Research Article

Study and Determination of elemental impurities and Trace metals by ICP-MS (Inductively Coupled Plasma Mass Spectrometer) in Buprenorphine Hydrochloride

Ms. Sandhya Solanki [1*], Dr. Ashutosh Tripathi [2], Mr. Bhavesh Mali [3], Dr. Siya Upadhyay [4]

[1]Student, PG, Derpartment of Chemistry, Institute of Sciences, SAGE University, Indore;
[2] Dean, The Department of Life, Health and Allied Science, ITM Vocational University, Vadodra, Gujarat;
[3]Student, PG, Institute of Forensic Science, Mumbai University, Mumbai;
[4]Professor, Department of Chemistry, Institute of Sciences, SAGE University, Indore;

Abstract: The main objective of the current study and clinical examination on the Buprenorphine Hydrochloride drug is to examine the different types of elemental impurities present in that drug. It is a well known fact that this drug is a synthetic phenanthrene with narcotic analgesic activity. It attacks the central nervous system and acts as a depressants. It is hihgly toxic in nature too as it acts as a chronic poison. It can be administered in body through all the routes of administration. The current study is comparing and studying the elemental impurities in **Buprenorphine Hydrochloride.**

Keywords: Buprenorphine Hydrochloride, Deprassants, Elemental Impurities, Narcotic Analgesic and Synthetic Phenanthrene.

1. Introduction

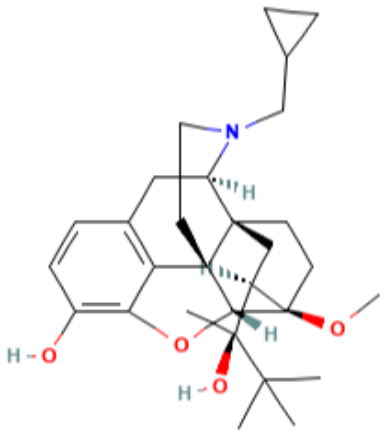
Buprenorphine Hydrochloride:-

Buprenorphine Hydrochloride is the hydrochloride salt form of buprenorphine, a **synthetic phenanthrene with narcotic analgesic activity**. Buprenorphine hydrochloride is a partial agonist at the mu-opioid receptor and an antagonist at the kapa-opioid receptor in the central nervous system.

Toxicity:-

Buprenorphine therapy has been associated with a low rate of serum enzyme elevations during treatment, although the populations studied (opioid dependent) often have coexisting chronic liver diseases which complicate such assessments. As, Cd, Hg and Pb are significantly toxic across all routes of administrations .Typically , they have limited or no use in the manufacture of pharmaceuticals but can be present as impurities in commonly used materials and cannot be readily removed from the material.Because of their unique nature , these four elemental impurities require consideration during the risk assessment across all potential sources of elemental impurities .

Structure of Buprenorphine Hydrochloride



Side Effects :- Constipation, Headache, Nausea, Dizziness, Drowsiness and difficulty in breathing, blurred vision

Objective:- To compare the elemental impurities (As, Cd, Pb, Hg, Al) in Buprenorphine hydrochloride by ICP-MS .

Scope:- This report is application to study of Comparison study of elemental impurities (As ,Cd, Pb ,Hg, Al) in Buprenorphine hydrochloride by ICPMS.

Sample:- Drug substance.

2. Materials and Methods

Instrumentation :- ICPMS (Inductively coupled plasma mass spectrometer)

Reagents /chemicals:- Nitric acid concentrated (ultrapure)**,** Hydrochloric Acid concentrated (ultrapure) and Millipore Water

Standard Used :- Multi elements standard and Gold and internal standard

Preparation of diluent :- Taken 10 ml of Nitric acid and 2.5 ml of hcl in 500 ml of purified water.

Preparation of blank:- Use diluents as a blank .

Preparation of standard stock solution(50 ppm):- Take 0.5 ml of 1000ppm all standard solution in 10 ml tarson tubes and dilute with water to volume .

Prearation of linearity stock solution :- Pipette out 2.0 ml of aluminium standard , 0.3 ml of cadmium standard ,0.5 ml of lead standard ,0.3 ml of chromium standard and ml of arsenic standard from standard stock solution in 50 ml of tarson tubes .

Preparation of internal standard :- Stock solution (1000ppm) :- scandium, yttrium ,bismuth , germanium , and gold .

Intermediate stock solution (5ppm):- Take 0.25 ml from 1000ppm stock solution of scandium ,yttrium ,bismuth and germanium in 50 ml of tarson tube with dilute with water to volume .

Preparation of stock solution of gold (10ppm):- Take 0.5 ml of 1000ppm gold stock solution of gold in 50 ml of tarson tube and dilute with water to volume .

Mix internal standard solution :- Take 0.25 ml of 5ppm scandium , yttrium , germanium , bismuth and 0.25 ml of 10ppm of gold standard in 25 ml of tarson tubes and dilute with water to volume .mix it well .

Preparation of linearity solution :-

- 25% linearity :- pipette out 0.125 ml of linearity mix solution and add 0.5 ml of internal standard and dilute to 50 ml with diluent to volume .
- 50% linearity :- pipette out 0.250 ml of linearity mix solution and add 0.5 ml of internal standard and dilute to 50 ml with diluent to volume .
- 100% linearity :- pipette out 0.500 ml of linearity mix solution and add 0.5 ml of internal standard and dilute to 50 ml with diluent to volume .
- 150% linearity :- pipette out 0.750 ml of linearity mix solution and add 0.5 ml of internal standard and dilute to 50 ml with diluent to volume.
- 200% linearity :- pipette out 1.000 ml of linearity mix solution and add 0.5 ml of internal standard and dilute to 50 ml with diluent to volume

Preparation of as such sample :- Weigh about 500 mg to sample and transfer in vessel liner. Add 5ml of nitric acid and 0.25 ml hcl to it . Add 5ml of diluent and keep for 30 min in fume hood for pre-digestion .Cover vessel liner with vessel cover and load disk . Insert vessel liner in vessel assembly and place in turntable for further digestion . After completing digestion cycle ,open the digestion vessel slowly to remove gases , then transfer digested sample in 50 ml polypropylene volumetric flask and make up the volume up to 50ml with diluent.

Preparation of Spike sample :- Weigh about 500 mg to sample and transfer in vessel liner and pipette out 0.500ml of linearity mix standard . Add 5ml of nitric acid and 0.25 ml hcl to it . Add 5ml of diluent and keep for 30 min in fume hood for pre-digestion .Cover vessel liner with vessel cover and load disk . Insert vessel liner in vessel assembly and place in turntable for further digestion . After completing digestion cycle ,open the digestion vessel slowly to remove gases , then transfer digested sample in 50 ml polypropylene volumetric flask and make up the volume up to 50ml with diluent.

Preparation of blank sample solution:- Prepare sample blank in same manner as sample preparation with without the adding the sample .

Procedure :- Place the sipper probe in the blank solution ,standard solution and sample solution .

Sequence of Blank, Standard and Sample :-

S.No.	Name	Number of Injection
1	Blank	2
2	Linearity Level_1	1
3	Linearity Level_2	1
4	Linearity Level_3	1
5	Linearity Level_4	1
6	Linearity Level_5	1
7	Sample Blank	1
8	Sample solution	2
9	Sample Spike solution	2
10	Blank	2
11	Linearity Level_3_BKT	1
12	Washing (with 1% Nitric acid solution)	2

System Suitability

- Correlation coefficient shall be not less than 0.98
- % RSD of Linearity level should not be more than 15%.
- Bracketing % RSD of Linearity level should not be more than 15%.

Calculations:-

Calibration curve is plotted using Concentration against Response usingequation of line:

Y = mx + C

Where in from

m = slope and C = Intercept; Concentration x of the Unknown sample is calculated.

Feasibility Parameters

- Specificity
- Determination of LOD and LOQ
- Linearity

Specificity:- Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the samples. The specificity (selectivity) of the method shall be determined for digested blank at particular wavelength of each element and potential interference of other analyte element

Limit of Detection (LOD): Detection limit is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantified. Under the stated experimental conditions.

Limit of Quantitation (LOQ): Quantitation limit is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Method's capability to remain unaffected. Limit of Detection and Limit of Quantitation can be performed by using standard deviation of response and slope through regression technique.

Calculate the limit of Quantitation by using the following formula

LOD (in ppm)

$$= \frac{3.3 \text{ X Standard deviation} \times 1000}{\text{Slope of Linearity curve}}$$

LOQ (in ppm)

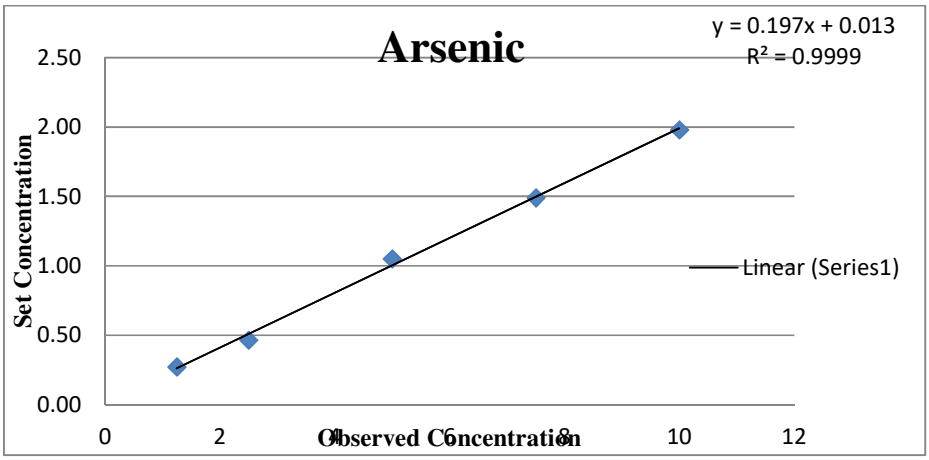
$$= \frac{10 \text{ X Standard deviation} \times 1000}{\text{Slope of Linearity curve}}$$

Linearity:- Linearity of analytical method is its ability to elicit test results that are directly, or by well-defined mathematical transformation, proportional to concentration of analyte in samples within given range

Observation: Obtained concentration of all elements summarized in table:-

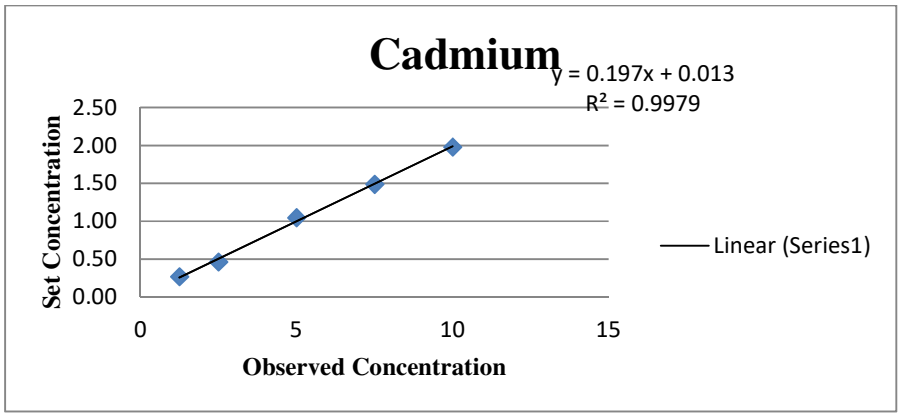
1)Arsenic:-

S.No.	Number of Injection	Set concentration (ppb)	Observed concentration ppb
1.	1	0.75 ppb	0.50 ppb
2.	1	1.50 ppb	1.00 ppb
3.	1	3.00 ppb	2.00 ppb
4.	1	4.50 ppb	3.00 ppb
5.	1	6.00 ppb	4.00 ppb



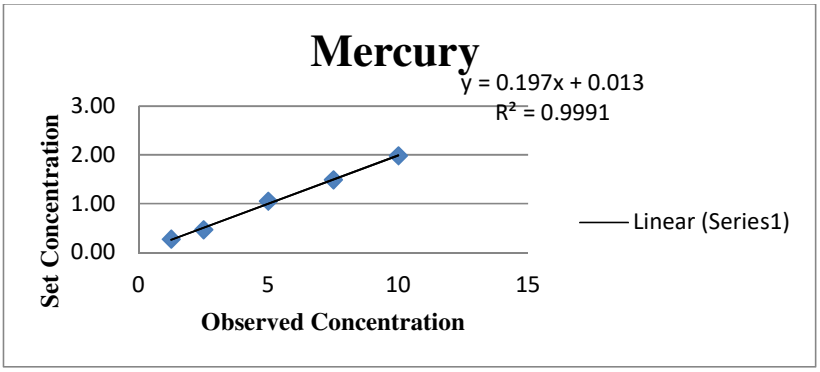
2) Cadmium

S.No.	Number of Injection	Set concentration (ppb)	Observed concentration ppb
1.	1	0.75 ppb	0.74 ppb
2.	1	1.50 ppb	1.48 ppb
3.	1	3.00 ppb	3.05 ppb
4.	1	4.50 ppb	4.48 ppb
5.	1	6.00 ppb	5.99 ppb



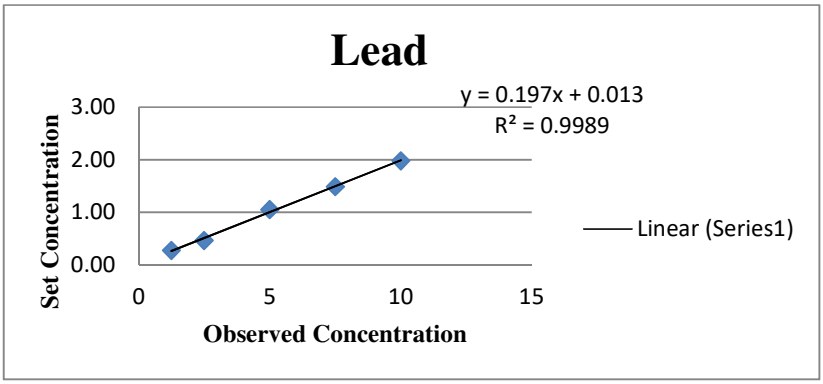
3) Mercury

S.No.	Number of Injection	Set concentration (ppb)	Observed concentration ppb
1.	1	0.25ppb	0.18 ppb
2.	1	0.50 ppb	0.46 ppb
3.	1	1.00 ppb	1.04 ppb
4.	1	1.50 ppb	1.45 ppb
5.	1	2.00 ppb	2.08 ppb



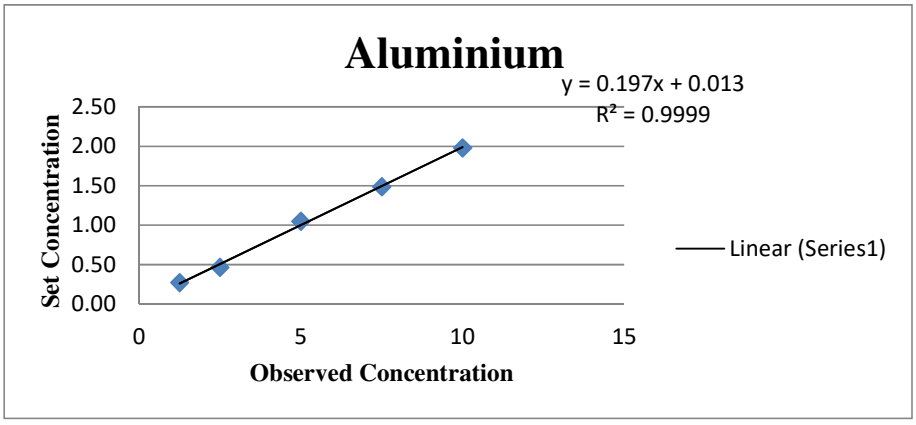
4) Lead:-

S.No.	Number of Injection	Set concentration (ppb)	Observed concentration ppb
1.	1	1.25 ppb	1.27 ppb
2.	1	2.50 ppb	2.57 ppb
3.	1	5.00 ppb	5.05 ppb
4.	1	7.50 ppb	7.22 ppb
5.	1	10.00 ppb	5.30 ppb



5) Aluminum:-

S.No.	Number of Injection	Set concentration (ppb)	Observed concentration ppb
1.	1	5.00 ppb	5.30 ppb
2.	1	10.00 ppb	9.98 ppb
3.	1	20.00 ppb	20.00 ppb
4.	1	30.00 ppb	30.00 ppb
5.	1	40.00 ppb	39.50 ppb



Correlation Coefficient:-

1. Arsenic – 0.9999
2. Cadmium – 0.9979
3. Mercury -0.9991
4. Lead – 0.99989
5. Aluminium – 0.9999

Note:- Correlation coefficient of calibration standard solution should be not less than 0.99 for each element

Batch Analysis First we performed the Buprenorphine Hydrochloride drug substance on different element and further performed the batch analysis of same drug substance .

S.No.	Sample Name	Batch No.
1.	Buprenorine Hydrochloride	XYZ
2.	Buprenorine Hydrochloride	123
3.	Buprenorine Hydrochloride	ABC

Batch Analysis Results

S.No.	Elements	Observed Concentration (ppb) XYZ	Observed Concentration (ppb) 123	Observed Concentration (ppb) ABC
1.	As	0.07	0.03	0.13
2.	Cd	0.01	ND	ND
3.	Hg	ND	ND	0.04
4.	Pb	0.15	0.16	0.34
5.	Al	35.45	47.35	60.90

3. Results and Discussion

Introduction Of ICPMS (Inductively coupled plasma mass spectrometer):-

- Inductively coupled plasma mass spectrometer is an analytical technique in which we identify the ionizes chemical species and sorts the ions which based on their mass-to- charge ratio.
- ICPMS is a type of spectrometer which is able to detect the metals and several non- metals at low parts per millions to parts per trillions on non-interfered low background isotopes.
- In ICPMS , elemental analysis with wide elemental coverage. Fast analysis time in which we detect and identify overall 71 elements at once . It has simple spectrometer and high throughout and productivity.It has extremely low detection limit at ppm to ppt level (mg/l to ng/l).

Basic of Instrumentation:-

In ICPMS instrument :-

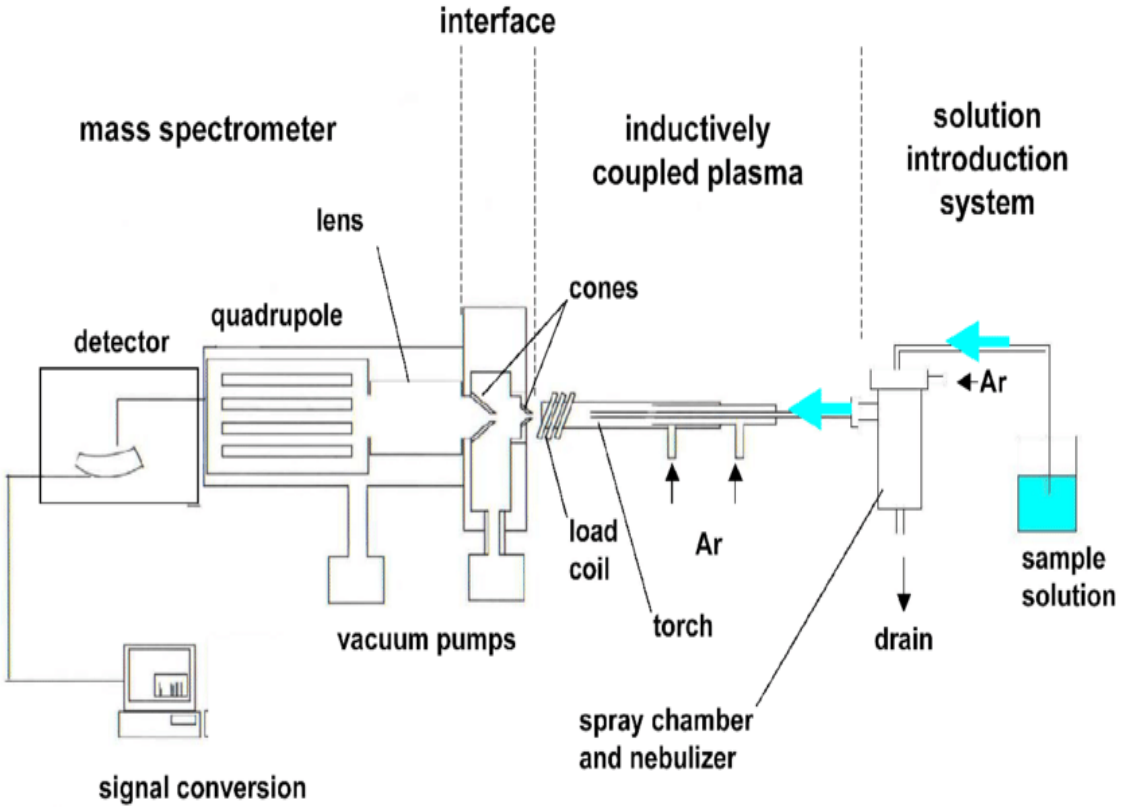
- First the sample introduced through nebulizer in which liquid sample converts toaerosol(liquid + gas flow) .
- Plasma ion source:- decomposes sample matrix and format ions .
- Ion Lenses:- Focuses ions and removes photon and neutrals .
- Collision and reaction cell:- Removes spectral interferences (polyatomic ions)
- Quadrupole mass spectra :- Separates ions by mass to charge ratio –unit mass resolution.
- Detector :- Detects ions and transfers counts to data system .
- In ICPMS Interface links the atmospheric pressure ICP ion source to the high vacuum MS.
- Vacuum system :- provides high vacuum for ions optics , Quadrupole and detector .
- Data handling and system controller:- controls all aspects of instruments control and data handling to obtain final concentration results .

Instrumentation:-

- **Peristaltic pump**:- ensures the constant flow of liquid sample and standards and blanks . Sample pumped at ml/mn
- **Micromist Nebulizer**:- liquid is converted into fine aerosol by pneumatic action of flow of argon gas (-1L/min) smashing the liquid into tiny droplets.
- **Doublepassspraychamber**:- Spray chamber only allows fine and small droplets(<10µm) to enter the plasma . Larger droplets get condensed and eventually fall out by gravity through the drain .
- **Ionplasma**:- ICPMS are formed by coupling energy produce by a RF generator to the plasma support gas with an electromagnetic field . The field is produced by applying an RF power (typically 700-1500 W) to load coil.
- **ICPInterfaceRegion** :- A section that connects the ionizing section at pressure to the mass spectrometer at high vacuum . Function is to export the ions produced in argon plasma and transport them to the mass spectrometer . Two metallic (nickel and platinum) cones with small orifices sampler and skimmer cones directs the expanded gas jet of ions into the MS .

- **IonFocussingRegion**:-one and more electrostatically controlled lens component made up of series of metallic plates or cylinders having a voltage on them . Ions are separated from photons and neutrals by MS off axis to the ion beam or using physical barrier.Out of a million ions generated in the plasma,only one actually reaches the detector due to higher concentration of matrix elementsthan analyts.Role of ions focusing is to transport maximum number of ions from the hostile environment of plasma to the MS.
- **Massanalyer (Quadrupole)**:- Quadrupole is a mass filter, which separates ions based on their m/z. Measurement of the m/z of the ion allows qualitative identification of the isotopes or molecules. Two pairs of parallel cyclindrical rods to which a varying AC voltage plus a DC voltage is applied.
- **Detectors**:- In ICPMS, Detector is EMT (electron multiplier Device) which can generates s measurable signal pulse from the impact of a signal ion. Each electron which strikes a dynode release several electrons from that surface and hence the device is called “electron multiplier”.

Block Diagram:-



Generation of Positively Charged ions:-

Nebulization:-In which liquid sample And Other type of sample convert to aerosol Solution .
Desolvation:- in which aerosol solution converts to particles .
Vaporization:- In Which , Gaseous Sample And liquid sample particles separates into individuals molecule.
Atomization:- The molecules now Breaking into atoms .

Ionization:- This atoms converts in ions in ionization generation And form a mass spectrum by mass analysis.

Liquid- Solid – Gas – atoms – Ions

Application:-

In Pharma Sector :- Routine heavy metals contamination, Drug substance and drug Discovery and Ions and metals in raw materials .

In food analysis:- Nutrition**,** Toxic elements and species and Heavy Metals like lead ,arsenic, Mercury etc

In Clinical analysis :- Blood ,Urine ,serum, Hair , tissues testing and Nutrition and vitamins deficiency

In Environmental :- Heavy metals in Drinking water, sea water, Soils ,solid waste and plant materials / agriculture

Daily Cleaning and routine maintenance:-

- First check the rotary pump start and check the purity of argon gas (Auxillary and plasma gas) (99.9%).
- Check the skimmer and sample cone, there is no blocking and deposition of sample .
- Clean the cones and extantion tube with 1% nitric acid and plasma torch with 5% nitric acid .
- Check Drain of spray chamber must function properly .malfunctioning or leaking drain can produce changes in the spray chamber backpressure .
- Staining and discoloration of the outer tube of the quarts torch because of heat and the corrosiveness of the liquid sample can cause electrical arcing .
- Tip of nebulizer should not get blocked .
- Constant motion and pressure of the pump rollers on the pump tubing
- Check the TDS of sample solution , TDS (total dissolved solids) must be kept below 0.2 %.
- Common dissolution agents are nitric acid , perchloric acid ,hydrofluoric acid or various mixture of these the acid concentration in the final solution should be 2-3% maximum.
- Ultrapure reagents and Millipore water should be used for preparation and dilution .
- Reagents should not contaminate or interfere with the analysis.

- Before sample analysis , daily calibrate the instrument with calibration solution and according to instructions
- Shelf life of all chemicals should be noted .use ultra high purity grades of chemicals ,acids .
- Analyst must be careful about some common trace element containments found in human body.

3. Conclusions

•ICP-MS is excellent detector for HPLc in bioanalysis.

•Orthogonal to other Detector

•Rapid and efficient method for metabolism studies.

•Polyatomic interferences from high matrix samples are a major challenge.

On the basis of this study, it is concluded that the proposed analytical method for Study and Determination of elemental impurities and Trace metals in Buprenorphine Hydrochloride in Lead, Arsenic, Cadmium, Mercury and Aluminum by by ICP-MS (Inductively Coupled Plasma Mass Spectrometer) is validated for its intended use.

Funding

None

Acknowledgments

We are wholeheartedly thankful to Dr. Ashutosh Tripathi, HOD, Institute of Sciences, SAGE University Indore for supervising and guiding us regarding this research work. We are also thankful to Dr. Siya Upadhyay, Professor, Institute of Sciences, SAGE University Indore for helping us throughout the research project.

Conflicts of Interest

None

References

- Dobkin, A. B. (1977). Buprenorphine hydrochloride: determination of analgesic potency. Canadian Anaesthetists' Society Journal, 24, 186-194.
- Cordery, S. F., Husbands, S. M., Bailey, C. P., Guy, R. H., & Delgado-Charro, M. B. (2019). Simultaneous transdermal delivery of buprenorphine hydrochloride and naltrexone hydrochloride by iontophoresis. Molecular Pharmaceutics, 16(6), 2808-2816.
- Koocheki, S., Madaeni, S. S., & Niroomandi, P. (2011). Development of an enhanced formulation for delivering sustained release of buprenorphine hydrochloride. Saudi Pharmaceutical Journal, 19(4), 255-262.
- Love, E. J., Taylor, P. M., Whay, H. R., & Murrell, J. (2013). Postcastration analgesia in ponies using buprenorphine hydrochloride. Veterinary Record, 172(24), 635-635.

- Kugawa, F., Arae, K., Ueno, A., & Aoki, M. (1998). Buprenorphine hydrochloride induces apoptosis in NG108-15 nerve cells. European journal of pharmacology, 347(1), 105-112.
- Mostafavi, A., Abedi, G., Jamshidi, A., Afzali, D., & Talebi, M. (2009). Development and validation of a HPLC method for the determination of buprenorphine hydrochloride, naloxone hydrochloride and noroxymorphone in a tablet formulation. Talanta, 77(4), 1415-1419.
- Jones, H. E. (2004). Practical considerations for the clinical use of buprenorphine. Sci Pract Perspect, 2(2), 4-20.