QUANTITATIVE DETERMINATION AND STANDARDIZATION OF ELECTROLYTES BY INDUCTIVELY COUPLED PLASMA MASS-SPECTROMETRY (ICP-MS) FROM BLOOD PRESSURE CONTROLLER CAPSULE

Raju P. Suryawanshi[1]* and Prafullachandra P. Tekale[2]

[1]Dept. of Chemistry, Government of Maharashtra's Rajaram College, Kolhapur -416004; Maharashtra, India.

[2]Dept. of Chemistry, Shiromani Gurudwara Prabandhak Committee's Guru Nanak Khalsa College of Arts, Science and Commerce, Matunga, Mumbai-19; India.

*Corresponding Author

Raju P. Suryawanshi

Dept. of Chemistry,

Government of

Maharashtra's Rajaram

College, Kolhapur -416004;

Maharashtra, India.

ABSTRACT

B.P.C. (Blood Pressure Controller) Capsule is one of the most popular herbal medicines in Indian market for Blood Pressure patients. It contains Electrolytes, Trace elements, Toxic, Essential, Trace metals, Minerals, Alkaloids, Steroids and organic compounds, enzymes, proteins and other Inorganic elements like Calcium and manganese, which are naturally present in herbal medicines. The B.P.C**.** Capsule contain, Calcium (Ca). Manganese (Mn). Though they are essential elements, it is utmost necessary to determine the quantitatively because it affects mostly kidney and blood pressure. The daily requirements of Ca and Mn is in micrograms. It is necessary to establish an accurate, precise, and easy method to detect these elements from herbal formulations. Popularity of herbal medicines is growing worldwide because of their minimal side effects. Herbal medicines required standardization, with implementation and constant review of technical standards of production and effective quality control methods. It is necessary to promote this study in the view of the importance of results of both individual and social field. Diseases occur due to their deficiency and toxicity due to overdose. These Elements can bind to vital cellular components and interfere with their normal functions**.** Human being, these elements can cause severe physiological and health effects. Therefore, it is necessary to check the efficacy and standardize the levels of these elements in terms of their values. These Elements can be determined quantitatively and

validated by using modern technique such as ICP-MS (Inductively Coupled Plasma Mass-Spectrometry)

KEYWORD: B. P. C. Capsule, Standardization, Herbal medicine, Standardization, ICP-MS, Calcium **(**Ca**)**, Manganese (Mn).

INTRODUCTION

ICP-MS (Inductively Coupled Plasma Spectrometry) used to determine the accurate concentration of Calcium (Ca). Manganese (Mn) present in B.P.C. (Blood Pressure Controller) Capsule of herbal medicines. These elements are needed for metabolism, enzymatic reactions, normal cellular activities and help to maintain the acid-base balance. Herbal tablets of B.P.C. Capsule were obtained from Peekay pharma and details are given in Table Number -01. Herbal medicine in Ayurveda is derived from roots, leaves, fruits, bark, seeds, etc. Various parameters such as dosage, stability, toxicity, chemical factors such as pesticide residues, aflatoxin contents and heavy metals contamination and patient's age (Adult or Children) should be taken in to consideration**.** World Health Organization (WHO) states that around 85-95% of the world population uses traditional herbal medicines.[1] Most of the people use herbal medicines for less toxicity and minimum side effects. These medicines are now available in different forms like tablets, elixirs, Tonic and powders.[2] Herbal medicines have become more popular as alternative and supplementary remedies in recent years. Contamination or adulteration of herbal medicines with toxic metals, essential elements, trace elements and insect debris[3] are of major concern. The poor-quality control of these medicines causes health hazards like anemia due to destruction of red blood cells. World health Organization gives some guidelines[4] for the preparation of herbal medicines and listed some methods for the standardization of herbal medicines[4] and give maximum permissible limit of heavy metals[5] and quality-controlled norms. It is important to follow the quality control norms to standardize the herbal medicines. Various instrumental methods like HPLC -High –Performance Liquid Chromatographic techniques,[6] GC-Gas Chromatography[6] electrophoresis and TLC -Thin Layer Chromatography,[6] XRPD[7] are reported for the standardization of herbal medicines in maintaining the quality and well-defined constituents are required for reliable beneficial therapeutic effects. Therefor ICP-MS methods are developed which has high degree of sensitivity and specificity with the elements presents.

MATERIAL AND METHODS

For ICP-MS

Chemicals: Yttrium as internal standard, de-ionized water solution of 0.5% nitric acid and 2 ppm gold. (Thermo – fisher ICP-MS iCap model.)

Table 01: Tablet name with company name and plant constituents as per label.

Sr. No	Brand and Company Name	Medicines Name	Plants as per label*
1	Peekay pharma (Mfg. Lic No- 25D/10/88)	B.P.C. capsule	Sarpgandha, Lahasun, Arjunchhal Ex, Guggul Ashwag Jatamansi, Naandha, Isabgol, Brahmi, Jatamansi, Nagarmotha, Shankpushi, Kapoor kachri, Badi ilaichi

Sampling*:* In the present study, the marketed herbal tablets B.P.C. Capsule, was selected for the analysis. The brand names of the medicines, license number and the plants used as per company's label are included (Table 01).

Figure 01: B. P. C. Capsule sample.

METHODS

Samples: B. P. C. Capsule, here after labelled F. By taking the weight of Capsule contents on digital balance. Contents of Capsule is gently ground to fine powder using mortar and pestle and packed in butter paper until analysis. Quantitative multi-elemental analysis was carried out by inductively coupled plasma (ICP) iCap-Q spectrometry depends on a complete digestion of solid samples. To determine each inorganic metal concentration, 0.125 mL internal standard and 4.675 mL of diluent added in to 0.2 mL sample solution. De-ionized water solution of 0.5% nitric acid and 2 ppm gold was used as a diluent.

Table 02: Sample weight and dilution.

Sr. No.	Samples	Weight in grams	Dilution
1	B. P. C. Capsule (F)	0.11798	100 ml in 1 % HNO_3

Table 03: Standard preparation.

Concentration	Yttrium 1 ppm	MES	MES + Hg (20 ppb)	Final Volume (mL)
Std .05 ppb	750 µL	-	75 µL	30
Std 0.5 ppb	750 µL	-	750 µL	30
Std 1.0 ppb	750 µL	-	1500 µL	30
Std 2.0 ppb	750 µL	-	3000 µL	30
Std 5.0 ppb	750 µL	150 µL	-	30
Std 20 ppb	750 µL	600 µL	-	30
Std 50 ppb	750 µL	1500 µL	-	30
Std 100 ppb	750 µL	3000 µL	-	30
Std 200 ppb	750 µL	6000 µL	-	30

Instrument configuration

Thermo – Fisher ICP-MS iCap model was used for all measurements. The instrument was operated in a single collision cell mode with kinetic energy discrimination (KED), using pure Helium as collisions gas. The general analytical condition set for the ICP-MS are given in Table Number 04.

Table 04: General analytical conditions for ICP-MS.

Sr. No.	Parameter	Value
1	Spray Chamber Temperature	$2.7\,^0C$
2	Cool Flow	14 L/Min
3	Sampling Depth	5 mm
4	Plasma Power	1550 Mpa
5	Auxiliary Flow	0.8 L/Min
6	Nebulizer Flow	1.0079 L/Min
7	Peristatic Pump Speed	25 rpm

Table 05: Calibration correlation coefficient R and BEC (ppb) data.

Sr. No	Isotope	R	BEC (ppb)
1	^{40}Ca	0.995	-1.230
2	^{55}Mn	0.993	0.612

Table 06: Observed elemental concentrations in ppm by ICP-MS.

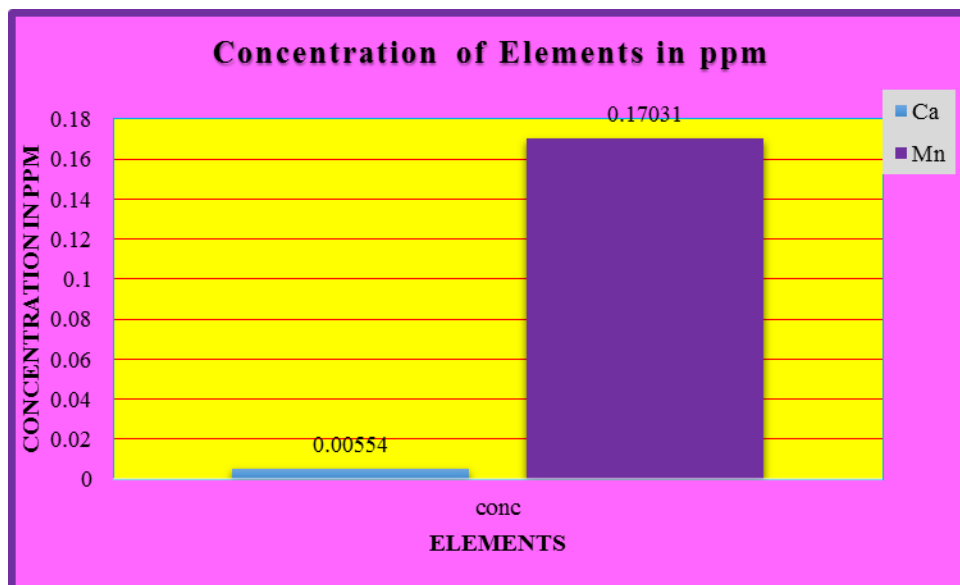| Sr. No	Samples	Elemental concentration in ppm	
		Ca	Mn
1	B.P.C. Capsule	0.00554	0.17031

Figure 02: Graphical representation of concentration of elements.
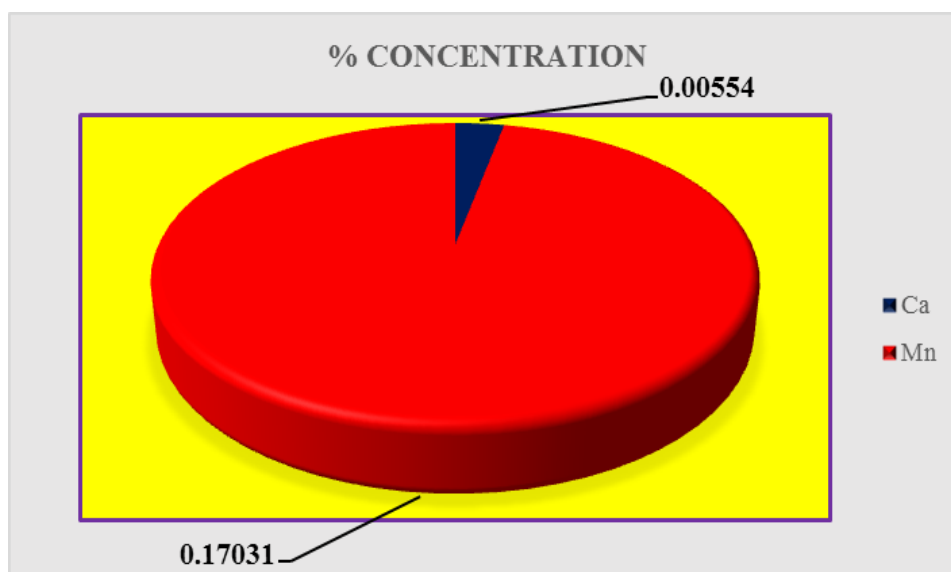

Figure 03: Graphical representation of percentage concentration of elements.

RESULT AND DISCUSSION

Biological application[8]

Manganese

Manganese is an important electrolyte for human health, essential for development of metabolism, and the antioxidant system.

Calcium

Calcium is not only necessary for the formation of bones and teeth, but also critical for transmission of nerve impulses, blood clotting, and muscle contraction. The excess calcium

in the body, is uncommon, but can come from excessive consumption of calcium-rich foods, certain bone diseases or extreme inactivity e.g., quadriplegic/paraplegic conditions where the bones bear no weight. Symptoms may include digestive problems and nausea in minor cases, but can cause brain dysfunction, coma or even death in extreme instances. Deficiency of calcium may not cause immediate symptoms, but over time can also affect the brain, leading to delirium, memory loss and depression; severe cases may lead to muscle spasms, seizures and abnormal heart rhythms.

Table 07: LD_{50} of the elements (The merck index, 1989).[9]

Sr. No	Elements	Compounds	LD_{50}
1	Calcium (Ca)	Calcium acetate	04.28 g/kg orally on rat
2	Manganese (Mn)	Manganese dioxide	45 mg/kg in rabbit

Table 08: Airborne threshold limit of elements.[10]

Sr. No	Elements	Air born threshold limits
1	Calcium	2.0 mg/m^3
2	Manganese	0.02 mg/m^3

Table 09: Approximate elementary composition of the human body (Dry Weight Basis).[11]

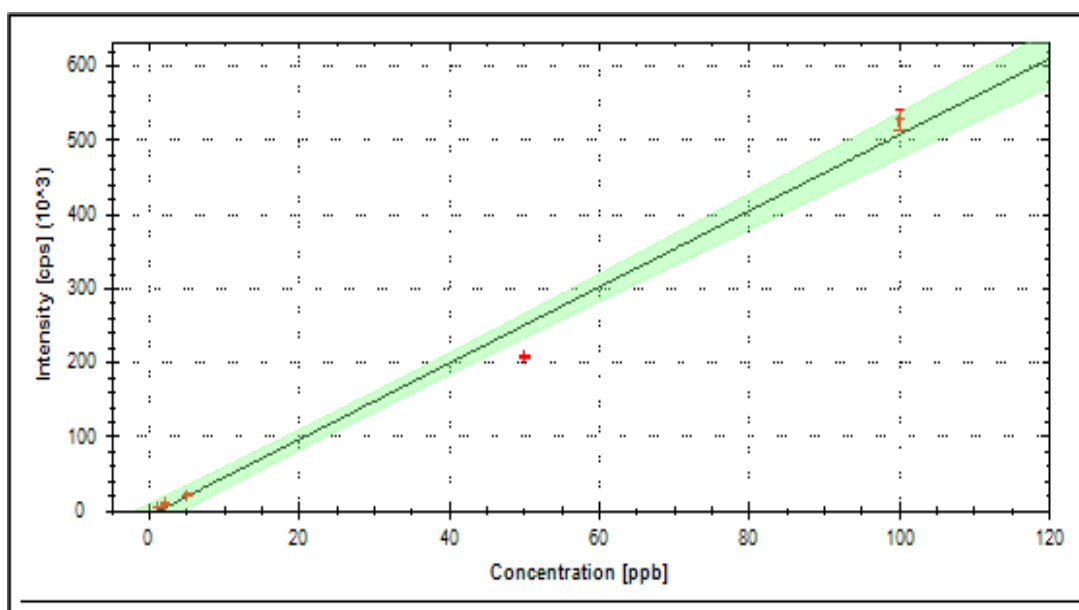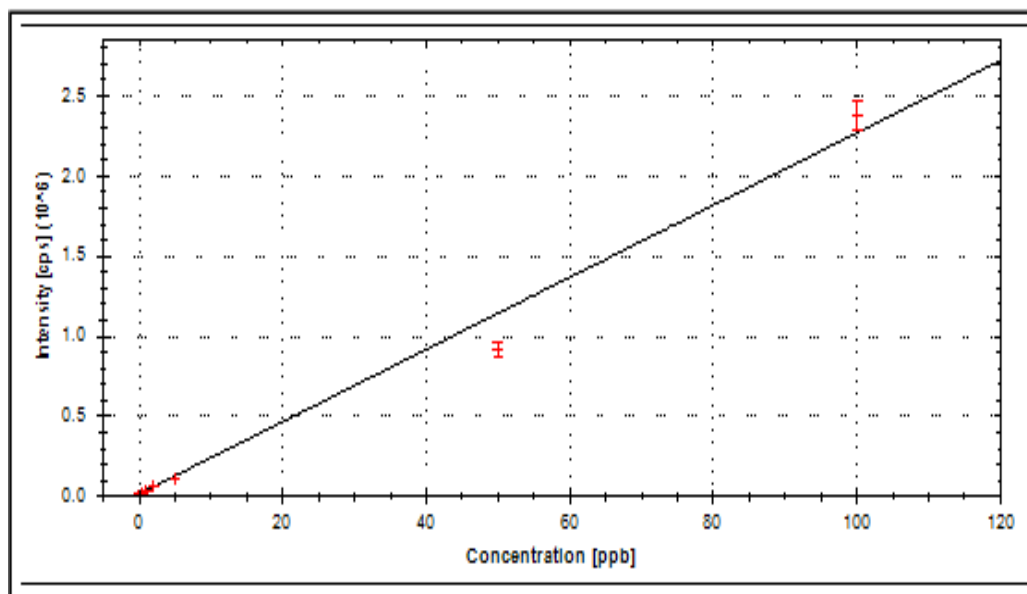| Sr. No	Element	Percentage
1	Calcium (Ca)	4.00
2	Manganese (Mn)	0.001

Figure 04: Calibration curve for calcium (Ca).

Figure 05: Calibration curve for manganese (Mn).

Limit of detection (LOD) and Limit of quantification (LOQ) for the instrument

Development of Analytical method and validation are the most important factors for the preparation of drugs in pharmaceutical industry. Limit of detection and limit of quantification are two importance parameters in method of validation.[12] Ayurvedic medicinal Sample F, B.P.C. Capsule a wet digestion and diluted to 100 ml in 1% HNO_3. This sample is directly run through ICP-MS for the detection of metals.

$$LOD = \frac{3SD}{b}$$

$$LOQ = \frac{10SD}{b}$$

Where- LOD = Limit of Detection

LOQ = Limit of Quantification

SD = Standard deviation

b = Slope

$$LOD = \frac{3\ SD}{Slope}$$

$$= \frac{3 \times 0.208494604246}{5.133}$$

$$= 0.1218554086 \text{ ppb}$$

$$= 0.000121855 \text{ ppm}$$

$$LOQ = \frac{10SD}{Slope}$$

$$= \frac{10 \times 0.208494604246}{5.133}$$

= 0.4061846955 ppb

= 0.0004061 ppm

From above calculation LOD for Calcium is 0.0001218 ppm and LOQ is 0.0004061 ppm. The actual detected concentration of Calcium is 0.00554 it means the detected concentrations of elements between the detection limit.

Table 10: Validation and Confidence limit with standard deviation.

Sr. No	Observations	Concentration of Elements in ppm	
		Ca	Mn
1	Element	Ca	Mn
2	Mean	0.00543	0.17048
3	SD	± 7.94984E-5	± 0.0135023
4	95%	± 0.00000912	± 0.01552476
5	99%	± 0.00001431	±0.02243468

The elements Calcium (Ca), Manganese (Mn), are of great importance in living organism and these elements plays an important role in biochemical reactions. The requirement of these elements for human being is in grams or in micro grams in the form of essential elements and Minerals. The detected concentration of Calcium (Ca) was 0.00554 ppm, Manganese (Mn) was 0.17031 ppm. The detected concentration of these elements is below the LD_{50} and below the air borne threshold limit and are not hazardous to humans.

CONCLUSION

Results obtained from ICP-MS analysis of tablet samples detected the accurate values of elemental concentration in ppm. The detected values of elements are below LD_{50}. The content of these elements did not indicate the label. Elemental analysis by ICP-MS is a recent technique which gives more accurate concentration of these elements contained in the samples which is not previously reported by researchers in this tablet. Quantitative estimation of metals is done by atomic absorption spectroscopy in bhasma only, not in tablets, therefore, the concentration of these elements is below the hazardous levels to the patient. The sensitive instrumental techniques of ICP-MS are used in the present study can be made mandatory for the quality control of Herbal medicines.

ACKNOWLEDGEMENTS

Prabandhak Committee's, Guru Nanak Khalsa College of Arts, Science and Commerce, Matunga, Mumbai-19.

REFERENCES

1. World Health Organization (WHO) WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, WHO publication, Switzerland, 2007.

2. Lozak, A et al. P. Determination of selected trace elements in dietary supplements containing plant materials. Pol. J. Food Nutr. Sci, 2012; 62(2): 97-1002.

3. Amol, V. Application of modern analytical methods in herbal drug analysis, Ph. D thesis Mumbai University, 2006; 06: 65-89.

4. World Health Organization (WHO) Quality control methods for herbal materials. Updated edition of Quality control methods for medicinal plant materials, 1998; 2011.

5. The Merck Index. Eleventh edition. An Encyclopaedia of Chemicals, Drugs and Biologicals. Merck & co. Inc. Rahway. New Jersy. USA, 1989.

6. World Health Organisation (WHO). WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, WHO publication, Switzerland, 2007; 19-20.

7. Sunil, S. Application of thermal methods and X-ray powder diffraction methods in developing fingerprints of some herbal and synthetic drugs, Ph.D. Thesis, Mumbai University, 2009; 10: 169-239.

8. Melissa Conrad Stoppler, William C. Shiel Jr. Electrolytes. MedicineNet.com.

9. The Merck Index. Eleventh edition. An Encyclopaedia of Chemicals, Drugs and Biologicals. Merck & Co. Inc. Rahway. NewJersy. USA, 1989.

10. Harper's Biochemistry Twenty Second edition by Robert K. Murray, Prentice Hall, USA, 1988; 07.

11. Alankar Shrivastava, Vipin B. Gupta. Methods for the determination of limit of detection and limit of quantification of the analytical methods. Chronicles of Young Scientists, 2011; 2(1): 21-25.