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COMBINING SYNERGY OF INDUCTIVELY COUPLED PLASMA MASS-SPECTROPHOTOMETRY (ICP-MS) AND X-RAY POWDER DIFFRACTION (XRPD) FOR HERBAL MEDICINAL PRODUCT OF B. P. C. CAPSULE.

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ABSTRACT
BPC Capsule is one of the most popular herbal medicines in Indian market for Blood Pressure patients**.** Electrolytes, Trace elements, Toxic, Essential, Trace metals, Minerals, Alkaloids, Steroids and organic compounds, besides that it also contains enzymes, proteins, and other Inorganic elements are naturally present in herbal medicines**.** The BPC**.** Capsule contain, Calcium**(**Ca**)**, Manganese(Mn) and zinc**(**Zn**).** Though they are essential elements it is utmost necessary to determine the quantitatively because it most effects on Blood pressure and kidney**.** The daily requirement of Calcium**(**Ca**)**, Manganese(Mn) and zinc**(**Zn**)** is in micrograms, but there is no proper method to detect the accurate concentration of essential elements, minerals, electrolytes and Inorganic elements present in herbal medicines**.** 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**.** In human being, these elements can cause severe physiological and health effects**.** Therefore, it is thought necessary about the efficacy and standardization of BPC Capsule**.** These Elements can determine by quantitatively and validated by using modern technique such as ICP-MS **(**Inductively Coupled Plasma Mass-Spectrophotometry**)** and XRPD**. (**X-Ray Powder Diffraction**).**

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

INTRODUCTION
ICP-MS (Inductively Coupled Plasma Spectrophotometry) used to determine the accurate concentration of Calcium(Ca), Manganese(Mn) and zinc(Zn) present in BPC (Blood Pressure Controller) Capsule of herbal medicines**.** The rapid development of metro cities in developing as well as in developed countries it causes adverse life style effects like unhealthy food, pollutions and a sedentary job, and transportation mode are likely to be risk factors for Blood Pressure, Bone diseases and cardiac diseases**.** These elements are needed for metabolism, enzymatic reaction, normal cellular activities and help to maintain the acid-base balance. X-Ray Powder Diffraction (XRPD) shows the direct information regarding the elements and their physicochemical properties. It is very important technique in the pharmaceutical industry, sensitive method as a phase characterization, polymorphism. Particle size affect the absorption, efficacy of the herbal drugs. Herbal Tablet of BPC. Capsule, samples (Details Given in table number -1.) were scanned for the X-Ray powder diffraction by using X-ray diffractometer (Rigaku, Model-Miniflex II). Inorganic element studied by X-Ray Powder Diffraction by using inorganic oxides as a standard and to study the XRPD pattern by comparing with standard pattern. Several modern technologies are used to know the showing 2 θ (°) (Two Theta Degree) value of three strongest peaks of material characterization of BPC Capsule and can be used as fingerprint in quality control of BPC Capsule. Herbal medicine in Ayurveda 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 [1]. 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 [2] are of major concern. The poor-quality control of these medicines causes health hazards like anemia due to destruction of red blood cells. Particle size affect the absorption, efficacy of the herbal drugs, therefor XRPD analysis is essential Herbal tablet is safe for human consumption. World health Organization gives some guidelines [3] for the preparation of herbal medicines and listed some methods for the standardization of herbal medicines [3] and give maximum permissible limit of heavy metals [4] and quality controlled norms. It is important to follows the quality control norms to standardize the herbal medicines. Varies instrumental methods like HPLC -high –performance chromatographic techniques [5], GC-gas chromatography [5], electrophoresis and TLC -thin layer chromatography [5], XRPD [6]. Are reported for the standardization of herbal medicines maintained the quality and containing well defined constituents are required for reliable beneficial therapeutic effects. Therefor ICP-MS and XRPD methods are developed which has high degree of sensitivity and specificity.

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: 1. Tablet name with company name and plants as per label.

Sr. No	Brand and Company Name	Medicines Name	Plants as per label *
1	Peekay pharma (Mfg. Lic No-25D/10/88)	BPC 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 BPC 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 1).

Experimental design

Methods

Samples

BPC 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 by inductively coupled plasma (ICP) Icap-Q spectrometry depends on a complete digestion of solid samples. However, fast and thorough sample digestion is a challenging analytical task in modern multi-elemental analysis. To determine each heavy metal concentration, 0.125 mL internal standard and 4.675 mL of diluent added in to 0.2 mL sample solution. De-ionzed water solution of 0.5% nitric acid and 2 ppm gold was used as a diluent.

Table: 2 Sample weigh and dilution.

Sr. No.	Samples	Weight in grams	Dilution to
1	B. P. C. Capsule(F)	0.11798	100 ml in 1 % HNO3

Table: 3. Standard Preparation:Stock Standards Available of 10 ppm Multi Elemental Standards and Mercury Analysis

Concentration	Yttrium 1 ppm	MES	MES + Hg (20 ppb)	Final Volume (mL)
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

***MES- Multi Elemental Standards**

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 He as collision gas. The general analytical condition set for the ICP-MS are given in table number 2.

Table: 4. General Analytical Conditions for ICP-MS

Sr. No	Parameter	Value
1	Spray Chamber Temperature	2.7
2	Cool Flow	14
3	Sampling Depth	5
4	Plasma Power	1550
5	Auxiliary Flow	0.8
6	Nebulizer Flow	1.0079
7	Spray Chamber Temperature	2.7
8	Peristaltic Pump Speed	25

Table: 5. Calibration correlation coefficient R and BEC (ppb) data.

Sr. No	Isotope	R	BEC(ppb)
1	^{40}Ca	0.995	-1.230
2	^{66}Zn	0.995	3.633
3	^{55}Mn	0.993	0.612

Table: 6. Elemental concentration in ppm by ICP-MS.

Sr. No	Samples	Elemental concentration in ppm		
		Ca	Zn	Mn
1	B.P.C. Capsule	0.00576	ND	0.17642

For XRPD-

Samples

By taking the weight of B. P. C. Capsule contents on digital balance is gently ground to fine powder using mortar and pestle at room temperature and packed in butter paper until analysis. X-ray diffractometer (***Rigaku, Model-Miniflex II***) instrument was set up as follow then sample of BPC Capsule was scanned. Parameters are given in table number -1.

TABLE: 7. XRD PARAMETR

Sr. No	Parameter	Values
1	X-ray	Cu / 30 kV / 15 mA
2	Div Slit	1.25 deg
3	Sct Slit	1.25 deg.
4	Rec Slit	0.3mm
5	Scan mode	Continuous
6	Scan speed	5.000 deg./min
7	Sampling width	0.020 deg.
8	Scan axis	2theta/theta
9	Scan range	10.000 -> 80.000 deg.
10	Theta offset	0.000 deg.

Figure No-1 B. P. C. Capsule sample

RESULT AND DISCUSSION

Biological Application of Calcium, Zinc and Manganese.

Calcium(Ca)

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.[8]

Zinc(Zn)

Zinc is essential for the normal growth and development of mammals and present to extent of 1.4 2.3 g in human body. Zinc complexes are good buffers and are used to control the PH.[7] Zinc interact with wide range of organic ligands and has roles in the metabolism of RNA and DNA. zinc can be part of an effective treatment for age related macular degeneration. zinc is in the brain, muscle, bones, kidney and liver, with the highest concentrations in the prostate and parts of the eye. Zinc is useful to making catalytic agent in hydroxylation and other enzymatic reactions.

Manganese(Mn)

Manganese is required for Photosynthetic Oxygen evolution and plays an important role in several metabolic processes such as bone growth, glucose tolerance, reproduction and development of inner ear.[7] Manganese is an important element for human health, essential for development of metabolism, and the antioxidant system. It is also important in photosynthetic.[8]

Table: 8. LD 50 of the elements (The Merck Index, 1989).

Sr. No	Elements	Compounds	LD50
1	Calcium	Calcium acetate	04.28 g/kg orally on rat
2	Zinc	Zinc acetate	2.46 g / kg orally in rats
3	Manganese	Manganese dioxide	45 mg/kg in rabbit

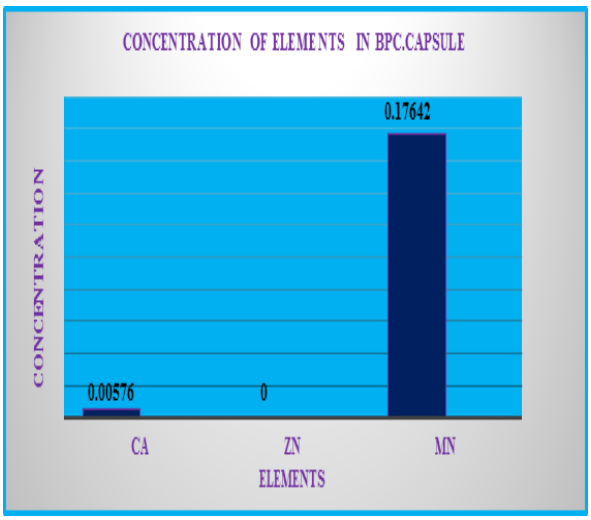

Figure: 2. Graphical representation of concentration of elements

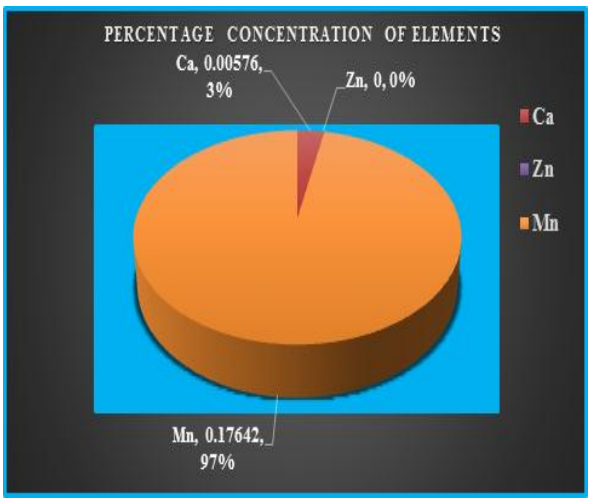

Figure: 3. Graphical representation of Percentage concentration of elements

Table: 9. Airborne threshold limit of elements.[9]

Sr. No	Elements	Air Born Threshold Limits
1	Calcium	2 mg/m^3
2	Zinc	2 mg/m^3
3	Manganese	0.02mg/m^3

Table: 10 Approximate elementary composition of the human body (dry weight basis)[10]

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

Table: 11. Ion Concentration in Extracellular Blood Plasma.[7]

Sr. No	Ion	Ion Concentration in Blood Plasma
1	Ca (calcium)	3Mm
2	Zinc	0.02Mm

XRPD measurements are performed for Sample F, B. P. C. Capsule an analytical instrument with Cu Kα1 radiation with highly sensitive solid state detector. The X-ray tube operated at Cu / 30kv and 15mA. XRPD analysis of B. P. C. Capsule F1(One Capsule) Shows the highest peak at 26.58 and 31.01 Two theta degree and B. P. C. Capsule F3(Three Capsule) Shows the highest peak at 26.66 and 30.96 two theta degree as shows in figure - 4 XRPD Pattern of BPC Capsule.

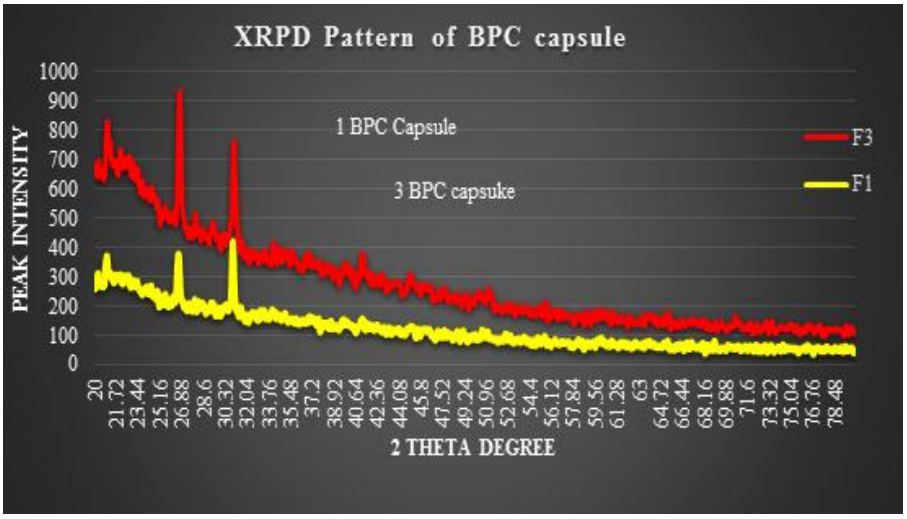

Figure: 4. XRPD Pattern for B. P. C. Capsule

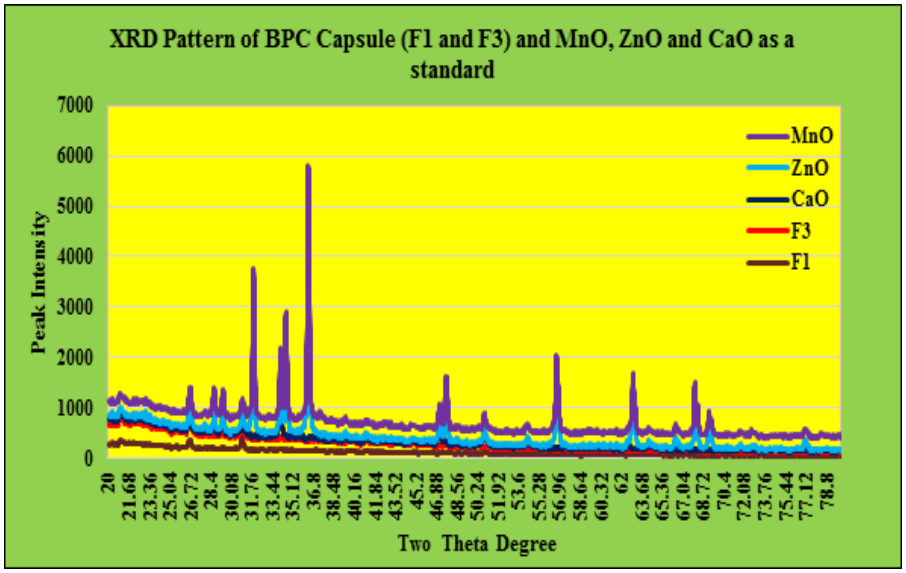

Figure: 5. XRPD Pattern of BPC Capsule and Calcium Oxide, Zinc Oxide and Manganese Oxides as a standard

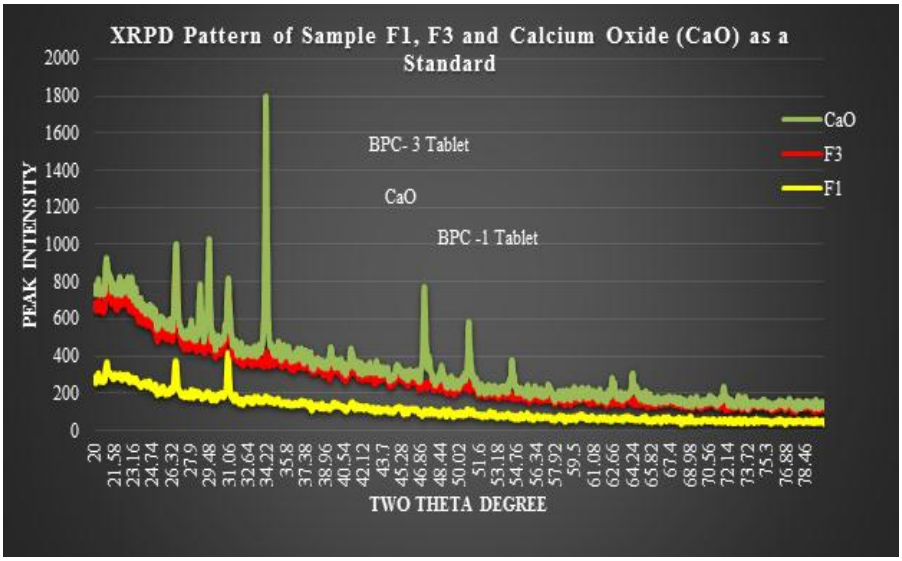

Figure: 6. XRPD Pattern of BPC Capsule Sample and Calcium Oxide

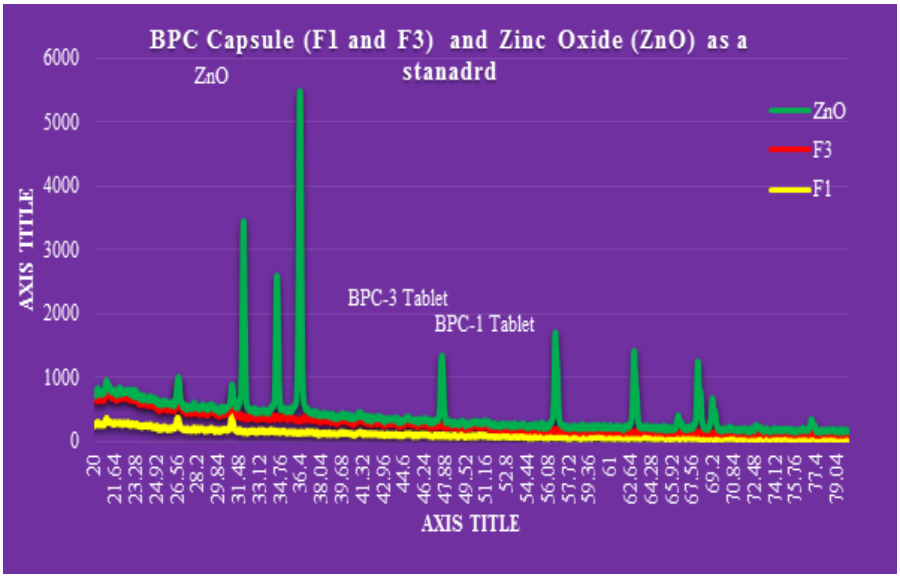

Figure: 7. XRPD Pattern of BPC. Capsule (F1 and F3) sample and Zinc Oxide (ZnO) as a standard

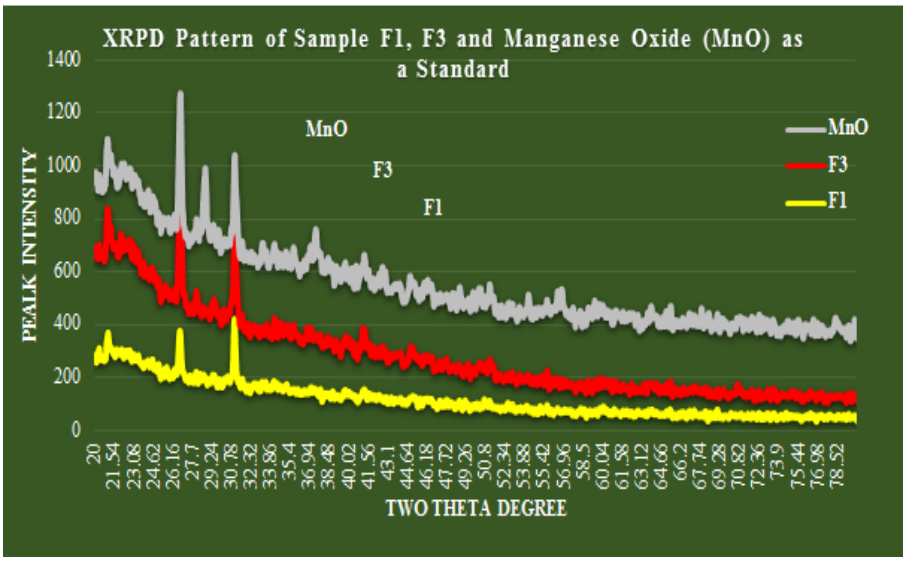

Figure: 8. XRPD pattern of BPC. Capsule (F1and F3) sample and Manganese Oxide (MnO) as a standard.

The elements Calcium(Ca), Zinc(Zn) and 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 **0.00576 ppm**, Zinc(Zn)was **not** detected and Manganese(Mn) was **0.17642 ppm**. The detected concentration of these elements is below the LD50 and below the air borne threshold limit and are not hazardous to humans.

These elements also detected by using modern technique XRPD (X-Ray Powder Diffraction).

X-ray diffractometer indicate the presence Calcium(Ca) and Manganese (Mn) in the BPC Capsule of Herbal medicine. By using this X-Ray Powder diffractometer to avoid the laborious laboratory work and to get instant result regarding the presence of inorganic elements.

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 LD50. The content of these elements is not indicated 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. Quantitative estimation of metals is done by atomic absorption spectrophotometer 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 and XRPD are used in the present study can be made mandatory for the quality control of Herbal medicines.

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