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Original Article

A Quantitative Approach to Estimate both Essential and Non-essential Elements in Some Commercial Samples of *Triphala churna* by using WD-XRF Spectrometry

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ABSTRACT

Introduction: The need for quality control of herbal drugs is in demand in order to ensure the purity, safety and efficacy of herbal products. A total of 19 elements including essential and non-essential elements were characterized in five commercial samples of *Triphala churna* using WD-XRF spectrometry. **Method:** The WD-XRF method was validated for each element by a pre-calibrated program using five Chinese certified reference materials of vegetable standards (NCS ZC73012, NCS ZC73013, NCS ZC73017, NCS ZC85006 and NCS DC73348). **Results:** The following elements were detected in all the samples out of 19 elements tested with increasing order of concentrations (mg/kg): Cr (3) < Cu (7) < Ba (24) < Zn (31) < Pb (46) < Mn (57) < S (700) < Na (1064) < Mg (1250) < Fe (1329) < P (1400) < Cl (2960) < Ca (3110) < Si (4350) < K (15130). Lead (41-46 mg/kg), a non-essential element was found above its PDE limit (≤ 10 mg/kg). **Conclusion:** WD-XRF method was found simple, rapid, reliable and non-destructive technique to investigate the elemental concentrations in herbal drugs.
Key words: Elemental analysis, WD-XRF, ICP-MS, *Triphala churna*, *Ayurveda*, ISM

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INTRODUCTION

Ayurvedic medicines are one of the important ancient medical systems of India. About 80% of the world population still primarily rely on herbal drugs.[1] In India, *Ayurvedic* medicines were widely considered due to affordability and availability in local markets. Also, the demand for *Ayurveda* drugs in Western market has increased tremendously which draw attention to the need of quality of *Ayurvedic* products. Especially, the presence of heavy metals at high concentrations in these drugs are potentially harmful to consumers and causing severe adverse health effects. Hence, the screening of these herbal drugs is given preference in scientific studies and the WHO in number of resolutions has emphasized the need of quality control of plant products by using modern techniques and suitable analytical methods.[2] Therefore, the present study was focused to carry out to analyse essential and non-essential elements using WD-XRF spectrometry in five commercially important *Triphala churna* drugs. *Triphala churna* is a mixture of three dried fruits (Haritaki, Bibhitaki and Amalaki) in equal proportions (1:1:1) and a variety of therapeutic applications have been described in ISM.[3]

MATERIALS AND METHODS

Sample collection

Triphala churna drugs were procured from local registered *Ayurvedic* pharmacies at Visakhapatnam, India. The batch number, date of manufacture and date of expiry are recorded and kept available in the Centre for Chemical Analysis, Central Research Laboratory, GITAM University, Visakhapatnam, India. The brand names were blinded and coded in sequential order i.e. TPC1, TPC2, TPC3, TPC4 and TPC5.

Experimental and Instrumental Details

Pellets of each drug sample and standard were made by taking three grams of powder sample in an aluminum cup and pressing using a hydraulic press (HERZOG, TP40/2D) at 15 tons to obtain a disc shape with 40 mm diameter. These pellets were subjected to WD-XRF spectrometer and characterized the elemental composition as described by Swamy and Sivanarayanan (2014).[4]

RESULTS AND DISCUSSION

In the present study, the levels of 19 essential and non-essential elements such as Na, Mg, Al, Si, P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, As, Ba, Cd, Hg and Pb were determined using WD-XRF spectrometry in five samples of *Triphala churna* are summarized in Table 1 and they are the mean of three replicates. The WD-XRF method was validated for each element and good agreements were achieved between present values and published data.[4] The available specific standards for few trace elements (Pb ≤ 10 ppm, Cd ≤ 0.3 ppm, As ≤ 10 ppm and Hg ≤ 1 ppm) by WHO[5] and tolerance limits of elements in food or

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dietary intake published by CEC[6] are considered for quality regulation of *Ayurvedic* drugs tested in this study (Table 1).

Interpretation of WD-XRF data

Out of 19 elements analysed, Na, K, Mg, Ca, P, S and Cl are essential nutrients and they were present in substantial quantities in all remedies and most of them were within tolerable upper intake level (Table 1). Elements such as Ba, Cr, Cu, Al, Mn, Fe, Zn and Si are considered as micronutrients and exhibit relatively low toxicity with high PDE.[7] The concentration of 'Ba' in the present study was ranged from 20 to 24 mg/kg and it was within the permissible limits according to WHO guidelines (2004) i.e. the suggested mean daily intake of Ba from food, water and air is about 100 mg/kg.[8] Trivalent Cr has a low level of toxicity at high concentrations, but, hexavalent Cr is more toxic and causes renal and hepatic necrosis and growth retardation.[6] In the present study, Cr was found to be present in all samples ranging from 1 to 3 mg/kg, which is below the PDE limit i.e. 15 mg/kg as per USP guidelines (2009).[9] In all the samples, Cu was detected within PDE limit with concentration between 5 to 7 mg/kg and Zn was ranged 27 to 31 mg/kg which is about 30 times lesser than the recommended PDE limit by CEC (Table 1). The concentration of 'Fe' in the present study was found within PDE limit in all the samples except TPC1 (1329 mg/kg, Table 1). The excess iron levels in the body can damages cells in the heart and liver which can cause cancer, diabetes, coma, metabolic acidosis, liver failure, cardiac failure, circulatory shock and long-term organ damages.[10] An excess of Mn can lead to poor iron absorption and its deficiency can cause poor bone formation, affect fertility and ability of blood to clot.[10] In the present study, Mn was found below the PDE limit in all the samples within the range of 30 to 57 mg/kg (Table 1). Aluminum intake even in small amounts may lead to bio-accumulation in consumer's body, which is so difficult to get-rid-off and further, cumulative Al in the body can reduce the absorption of a number of elements including essential elements Fe, P, Ca, etc.[10,11] In the present study, Al was detected in all the samples within the range of 362 to 1140 mg/kg. Silicon was ranged from 1590 to 4350 mg/kg, an essential mineral required for human body to grow and maintain strong bones, hair, fingernails, connective tissues, skin, blood vessels and healthy artery walls.[10,11] It helps to counter the effects of high accumulation of Al in the body, but, an excess of Si may cause irreversible kidney damage.

The other elements that are analyzed, As, Cd, Hg and Pb are not useful role in human physiology and therefore considered to be non-essential elements. Of these, presence of Pb was detected in all market samples tested in the present study with a concentration range of 41 - 46 mg/kg (Table 1), which is almost 4 folds higher than its PDE (≤ 10 mg/kg). In fact, Pb existence in herbal drugs above its permissible limit is predictable and reported in many earlier studies. However, high dose of 'Pb' detected in current study in all samples may cause harmful health effects[12] and alarming the safety concern of these herbal products. As, Cd and Hg were not detected due to they maybe present below its detection limit of XRF and in such cases, XRF should be used in conjunction with a more sensitive technique, AAS / ICP.

Comparison of XRF data with ICP Data

The WD-XRF technique has adequate sensitivity to determine essential as well as trace elements and was well proved in earlier studies.[4] However, the results obtained in the present study by WD-XRF spectrometry were cross checked with available ICP-MS data (Table 2).[3] The Cu mean values determined by the WD-XRF method was slightly higher than that determined by the ICP-MS method with good RSD (3-28%) and only TPC1 sample was noticed with a higher ICP value with 28% RSD. A significant difference was observed for Zn i.e. almost two fold higher XRF values were noticed when compared with ICP data with 32-40% RSD. Agreement, typically 22-50% (RSD) was noticed between methods WD-XRF and ICP-MS for Cr. The mean values determined by the WD-XRF method was noticed to be slightly higher for elements Ba, Ca, Fe, K, Mg, Mn, Na when compared with reported ICP-MS data and they are close to each other with good agreement i.e. RSD (%), 19-25, 8-10, 12-19, 4-12, 14-17, 16-20 and 22-25, respectively. These variations in elemental con-

Table 1: Levels of elements in various Triphala Churna drugs by WD-XRF

Elements (mg/kg)	PDE / UL		Triphala Churna					Range	LLD
	AYUSH	CEC	TPC 1	TPC 2	TPC3	TPC 4	TPC 5		
Pb	≤ 10	3.0	41	46	43	42	43	41- 46	< 2
Ba	NA	NA	24	20	20	21	20	20 - 24	2
Al	NA	NA	1140	362	577	547	561	362 - 1140	5
Ca	NA	70000	2930	3000	3030	2130	3110	2130 - 3110	5
Cr	NA	NA	2	3	2	2	1	1 - 3	< 2
Cu	NA	110	5	7	6	5	5	5 - 7	2
Fe	NA	900	1329	465	792	869	918	465 - 1329	2
K	NA	310000	14760	13180	14560	15130	13330	13180 - 15130	5
Mg	NA	50000	1250	1220	1210	1220	1200	1200 - 1250	5
Mn	NA	1000	57	30	44	37	35	30 - 57	2
Na	NA	350000	1064	684	656	740	758	656 - 1064	5
Zn	NA	950	27	29	31	27	29	27 - 31	2
P	NA	55000	1070	1400	1170	1170	1110	1070 - 1400	2
S	NA	NA	620	700	630	630	560	620 - 700	2
Si	NA	NA	4350	1590	2210	2560	2350	1590 - 4350	2
Cl	NA	540000	2960	2180	2450	2270	2420	2180 - 2960	5

AYUSH: Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy ; CEC: Commission of the European Communities, UL: upper intake level, NA: not available, LLD: lower limit of detection, PDE: permitted daily exposure

Table 2. Comparison of elemental concentrations (mg/kg) by WD-XRF technique with published ICP-MS data

Element (mg/kg)	TPC1				TPC2				TPC3			
	XRF	ICP*	SD	RSD	XRF	ICP*	SD	RSD	XRF	ICP*	SD	RSD
Ba	24	16.8	5	25	20	14.8	4	21	20	15.2	3	19
Cr	2	2.7	1	22	3	5.1	1	36	2	1.3	0	28
Cu	5	7.5	2	28	7	6.0	1	11	6	4.3	1	23
Zn	27	16.1	8	36	29	16.2	9	40	31	19.6	8	32
Mn	57	45.2	8	16	30	22.8	5	19	44	32.9	8	20
Fe	1329	1026	214	18	465	357	77	19	792	668	87	12
Na	1064	762	213	23	684	490	137	23	656	456	141	25
K	14760	12930	1294	9	13180	11057	1501	12	14560	13230	940	7
Mg	1250	998	178	16	1220	960	184	17	1210	987	158	14
Ca	2930	2536	279	10	3000	2672	232	8	3030	2721	218	8

Table 2. contd.

TPC4				TPC5				
XRF	ICP*	SD	RSD	XRF	ICP*	SD	RSD	RSD Range
21	16.1	3	19	20	14.2	4	24	19-25
2	3.8	1	45	1	2.1	1	50	22-50
5	5.2	0	3	5	4.2	1	12	3-23
27	16.5	7	34	29	17.1	8	37	32-40
37	29.2	6	17	35	27.6	5	17	16-20
869	711	111	14	918	730	133	16	12-19
740	541	141	22	758	529	162	25	22-25
15130	14220	643	4	13330	12480	601	5	4-12
1220	988	164	15	1200	948	178	17	14-17
2130	1888	171	9	3110	2729	269	9	8-10

**published data[3]; SD: standard deviation; RSD: relative standard deviation*

centrations i.e. low values were noticed by ICP-MS method3 with respect to XRF data, may be due to multi-elemental standards, different sample extraction procedures and various dilution concentrations are part of ICP analysis whereas XRF is a non-destructive technique. In hypothesis, a specific digestion method only recovers certain elements absolutely that are easily soluble and not structurally complicated, especially present in plant materials. In the opposite case, technique XRF also suffers from significant matrix effects present in plant materials. However, the XRF technique penetrates deeper into the sample bonds than the chemical extraction procedure for ICP-MS, may be showing higher concentrations for the same sample.

CONCLUSION

XRF is a non-destructive sample technique and the results obtained by WD-XRF spectrometry in present study were found to be compatible with reported ICP-MS data which suggests that the XRF technique can be a good choice for estimation of elemental concentrations in plant / herbal / Ayurvedic samples.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interests.

ABBREVIATIONS USED

WD-XRF: Wavelength Dispersive X-ray Fluorescence Spectrometry; **ICP-MS:** Inductively Coupled Plasma Mass Spectrometry; **AAS:** Atomic Absorption Spectroscopy; **PDE:** Permitted Daily Exposure; **UL:** Upper-intake Level; **mg/kg:** milligram/kilogram; **WHO:** World Health Organization; **CEC:** Commission of the European Communities; **USP:** United State Pharmacopeial Convention; **SD**: standard deviation; **RSD**: relative standard deviation; **TPC:** *Triphala churna*; **ISM:** Indian System of Medicine AFI: Ayurvedic Formulary of India

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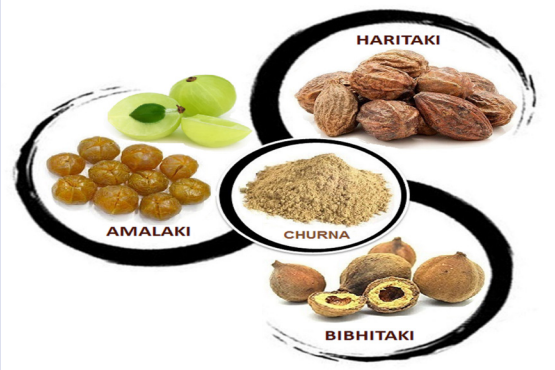
GRAPHICAL ABSTRACT



SUMMARY

- *Triphala churna* is one of the well known powder preparations of ISM being used in Ayurveda since ancient time. As per AFI, it is a polyherbal preparation comprised of 3 dried fruits viz. Haritaki (*Terminalia chebula* Gaertn Retz.), Bibhitaki (*Terminalia bellirica* Roxb.) and Amalaki (*Embellica officinalis* Gaertn.), in equal proportions (1:1:1).
- Elemental analysis in the present study has significance with respect to WD-XRF technique. The levels of 19 elemental concentrations (mg/kg) in 5 different branded TPC drugs have been found in a range of 1 (Cr) to 15130 (K) and a non-essential element, Pb (41-46 mg/kg) was found above its PDE limit (≤ 10 mg/kg).
- Good agreements were achieved between WD-XRF values and ICP-MS data.
- Results show that WD-XRF technique can be a good choice to estimate elemental concentrations in herbal drugs

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