAPSULE SHELL BY USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS).

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Abstract

A precise, linear, accurate, sensitive and selective eco friendly analytical method has been developed and validated using inductively coupled plasma mass spectrometry (ICP-MS) for the determination of heavy metal Arsenic (As), Mercury (Hg), Lead (Pb) and Cadmium (Cd) in hard gelatin capsule shell. Arsenic, Mercury, Lead and Cadmium are heavy metal and heavy metals are a genotoxic in nature. These heavy metals follows under class I category therefore ICH guidelines Q3D have control limit base on its risk assessment. The developed analytical method was selective and sensitive for capable detecting heavy metal as 0.006ppm As, 0.002ppm Hg, 0.019ppm Pb, 0.005ppm Cd and further quantified from 0.020ppm As, 0.012ppm Hg, 0.063ppm Pb, 0.017ppm Cd to 200 percent of limit concentration. The analytical method found to be linear with working concentration range from 0.986 ppb to 100 ppb for As, 0.856ppb to 50 ppb Cd, 0.302 ppb to 10 ppb Hg and 3.127 ppb to 100 ppb Pb with correlation coefficient 1.0000 As, 1.0000 Cd, 0.9999 Hg and 0.9998 Pb. The percentage recoveries of heavy metals at three different concentrations with spiking in samples of hard gelatin capsule were found to be an acceptable range as 70% to 130 %. The method was precise and robust and its relative standard deviation was below 25%. The actual % RSD in precision are 5.67% As, 5.19% Cd, 3.79% Hg and 5.34% Pb. Therefore the developed method can use for routinely quantitative determination of heavy metal in hard gelatin capsule shell to ensure the quality of capsule shell.

Introduction:

Gelatin is a natural product, a solid substance and it’s tasteless, colorless, and translucent obtained from partial hydrolysis of collagen. The gelatin can be made from materials that are rich in collagen such as skin, connective tissue, organs, intestines and bones of animals just as pigs, horses, cattle or other animals. However if made from leather and cow bone or other large animals, the process become longer and requires lot of water for washing, due to natural product and huge water washing in manufacturing process heavy metals may present in gelatin. Such heavy metals need to be identified and determine any of analytical technique. Hence method development need for those heavy metals. Heavy metals are a genotoxic in nature and its follows under class I category therefore ICH guidelines

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Q3D have given control stringent limit base on risk assessment. The graphical representations of material used in gelatin production are as follows.

![Materials Used in Gelatin Production](image)

Fig.1: Gelatin material composition.

Gelatin capsule was first patented by Mr.F.A.B. Mothes, students and Dublanc, a pharmacist, they obtained the patent in 1834, cover a method for producing gelatin capsules consisting of one section, oval-shaped, and covered with a drop of hot concentrated solution of gelatin after charging. The uses of gelatin capsules are spread even produced by many countries in Europe and America restricted they use gelatin capsules patent on a particular company, sparked two new capsule forms. In 1839 in Paris, Garot created a thin layer coated products, gelatin-coated pills. In 1846 another pharmacist, J.C. Lehubby patented capsule 2 parts which is still used. Many medications enclosed in capsule shell are administered orally. The Pharmacopoeia of the People’s Republic of China (2010 version) sets a clear standard for the grade of gelatin that can be used for drug capsule production and requires that pharmaceutical companies only purchase capsules from manufacturers that are licensed. There have been recent reports that some companies in eastern China have been making and selling capsules made from cheaper industrial gelatin prepared from discarded leather. Heavy metal like Chromium, which is a known carcinogen, and can be toxic if ingested in large quantities, is used in the leather tanning process. Consequently 20 to 90 times more Cr was typically found in the leather-derived gelatin than in pharmaceutical/edible grade gelatin.

In current pharmacopeia heavy metals control by heavy metal test by visual observation no any specific instrument technique like flame photometric, atomic absorption spectroscopic, inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma mass spectroscopy was given. In most of Active pharmaceutical ingredient and excipients pharmacopeia mentions that heavy metals to be perform by any of analytical instruments. This instrumentation technique will compulsory applied by pharmacopeia from year 2018. In current scenario most of literatures are given on inductively coupled plasma atomic emission spectroscopy (ICP-AES), however an extensive survey revealed that there were no any quantitative methods for determination of genotoxic heavy metal by inductively coupled plasma mass spectrometry (ICP-MS) in hard gelatin capsule. Hence it was felt necessary to develop an accurate, rapid, sensitive, and specific method for the determination of heavy metals in hard gelatin capsule. We developed simple, fast, linear, accurate, reproducible and robust ICP-MS method. The method was validated by following ICH guideline parameter.

**Materials and Methods:-**

**Chemical and reagents:-**

| Table.1: Chemical and Reagents |
|------------------------------|
| Name                        | Make          | Batch no.          |
| Nitric Acid (69%)           | Fluka         | BCBQ1240W          |
| Hydrogen Peroxide           | Merck AR grade| CC3C630119         |
| Water, Milli Q              | Millipore     | - NA-              |
Equipments:
The heavy metals analysis was carried out by using a Thermo; Inductively Coupled Plasma Mass spectrometry (ICP-MS) modal iCAP Q with Anton paar; Microwave Reaction System, modal Multi PRO. The whole analysis data was process through Qutegra software and software was 21CFR part 11 comply.

Instrument conditions:

Microwave Reaction System:

Method Parameter:

| Parameter                        | Value          |
|----------------------------------|----------------|
| Max. Pressure increases Rate     | 0.5 bar/s      |
| Max. Pressure                    | 40 bar         |
| Max. Microwave power             | 1200 W         |
| IR temperature Limit             | 210 °C         |
| Internal temperature Limit       | 240 °C         |

Digestion Program:

| Step               | Temp.(°C) | Power (W) | Time (min) | Fan Level |
|--------------------|-----------|-----------|------------|-----------|
| Power ramp         |          | 1200      | 10         | 1         |
| Power hold         |          | 1200      | 15         | 1         |
| Cooling            | 50        | --        | --         | 3         |

ICP-MS Parameter:

| Parameter               | Value          |
|-------------------------|----------------|
| Plasma power            | 1550W          |
| Carrier Gas 1 / Flow Rate| Argon (14 mL/min.) |
| Carrier Gas 2           | Helium         |
| Analysis mode           | KED (Kinetic Energy Discrimination) |
| No. of Sweeps           | 10             |
| Main Runs               | 6              |
| Dwell time              | 0.1            |
| Peristaltic pump speed  | 40rpm          |
| Up take time            | 30 seconds     |
| Wash time               | 30 seconds     |

Preparation of solutions:

Diluent:
Transfer about 40 mL of Concentrated (69%) Nitric acid in to a 500mL flask containing 300 mL of water and dilute to volume with water.

Sample blank preparation:
Transfer 1.5 ml Nitric acid (69%), 0.8mL H₂O₂, 4 ml water and add 0.2ml of Gold Standard stock solution (10ppm) into the microwave digestion vessel; place the vessel in Microwave digester chamber and run digestion program. After completion of digestion transfer the Content into the 10 ml volumetric flask and dilute up to the mark with water. Centrifuge the sample blank and use supernatant for analysis.

Test Solution preparation:
Weigh and transfer accurately about 0.5g of sample into the microwave digestion vessel, add 1.5 ml Nitric acid (69%), 0.8 ml H₂O₂, 4 ml water and add 0.2ml of Gold Standard stock solution (10ppm); place the vessel in Microwave digester chamber and run digestion program. After completion of digestion, transfer the content into the 10 ml volumetric flask, and dilute up to the mark with water. Centrifuge the test sample and use supernatant for analysis.

Standard stock solution:

Arsenic Standard stock solution (10ppm):
Transfer 1 ml from 1000ppm of Arsenic standard into 100 ml volumetric flask and dilute up to the mark with diluent.
Mercury Standard stock solution (10ppm):-
Transfer 1 ml from 1000ppm of Mercury standard into 100 ml volumetric flask and dilute up to the mark with diluent.

Lead Standard stock solution (10ppm):-
Transfer 1 ml from 1000ppm of Lead standard into 100 ml volumetric flask and dilute up to the mark with diluent.

Cadmium Standard stock solution (10ppm):
Transfer 1 ml from 1000ppm of Cadmium standard into 100 ml volumetric flask and dilute up to the mark with diluent.

Gold Standard stock solution (10ppm):
Transfer 1 ml from 1000ppm of Gold standard into 100 ml volumetric flask and dilute up to the mark with diluent.

Linearity Standard Stock Solution:-
Transfer 2.5 ml from Arsenic Standard stock solution (10ppm), 1.25mL of Cadmium Standard stock solution (10ppm), 0.25 ml from Mercury Standard stock solution (10ppm), 2.5 ml from Lead Standard stock solution (10ppm) in to 100 ml volumetric flask and dilute up to the mark with diluent.

Preparation of Linearity Standard Solutions:--
Table 2: Linearity Standard Solutions.

| STD Nos. | Volume taken in mL from Linearity Std Stock Soln (mL) | Volume of Gold Std stock Soln (10ppm) | Volume to be made (mL) | Conc. of elements in ppb |
|----------|-----------------------------------------------------|---------------------------------------|-----------------------|--------------------------|
| 1        | 2.50                                                | 0.5                                   | 25                    | As 25.0  Hg 2.50  Pb 25.0  Cd 12.50 |
| 2        | 3.75                                                | 0.5                                   | 25                    | As 37.5  Hg 3.75  Pb 37.5  Cd 18.75 |
| 3        | 5.00                                                | 0.5                                   | 25                    | As 50.0  Hg 5.00  Pb 50.0  Cd 25.00 |
| 4        | 7.50                                                | 0.5                                   | 25                    | As 75.0  Hg 7.50  Pb 75.0  Cd 37.50 |
| 5        | 10.00                                               | 0.5                                   | 25                    | As 100.0 Hg 10.00 Pb 100.0 Cd 50.00 |

Procedure:--
Keep the instrument ready as per instrument parameters given in instrument condition and run the sequence as blank, Std-1 to 5, sample blank, Test solution and finally bracketing standard in six times. Plot the Linearity standard solution graph as intensity response of element on Y-axis Vs Conc. of standards on X-axis and Calculate Intercept, Slope.

System Suitability Criteria:--
1. The correlation coefficient should not be less than 0.99
2. Cumulative %RSD of intensity response of Std-5 and Bracketing standard (Std-5) should not be more than 20.

Calculations:--
Calculate the concentration of element in sample as per following formula:
\[
\text{Element Content (ppm)} = \frac{I - C}{10} x \frac{1}{m} \times \frac{1}{\text{weight of Sample (g)}} \times \frac{1}{1000}
\]

Where,
- I = Intensity Response of element for Sample.
- C = Intercept of the linearity curve.
- m = Slope of the linearity curve.

Specificity:--
Specificity is the ability of a method to measure specifically or selectively the analyte in the presence of components which may be expected to be present in the sample. Specificity was established by analyzing the blank, Test blank, standard and Sample solutions in ICP-MS and observed the interference. The observed interference of blank and Test blank was less than 3.0% hence method is specific refer (Table 3).
Table 3: Specificity.

| Solution            | Arsenic (As) | Cadmium (Cd) | Mercury (Hg) | Lead (Pb) |
|---------------------|--------------|--------------|--------------|-----------|
| Blank- Intensity    | 29           | 49           | 257          | 43,858    |
| % Interference      | 0.03         | 0.02         | 0.24         | 0.38      |
| Test Blank-Intensity| 128          | 730          | 3,192        | 265,993   |
| % Interference      | 0.12         | 0.23         | 2.35         | 2.26      |

System suitability (system precision):
Six replicate of Linearity standard solutions 5 was run and find out relative standard deviation and System suitability run the Linearity standard solutions from 1 to 5 and check correlation coefficient. The %RSD of the six replicate run of Linearity standard solutions 5 was should be below 20% and correlation coefficient of Linearity standard from 1 to 5 should less than 0.99 refer (Table 4 and 5).

Table 4: System Precision.

| Run Nos. | Arsenic (As) | Cadmium (Cd) | Mercury (Hg) | Lead (Pb) |
|----------|--------------|--------------|--------------|-----------|
| 1        | 163,855      | 486,054      | 205,475      | 17,630,628|
| 2        | 163,146      | 486,261      | 204,698      | 17,643,338|
| 3        | 164,870      | 495,097      | 205,053      | 17,842,138|
| 4        | 165,454      | 492,956      | 206,045      | 17,963,836|
| 5        | 166,793      | 491,029      | 205,723      | 17,778,016|
| 6        | 167,647      | 493,071      | 207,724      | 18,121,590|
| Mean     | 162,726      | 485,314      | 203,988      | 17,652,338|
| SD       | 2967.00      | 6375.28      | 2407.64      | 247791.63 |
| % RSD    | 1.82         | 1.31         | 1.18         | 1.40      |

Table 5: System suitability.

| Standards | AS (ppb) | AS (Intensity) | Cd (ppb) | Cd (Intensity) | Hg (ppb) | Hg (Intensity) | Pb (ppb) | Pb (Intensity) |
|-----------|----------|----------------|----------|----------------|----------|----------------|----------|----------------|
| Blank     | NA       | NA             | NA       | NA             | NA       | NA             | NA       | NA             |
| Std 1     | 25.0     | 42,184         | 12.50    | 125,295        | 2.50     | 51,880         | 25.0     | 4,454,365      |
| Std 2     | 37.5     | 64,410         | 18.75    | 190,704        | 3.75     | 78,438         | 37.5     | 6,784,672      |
| Std 3     | 50.0     | 84,554         | 25.00    | 250,385        | 5.00     | 104,965        | 50.0     | 9,011,390      |
| Std 4     | 75.0     | 124,557        | 37.50    | 367,641        | 7.50     | 157,256        | 75.0     | 13,234,735     |
| Std 5     | 100.0    | 165,265        | 50.00    | 490,696        | 10.00    | 205,529        | 100.0    | 17,786,066     |
| Correlation Coefficient | 0.9999 | 0.9999 | 0.9999 | 0.9999 |

Limit of detection (LOD) and limit of quantitation (LOQ):
Five different concentrations of standard where run and find out the slope and STE_{XY}. Base on slope and STE_{XY} determined the LOD and LOQ. The LOD and LOQ for the element found to be 0.006ppm and 0.02ppm for As; 0.005ppm and 0.017 for Cd; 0.002ppm and 0.006 for Hg and 0.019ppm and 0.063ppm for Pb w.r.t test concentration refer (Table 6).
Table 6: Determination of LOD and LOQ: Linearity.

| Standards    | AS (ppb) | AS (Intensity) | Cd (ppb) | Cd (Intensity) | Hg (ppb) | Hg (Intensity) | Pb (ppb) | Pb (Intensity) |
|--------------|----------|----------------|----------|----------------|----------|----------------|----------|----------------|
| Blank        | NA       | NA             | NA       | NA             | NA       | NA             | NA       | NA             |
| Std 1        | 10       | 16,881         | 5        | 48,457         | 1        | 19,152         | 10       | 1,726,324      |
| Std 2        | 20       | 34,415         | 10       | 99,472         | 2        | 38,981         | 20       | 3,445,657      |
| Std 3        | 30       | 51,332         | 15       | 148,883        | 3        | 58,820         | 30       | 5,185,435      |
| Std 4        | 40       | 68,474         | 20       | 198,773        | 4        | 78,784         | 40       | 6,950,560      |
| Std 5        | 50       | 85,873         | 25       | 251,050        | 5        | 100,287        | 50       | 8,827,399      |

Correlation Coefficient
- 1.0000
- 1.0000
- 0.9999
- 0.9999

Slope
- 1720.4300
- 10089.7400
- 20207.3000
- 177070.5300

Intercept
- -217.9000
- -2019.1000
- -1417.1000
- -85040.9000

STEYX
- 169.2189
- 863.5950
- 610.8578
- 55384.7152

LOD in ppm
- 0.0003
- 0.0003
- 0.0001
- 0.001

LOQ in ppm
- 0.0010
- 0.001
- 0.0003
- 0.003

LOD w.r.t. SPL
- 0.006
- 0.005
- 0.002
- 0.019

LOQ w.r.t. SPL
- 0.020
- 0.017
- 0.006
- 0.063

Precision at limit of quantitation level:
The LOQ precisions were evaluated by using six replicate of LOQ concentration and determine the %RSD. The obtained %RSD of the element was 4.37 % for As; 2.00 % for Cd; 2.92 % for Hg and 0.85 % for Pb (Table. 7).

Table 7: Precision at Limit of Quantitation

| Preparation Nos. | Arsenic (As) | Cadmium (Cd) | Mercury (Hg) | Lead (Pb) |
|------------------|--------------|--------------|--------------|-----------|
| LOQ Solution_1   | 1.429        | 8.058        | 24.130       | 509,657   |
| LOQ Solution_2   | 1.332        | 7.667        | 24.456       | 500,831   |
| LOQ Solution_3   | 1.328        | 7.683        | 24.125       | 497,147   |
| LOQ Solution_4   | 1.280        | 7.697        | 24.820       | 499,437   |
| LOQ Solution_5   | 1.272        | 7.694        | 24.971       | 501,845   |
| LOQ Solution_6   | 1.293        | 7.662        | 25.668       | 501,656   |
| Mean             | 1322.33      | 7743.50      | 589.88       | 4240.75   |
| SD               | 57.77        | 154.71       | 2.92         | 0.85      |
| % RSD            | 4.37         | 2.00         | 0.020        | 0.063     |

Precision/ruggedness:
The ruggedness of the method was evaluated by determine the content of element form six different test preparation and find out the %RSD. The ruggedness parameter was done by different analysis, different day and different instrument. The obtained %RSD of each element was 5.67% for As; 5.19% for Cd; 3.79% for Hg and 5.34% for Pb (Table. 8 and 9).

Table 8: Precision and Ruggedness (Day1, Analyst 1).

| SPL Id  | SPL (wt) | As (Int) | Cont. (ppm) | Cd (Int) | Cont. (ppm) | Hg (Int) | Cont. (ppm) | Pb (Int) | Cont. (ppm) |
|---------|----------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|
| Test_1  | 0.49958  | 80,137   | 1.1804      | 171,957  | 0.4902      | 102,032  | 0.1187      | 6,954,179| 1.0894      |
| Test_2  | 0.49927  | 78,149   | 1.1524      | 167,712  | 0.4786      | 100,183  | 0.1167      | 6,825,024| 1.0705      |
| Test_3  | 0.50039  | 75,267   | 1.1082      | 160,353  | 0.4571      | 96,796   | 0.1126      | 6,489,566| 1.0174      |
| Test_4  | 0.49766  | 73,004   | 1.0815      | 157,163  | 0.4507      | 96,209   | 0.1126      | 6,462,042| 1.0188      |
| Test_5  | 0.49471  | 69,842   | 1.0418      | 151,398  | 0.4372      | 92,203   | 0.1087      | 6,058,172| 0.9631      |
| Test_6  | 0.49687  | 68,657   | 1.0200      | 148,929  | 0.4284      | 91,847   | 0.1078      | 6,032,597| 0.9550      |
| Average |          | 1.0974   | 0.4570      | 0.1129   |             | 1.0190   |             |          |             |
| SD      | 0.0622   | 0.0237   | 0.0043      | 0.0544   |             |          |             |          |             |
| % RSD   | 5.67     | 5.19     | 3.79        | 5.34     |             |          |             |          |             |
Table 9: Precision and ruggedness (Day2, Analyst 2).

| SPL Id | SPL (wt) | As (Int) | Cont. (ppm) | Cd (Int) | Cont. (ppm) | Hg (Int) | Cont. (ppm) | Pb (Int) | Cont. (ppm) |
|--------|----------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|
| Test_1 | 0.51187  | 68,829   | 1.0064      | 173,524  | 0.4536      | 98,458   | 0.1087      | 7,329,192| 0.9387      |
| Test_2 | 0.50249  | 67,886   | 1.0111      | 171,149  | 0.4557      | 97,137   | 0.1093      | 7,241,401| 0.9445      |
| Test_3 | 0.50804  | 67,794   | 0.9987      | 169,307  | 0.4458      | 95,871   | 0.1067      | 7,145,229| 0.9214      |
| Test_4 | 0.50537  | 67,643   | 1.0017      | 169,371  | 0.4483      | 96,535   | 0.1080      | 7,168,941| 0.9294      |
| Test_5 | 0.50902  | 67,616   | 0.9941      | 170,065  | 0.4470      | 96,879   | 0.1076      | 7,244,084| 0.9327      |
| Test_6 | 0.51518  | 67,795   | 0.9849      | 171,993  | 0.4467      | 97,786   | 0.1073      | 7,246,896| 0.9219      |
| Average|          |          |             |          |             |          |             |          |             |
|        | 0.9995   |          |             | 0.4495   |             | 0.1079   |             | 0.9315   |             |
| SD     |          |          |             |          |             |          |             | 0.0093   | 0.0041      |
| % RSD  | 0.93     | 0.92     | 0.88        | 0.98     |             |          |             |          |             |

Linearity:-
Under the optimized working conditions, five different concentration of standards were run and plotted calibration curve over the range from LOQ to 200%. The squared correlation coefficient was found to be 1.000 for As; 1.000 for Cd; 0.999 for Hg and 0.999 for Pb. For linearity curve refer (Table 6; Fig 2)

Fig 2: Linearity Graph for Arsenic, Cadmium, Mercury and Lead.

Accuracy/ Recovery study:-
Accuracy of method was determined by doping the respective concentration solution of element in test preparation and find the content of elements from test preparation. Recovery studies were carried out at concentration LOQ, 50%, 100%, and 200%. The obtained % recovery was well within the limit 70% to 150%. For accuracy refer (Table 10 and 11)
Table 10: Accuracy (As and Cd)

| Spiked SPL Id | SPL (wt)  | As (Int) | As Cont. (ppm) | As Dop. (ppm) | % Accuarcy | Cd (Int) | Cd Cont. (ppm) | Cd Dop. (ppm) | % Accuarcy |
|---------------|-----------|----------|----------------|---------------|------------|----------|----------------|---------------|------------|
| LOQ 0.49952  | 3.360     | 0.0175   | 0.020          | 89.2          | 9.782      | 0.0217   | 0.017          | 126.6         |
| LOQ 0.50167  | 3.176     | 0.0149   | 0.020          | 75.8          | 9.544      | 0.0210   | 0.017          | 122.4         |
| LOQ 0.49944  | 3.275     | 0.0169   | 0.020          | 86.1          | 9.662      | 0.0217   | 0.017          | 126.6         |
| 50% 0.50024  | 52.076    | 0.6516   | 0.500          | 130.3         | 104.278    | 0.2511   | 0.250          | 100.4         |
| 50% 0.49390  | 51.304    | 0.6503   | 0.500          | 130.1         | 103.335    | 0.2522   | 0.250          | 100.9         |
| 50% 0.49495  | 51.286    | 0.6486   | 0.500          | 129.7         | 103.180    | 0.2512   | 0.250          | 100.5         |
| 100% 0.49225 | 89.977    | 1.1642   | 1.000          | 114.4         | 192.094    | 0.4637   | 0.500          | 93.6          |
| 100% 0.50091 | 90.025    | 1.1440   | 1.000          | 114.7         | 192.637    | 0.4692   | 0.500          | 93.8          |
| 100% 0.49655 | 89.443    | 1.1468   | 1.000          | 114.7         | 192.637    | 0.4692   | 0.500          | 93.8          |
| 200% 0.49926 | 172.061   | 2.2179   | 2.000          | 110.9         | 396.654    | 0.9631   | 1.000          | 96.3          |
| 200% 0.49095 | 171.916   | 2.2546   | 2.000          | 112.7         | 394.128    | 0.9735   | 1.000          | 97.4          |
| 200% 0.49711 | 172.143   | 2.2287   | 2.000          | 111.4         | 391.220    | 0.9540   | 1.000          | 95.4          |

Average 83.7%-130.0% 93.4%-125.2%

Table 11: Accuracy (Hg and Pb)

| Spiked SPL Id | SPL (wt)  | Hg (Int) | Hg Cont. (ppm) | Hg Dop. (ppm) | % Accuarcy | Pb (Int) | Pb Cont. (ppm) | Pb Dop. (ppm) | % Accuarcy |
|---------------|-----------|----------|----------------|---------------|------------|----------|----------------|---------------|------------|
| LOQ 0.49952  | 21.791    | 0.0100   | 0.0120         | 83.5          | 1,060,171  | 0.0875   | 0.063          | 139.9         |
| LOQ 0.50167  | 21.957    | 0.0103   | 0.0120         | 85.5          | 1,050,004  | 0.0854   | 0.063          | 136.5         |
| LOQ 0.49494  | 22.334    | 0.0106   | 0.0120         | 88.5          | 1,062,058  | 0.0892   | 0.063          | 142.7         |
| 50% 0.50024  | 52.751    | 0.0586   | 0.050          | 117.1         | 3,815,211  | 0.4671   | 0.500          | 93.4          |
| 50% 0.49390  | 51.060    | 0.0586   | 0.050          | 117.2         | 3,882,569  | 0.4834   | 0.500          | 96.7          |
| 50% 0.49495  | 52.603    | 0.0591   | 0.050          | 118.2         | 3,951,061  | 0.4918   | 0.500          | 98.4          |
| 100% 0.49225 | 92.818    | 0.1075   | 0.100          | 107.5         | 6,982,526  | 0.9196   | 1.000          | 92.0          |
| 100% 0.50091 | 93.945    | 0.1068   | 0.100          | 106.8         | 7,074,562  | 0.9151   | 1.000          | 91.5          |
| 100% 0.49655 | 94.825    | 0.1089   | 0.100          | 108.9         | 7,183,941  | 0.9390   | 1.000          | 93.9          |
| 200% 0.49926 | 197.569   | 0.2292   | 0.200          | 114.6         | 14,996,339 | 2.0127   | 2.000          | 100.6         |
| 200% 0.49095 | 196.162   | 0.2316   | 0.200          | 115.8         | 15,078,283 | 2.0599   | 2.000          | 103.0         |
| 200% 0.49711 | 194.947   | 0.2271   | 0.200          | 113.6         | 14,941,265 | 2.0140   | 2.000          | 100.7         |

Average 85.8%-117.5% 89.1%-139.7%

Robustness study:
The robustness study was carried out by varying the instrument parameter and find out the content of heavy metals.
The variation in parameters was change in Dwell time from to 0.1s to 0.11s; Power hold time from 15 min to 16.5 min and 15min to 13.5 min. The obtained results of element were shown in (Table.12, 13 and 14).

Table 12: Robustness (Change in Dwell time from to 0.1s to 0.11s)

| SPL Id | SPL (wt) | As (Int) | Cont. (ppm) | Cd (Int) | Cont. (ppm) | Hg (Int) | Cont. (ppm) | Pb (Int) | Cont. (ppm) |
|--------|----------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|
| Test_1 | 0.49958  | 82677    | 1.2197      | 167641   | 0.4645      | 88838    | 0.0984      | 5965193  | 0.8498      |
| Test_2 | 0.49927  | 84640    | 1.2496      | 169118   | 0.4689      | 88862    | 0.0985      | 5997955  | 0.8550      |
| Test_3 | 0.50039  | 84444    | 1.2439      | 167667   | 0.4638      | 88035    | 0.0973      | 6036505  | 0.8586      |

Average 1.2377 0.4688 0.0981 0.08544
SD 0.0159 0.0028 0.0006 0.0044
% RSD 1.29 0.59 0.65 0.52

Table 13: Robustness (Change in power hold time from 15 min to 16.5 min.)
Table 14: Robustness (Change in Power hold time from 15min to 13.5 min)

| SPL Id | SPL (wt) | As (Int) | Cont. (ppm) | Cd (Int) | Cont. (ppm) | Hg (Int) | Cont. (ppm) | Pb (Int) | Cont. (ppm) |
|--------|----------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|
| Test_1 | 0.49587  | 86818    | 1.2444      | 172074   | 0.4686      | 91598    | 0.0987      | 620932   | 0.8679      |
| Test_2 | 0.49789  | 84460    | 1.2037      | 168930   | 0.4574      | 89306    | 0.0956      | 605138   | 0.8605      |
| Test_3 | 0.49885  | 84490    | 1.2039      | 169016   | 0.4576      | 89685    | 0.0960      | 605429   | 0.8608      |
| Average|          | 1.2173   | 0.4612      |          | 0.0968      |          | 0.0158      |          |             |
| SD     |          | 0.0235   | 0.0064      |          | 0.0017      |          | 0.0158      |          |             |
| % RSD  |          | 1.93     | 1.40        |          | 1.71        |          | 1.81        |          |             |

Batch analysis:
The batch analysis was done in Triplicate as per method of analysis and found the results are well within limit. Refer (Table 15)

Table 15: Batch Analysis

| SPL Id | SPL (wt) | As (Int) | Cont. (ppm) | Cd (Int) | Cont. (ppm) | Hg (Int) | Cont. (ppm) | Pb (Int) | Cont. (ppm) |
|--------|----------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|
| Test_1 | 0.49952  | 2.033    | 0.0381      | 879      | 0.0105      | 3.128    | 0.0081      | 425763   | 0.0747      |
| Test_2 | 0.49783  | 2.015    | 0.0380      | 887      | 0.0106      | 2.865    | 0.0078      | 425699   | 0.0749      |
| Test_3 | 0.50088  | 1.992    | 0.0375      | 850      | 0.0104      | 2.732    | 0.0076      | 427781   | 0.0747      |
| Average|          | 0.0379   | 0.0105      |          | 0.0078      |          | 0.0748      |          |             |

Results and Discussion:
Heavy metals are toxic in nature and have to control in specified limit. In recent ICH Q3D guideline specific limits were given for each element. In current pharmacopeia heavy metals test procedure performed by chemically and observed by visual observation, there was no any specific instrument methods was given in pharmacopeia therefore pharmacopeia team has revised the USP General chapter <232> and <2232>. This change will effective in year 2018. In hard gelatin capsules manufacturing process lots of water and colour dyes were used therefore heavy metals present in sample. To control these heavy metals needs method development. In hard gelatin capsule Arsenic, Mercury, Lead and Cadmium are heavy metals. We tried to develop a heavy metal on atomic absorption spectroscopy (AAS) instrument but due to less sensitivity and stringent limits as well as some limitation of AAS, AAS technique was not feasible. Hence we tried to develop method on inductively coupled plasma mass spectrometer. The sample preparation technique of hard gelatin capsule is very difficult because metals are soluble in waters and gelatin was insoluble in water. Hence sample preparation technique was critical. We use concentrated hydrochloric and nitric acid for sample preparation but sample was not dissolving properly. In sample preparation some issues was observed therefore we digest the sample in microwave digester and run the sample in inductively coupled plasma mass spectrometer (ICP-MS). The results obtained of such sample are getting higher side where as blank inference was more than 3.0%. Therefore again method development was required. We developed new technique using hydrogen peroxide and digest the sample in microwave digester. In this technique the blank interfere was below 3.0%. Base on this technique we further validate the method for specificity, linearity, accuracy, precision and robustness and obtained result are within acceptance criteria. The method was validated by ICH Q2 (R1) guideline parameter.
Conclusion:
The developed analytical method for determination of Arsenic, Mercury, cadmium and Lead as heavy metal in hard gelatin capsule shell by using inductively coupled plasma mass spectroscopy (ICP-MS). The analytical method was specific, accurate, precision, reproducible, rugged, linear and robust method. The same method has been validated as per ICH guideline Q2 (R1). This method can be use for routine quality control sample analysis or can use for control monitor for heavy metals in the manufacturing process of hard gelatin capsule preparation.

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