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QUALITY STANDARDIZATION OF AYURVEDIC FORMULATIONS - *SANSHAMANI VATI* AND *MAHALAXMIVILAS RASA*

Priyanka Patil [2], Madhuree Gawhankar [2], Shivcharan Bidve [2] and Santosh Yadav [*1]

Shree Dhootapapeshwar Limited [1], Shree Dhootapapeshwar Ayurvedic Research Foundation [2], Veer Savarkar Chowk, Panvel, Navi Mumbai - 410206, Maharashtra, India.

Keywords:

Sanshamani Vati, Mahalaxmi vilas rasa, HPTLC, ICP-OES, XRD, Ayurveda

Correspondence to Author:
Mr. Santosh Yadav

QA/QC Manager,
Shree Dhootapapeshwar Limited,
Veer Savarkar Chowk, Panvel, Navi Mumbai - 410206, Maharashtra, India.

E-mail: santosh.yadav@sdlindia.com

ABSTRACT: Purpose: Sanshamani Vati (SHV) is useful in all types of Jwara, and Mahalaxmivilas Rasa (MLVR) is the best drug in various cardiovascular and respiratory disorders. To maintain the enormous trust in Ayurveda, it's necessary to ascertain the quality, efficacy & safety of Ayurvedic formulations on scientific lines using modern techniques. Present work attempts have been made to standardize the traditional Ayurvedic formulations - SHV and MLVR with modern techniques. **Method:** The formulations were standardized for physicochemical, Elemental and phytochemical screening. The XRD profiling was done for MLVR. Also the authentication of RM used in SHV was done using HPTLC. The validation of the methods for Elemental and phytochemical assays was performed as per ICH guidelines. **Results:** The Physico-chemical and phyto-chemical screening of Sanshamani Vati, and Mahalaxmivilas Rasa Tablets was done. The HPTLC profile confirms the presence of Berberine at Rf 0.25 ± 0.02 in SHV and Myristicin at Rf 0.50 ± 0.02 in MLVR. The elemental content of Gold (Au), Iron (Fe), Mercury (Hg) & Copper (Cu) in MLVR was estimated by ICP-OES. HPTLC Densitogram confirms the presence of authenticated *Tinospora cordifolia* RM in SHV. The XRD profile confirms the absence of free toxic metals – Hg & As. **Conclusion:** The present work will help understand therapeutic value concerning the quality parameters of the formulations. The standardization parameters presented in this research may serve as a standard reference to set the quality parameters for Sanshamani Vati and Mahalaxmivilas Rasa.

INTRODUCTION: Ayurveda is the world's oldest medical system believed to deal wide range of infections without causing any side effects [1]. This traditional Indian medicine network is entrusted since human existence and plays a vital role in combating and catering to global healthcare needs [2].

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Recent studies affirmed that about 70-80% of people, including developing countries, now rely on herbal medicines for their primary healthcare compared to modern allopathic drugs [3].

In India, the Ministry of Ayush (Ayurveda, Yoga, Unani, Siddha & Homeopathy) and an advisory coronavirus committee has also published some guidelines and recommendations with preventive management steps as per Ayurvedic practices through boosting immunity and several Ayurvedic medicines for symptomatic management of Coronavirus infection during the pandemic [4, 5]. In Ayurveda, it is mentioned that the Rasayana

Chikitsa or therapy promotes and rejuvenates physical as well as mental health of the body and produces resistance against diseases. As per modern science, the Rasayana therapy enhances immune responsiveness of an organism against pathogens by activating the immune system with immunomodulatory agents of plant origin [6]. In Ayurvedic Rasayana, many medicinal plants are valued for their therapeutic potential with immunomodulatory, anti-inflammatory, antioxidant, antidepressant activities and have been scientifically proven with promising effect based on the recommendation by Ministry of AYUSH, Govt of India [7, 8].

In Ayurveda, "Rasayana botanicals" such as Shatavari (*Asparagus racemosus*), Guduchi (*Tinospora cordifolia*) and Ashwagandha (*Withania somnifera*) known to modulate the immune system and possess antiviral activities are used for rejuvenation by boosting the immune system and alleviating disease condition [9-14]. With reference to Bhavprakash Nighantu (an Ayurvedic text), Guduchi (*Tinospora cordifolia)* is categorized as "Rasayana" [15] and used for its anti-inflammatory [16, 17, 24], immunomodulatory [17-20], anti-allergic [20], antipyretic [20], antidiabetic [17, 20, 21] properties. Sanshamani vati *i.e*. Guduchi Ghan (concentrated form of decoction) Vati an Ayurvedic preparation, is used as adjuvant in various hospital trials against COVID-19 due to its anti-inflammatory, antimicrobial and immuno-modulation properties [22-25].

In-silico studies also confirmed that the phytochemical compounds of Guduchi (*Tinospora cordifolia)*, Berberine and Sitosterol found as most powerful inhibitors against COVID-19 [26, 27]. *Tinospora cordifolia* is the accepted botanical source for Guduchi in Indian Ayurveda system. Despite of this fact, *Tinospora crispa* is used as substitute and sometimes as adulterant to *Tinospora cordifolia* in the formulations due to their similarities [27]. Many cases reveal that *Tinospora crispa* might have the negative effect of inducing hepatotoxicity [29]. To avoid such circumstances, a system must be developed to confirm *Tinospora cordifolia* species in the formulation instead of *Tinospora crispa.* In addition to Rasayan botanicals, the Ayurvedic *rasa-aushadhis* are also having qualities such as instant effectiveness, requirement in very small dosage and abundant therapeutic utility [30]. These *Rasa-aushadhis* have been used to treat chronic ailments since time immemorial [29]. Mahalaxmivilas Rasa, a herbo-mineral-metallic preparation, comes under the *Khalviya Rasayana* (A preparation method of *Rasa-aushadhis*) [30]. It has broader therapeutic activity in *Urdhwa Jatrugata rogas* (Upper Respiratory Disorders), *kasa* (Cough), *Pinasa* (Chronicrhinitis/sinusitis), *Gala Roga* (Diseases of throat), *Atisara* (Diarrhoea), *Nasa Roga* (Disease of nose), *Netraroga* (Eye disorder), *Mukha Roga* (Disease of mouth) [30, 31]. The *Rasa-aushadhis* are the Formulations made by mercury and incinerated metals and minerals [30]. Mercury is an extremely hazardous heavy metal known for its toxicity to human health [32]. It undergoes extensive detoxification procedures before being used in any formulation. The toxicity of Mercury is seen mainly due to its elemental and organic form and not due to inorganic form. Inorganic mercury is considered to be the least toxic among the different forms of mercury [33-34].

Considering the therapeutic effect of Sanshamani Vati and Mahalaxmi vilas rasa, the attempt of present work has been made to characterize and validate the formulations with modern techniques and to prove their safety of the formulations. The present work will help understand therapeutic value concerning the quality parameters of the formulations.

METHODS AND MATERIALS:

Chemicals: The Ayurvedic formulations Sanshamani Vati and Mahalaxmivilas Rasa Tablets were procured from Shree Dhootapapeshwar limited stockiest. Batch codes designated were Sample-1, Sample-2 and Sample-3 for each of three batches of these formulations. The manufacturing details of these batches are tabulated in **Table 1**. Myristicin of purity 98.0% (CAS No. CC60706) was procured from Natural Remedies Private Limited and Berberine of purity 98.0% (CAS No. 633658) was procured from Sigma Aldrich for chromatographic evaluations. The Certified reference standards of Elements Gold (Au) NIST SRM® 3121, Iron (Fe) NIST SRM® 3126a, Copper (Cu) NIST SRM® 3114 and Mercury (Hg) NIST SRM® 3133from Merck with the concentration of 1000 PPM were used for

Elemental analysis. All other chemicals and reagents (Butanol, Ethyl acetate, Methanol, Glacial acetic acid, Toluene, Formic acid, Hexane, Hydrochloric acid and Nitric acid) used in analysis were of Analytical grade of Merck.

TABLE 1: MANUFACTURING DETAILS OF MAHALAXMIVILAS RASA AND SANSHAMANI VATI

Product Name	Mahalaxmivilas Rasa	Sanshamani Vati
Manufacturing Reference	**Rasayogsagar 2/235**	**Ayurved Sar Sangrah**
Ingredients	Suvarna Bhasma 1 Part Abhrak Bhasma 16 Parts Shuddha Parad 2 Parts Shuddha Gandhak 8 Parts Vang Bhasma 4 Parts Tamra Bhasma 1 Part Shuddha Hartala 2 Parts Karpoor 2 Parts Jatiphal 2 Parts Jayapatri 2 Parts Shuddha Dhatturbeej 4 Parts Vruddhdaru Beej 4 Parts	Each tablet contains: Guduchi Ghana 125 mg

Organoleptic Evaluation: Organoleptic evaluations like colour, taste and texture of the samples of Sanshamani Vati and Mahalaxmi vilas Rasa Tablets were analyzed as preliminary quality check.

Physico-chemical Screening: In Physico-chemical screening, the samples of Sanshamani Vati and Mahalaxmivilas Rasa Tablets were analyzed for various Physico-chemical Parameters such as Hardness, Friability, Disintegration, weight variation, Loss on Drying (LOD), Ash, Acid Insoluble ash (AIA), Water soluble extractive (WSE) and Alcohol soluble extractive (ASE) as per The Ayurvedic Pharmacopoeia of India (API).

HPTLC Instrumentation and Experimental Conditions for Chromatographic Analysis: HPTLC Instrument Camag with Linomat 5, TLC Scanner 4 and Wincat Software was used for chromatographic analysis of Sanshamani Vati and Mahalaxmi vilas Rasa Tablets.

A twin trough chamber was used for the development of the HPTLC plate. A photo documentation cabinet fitted with High-Resolution camera was used for capturing images at different wavelengths.

Densitometer TLC Scanner 4 equipped with D2 and Tungsten (W) lamp was used to obtain spectra for the quantitative determination of the compound. The solvent systems, wavelengths and lamps used for estimation of Berberine and Myristicin, which gave good resolution are tabulated in **Table 2**.

TABLE 2: CHROMATOGRAPHIC CONDITIONS

#	Marker	Extraction solvent	Extraction technique	Solvent System	Visualization	Lamp Used	λ_{max}
1	Berberine	Methanol	Reflux on water bath	Butanol : Ethyl Acetate : Glacial acetic acid : Water (3 : 5 : 1 : 1) v/v	Under UV at 366 nm	Mercury (Hg)	366 nm
2	Myristicin	n-Hexane: Chloroform (1:1)	Cold maceration	Toluene : Ethyl Acetate (9.8 : 0.2) v/v	After derivatization with Anisaldehyde sulfuric acid reagent	Deuterium (D2) lamp	212 nm

Authentication of *Tinospora cordifolia* used in Sanshamani Vati: The Methanolic extract of stem of *Tinospora cordifolia*, *Tinospora crispa* & three batches of Sanshamani vati were applied on TLC plate and the plate was then developed in the mobile phase (Hexane: chloroform: Methanol: Formic acid:: 4: 4: 2: 0.1 v/v/v/v). After spraying with Anisaldehyde sulfuric acid reagent, the plate was Visualize for band, and heated at 110°C for 5 min. The retention factor (RF values) and color of the bands were noted.

Elemental Analysis by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES): The samples of Sanshamani Vati and Mahalaxmi vilas Rasa Tablets were digested in a MARS 6 microwave digestion system (CEM corp., USA) equipped with Teflon closed vessels (Easy Prep Plus vessel) for safe operation under 800 psi. The instrumental conditions used for digestion of samples are given in **Table 3**. After completion of digestions, elemental content (Au, Fe, Hg & Cu) were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using a Perkin Elmer model AVIO 200 with Syngestic software. The instrumental conditions of ICP-OES used for Elemental analysis are given in **Table 4**.

TABLE 3: CEM MARS 6 MICROWAVE DIGESTION SYSTEM OPERATING PARAMETERS

Instrumental Parameters	Gold (Au)	Iron (Fe)	Copper (Cu)	Mercury (Hg)
Acid used for Digestion	Aquaregia (HCl : HNO3 :: 3 :1)	HCL	Aquaregia (HCl : HNO3 :: 3 :1)	Aquaregia (HCl : HNO3:: 3 :1)
Method	Au	Fe	Cu	Hg
Temperature	170°C	170°C	190°C	170°C
Pressure	650 psi	650 psi	650 psi	650 psi
Ramp Time	15 min	20 min	20 min	20 min
Hold Time	1 min	10 min	15 min	10 min
Cooling Time	15 min	15 min	15 min	15 min

TABLE 4: ICP-OES OPERATING PARAMETERS

Element	Wavelength	Plasma (L/min)	AUX (L/min)	Neb (L/min)	Power (watts)	View Dist.	Plasma View
Gold (Au)	242.795	10	0.2	0.60	1300	15	Radial
Iron (Fe)	238.204	10	0.2	0.60	1300	15	Radial
Copper (Cu)	327.393	10	0.2	0.60	1300	15	Radial
Mercury (Hg)	253.652	10	0.2	0.60	1300	15	Radial

Validation of the Method: The Method validation was performed for Phytochemical Quantification by HPTLC and Elemental Analysis by ICP-OES as per standard ICH guidelines, which included linearity, precision, accuracy, LOD and LOQ [35]. The linearity of method was performed by plotting calibration curves. Precision was performed by Repeatability and by estimating intraday and interday readings and %RSD relative standard deviation. Accuracy of analytical methods was expressed as % recovery. This was estimated by adding known concentration of standard solution to pre-analyzed sample solution. Limit of detection (LOD) and limit of quantification (LOQ) were estimated as per formula:

$$LOD = 3.3 \times \sigma/S \text{ and } LOQ = 10 \times \sigma/S,$$

Where σ = Standard deviation, S = Slope.

X-ray Diffraction (XRD) Profile: X-ray diffraction (XRD) analysis of Mahalaxmi vilas Rasa was carried out using Rigaku Miniflex 600 X-ray diffract meter with operating at 40 kV and 30 mA. The XRD Pattern was recorded for angle ranging from 3^0 to 100^0 at a scanning rate of 3^0/ min. and scan step of 0.01^0. The absence of Mercury & Arsenic in free from was confirmed by matching d-spacing with the standard database ICDD PDF-2 2021 (International Center for Diffraction Data).

RESULTS AND DISCUSSION: Sanshamani Vati, and Mahalaxmi vilas Rasa Tablets were characterized as brownish black to black and Greenish gray to dark gray in colour, respectively with round coated biconvex shape. The Physico-chemical screening of both Sanshamani Vati, and Mahalaxmi vilas Rasa Tablets showed Friability less than 1%, Hardness greater than 1.5 kg/cm^2, Disintegration time less than 60 min and Loss on drying (LOD) less than 6 %. Sanshamani Vati Tablets showed Ash content less than 30 %, Acid insoluble ash (AIA) less than 2 %, Water soluble Extractive (WSE) more than 45 % and Alcohol soluble Extractive (ASE) more than 20 % **Table 5**. All the samples of Sanshamani Vati, and Mahalaxmi vilas Rasa were found to comply with the weight variation test as per API [36]. Weight variation is an important factor that is affected by the tooling of the compression machine, head pressure, machine speed and flow properties of the powder, powder or granulate density and particle

size. The friability test helps to determine the tablet's physical strength, which is attributed to the tablet breaking force. The disintegration test is a measure of the time required under specified conditions for the tablets to disintegrate into particles. Loss on drying (LOD) measures the amount of water and volatile matter in a sample when the sample is dried under specified conditions. This is the major factor responsible for the deterioration of the drugs and formulations. Low moisture content is always useful for higher stability of drugs [37-38].

TABLE 5: PL GIVE SPACING IN BETWEEN SANSHAMANIVATI & MAHALAXMIVILAS RASA ANALYSIS DATA

Product name	**SANSHAMANI VATI**		
Batch code	**Sample - 1**	**Sample - 2**	**Sample - 3**
Colour	Brownish black coated tablets	Brownish black coated tablets	Brownish black coated tablets
Shape	Round convex	Round convex	Round convex
Friability (% w/w)	0.001 % w/w	0.002 % w/w	0.001 % w/w
Disintegration Time (min.)	18 min.	15 min.	20 min.
Hardness (kg/cm^2)	2.7 kg/cm2	3.0 kg/cm2	2.0 kg/cm2
Thickness (mm)	3.48 mm	3.40 mm	3.33 mm
Diameter (mm)	6.48 mm	6.48 mm	6.6 mm
Average Weight (mg)	127 mg	127 mg	128 mg
Weight Variation	Not more than 2 tablets deviate by more than 5% of the average weight and none by more than 10% of the average weight.		
Loss on Drying (LOD) (% w/w)	4.58 % w/w	7.0 % w/w	6.26 % w/w
Ash (% w/w)	21.54 % w/w	20.1 % w/w	14.15 % w/w
Acid Insoluble Ash (AIA) (% w/w)	1.47 % w/w	0.92 % w/w	1.15 % w/w
Water Soluble Extractive (WSE) (% w/w)	76.50 % w/w	72.81 % w/w	77.60 % w/w
Alcohol Soluble Extractive (ASE) (% w/w)	22.2 % w/w	21.96 % w/w	29.7 % w/w
Phyto-chemical Analysis by HPTLC			
Berberine content in ppm	5 ppm	5 ppm	5 ppm
Product name		**MAHALAKSHMI VILAS RASA**	
Batch code	**Sample - 1**	**Sample - 2**	**Sample - 3**
Colour	Greenish gray coated tablets	Greenish gray coated tablets	Greenish gray coated tablets
Shape	Round convex	Round convex	Round convex
Friability (% w/w)	0.006 % w/w	0.05 % w/w	0.02 % w/w
Disintegration Time (min.)	44 min.	23 min.	21 min.
Hardness (kg/cm^2)	3.0 kg/cm2	4.33 kg/cm2	4.0 kg/cm2
Thickness (mm)	3.46 mm	3.56 mm	3.57 mm
Diameter (mm)	6.65 mm	6.65 mm	6.67 mm
Average Weight (mg)	162.7 mg	166.3 mg	158.4 mg
Weight Variation	Not more than 2 tablets deviate by more than 5% of the average weight and none by more than 10% of the average weight.		
Loss on Drying (LOD) (% w/w)	5.81 % w/w	4.08 % w/w	5.16 % w/w
Elemental Analysis by ICP-OES			
Gold (Au) (mg/tab)	1.50 mg/tab	1.43 mg/tab	1.26 mg/tab
Iron (Fe) (mg/tab)	3.05 mg/tab	2.96 mg/tab	2.71 mg/tab
Mercury (Hg) (mg/tab)	2.27 mg/tab	2.80 mg/tab	2.23 mg/tab
Copper (Cu) (mg/tab)	0.75 mg/tab	0.86 mg/tab	0.88 mg/tab
Phytochemical Analysis by HPTLC			
Myristicin content in ppb	60 ppb	60 ppb	60 ppb

The HPTLC profile confirms the presence of Berberine at Rf 0.25 ± 0.02 And Myristicin at Rf 0.50 ± 0.02 in Sanshamani Vati and Mahalaxmi vilas rasa, respectively. The Herbal RM ingredients

Jayapatri & Jayphala (*Myristica fragrans*) contributed to presence of Myristin and Guduchi (*Tinospora cordifolia*) to Berberine.

The metal & mineral ingredients of Mahalaxmivilas rasa, Suvarna bhasma, Abhrak Bhasma, Shodhit Parad & Tamra Bhasma contributed to the content of Gold (Au), Iron (Fe), Mercury (Hg) & Copper (Cu) respectively. The contents of Gold (Au), Iron (Fe), Mercury (Hg) & Copper (Cu) in samples of Mahalaxmi vilas rasa were determined by ICP-OES and results were tabulated in **Table 5**.

Authentication of *Tinospora cordifolia* used in Sanshamani Vati: As shown in **Fig. 1, 2** & **3**, the methanolic extracts of Sanshamani Vati and *Tinospora cordifolia* confirm the presence of similar major bands at Rf 0.26 (greenish blue), 0.36 (light brown), 0.42 (Gray colour), 0.47 (Purple), 0.52 (Light gray colour) and 0.56 (Light purple), whereas the methanolic extract of *Tinospora crispa* does not show presence of similar band pattern. It confirms the authenticity of raw material used in Sanshamani vati as *Tinospora cordifolia*.

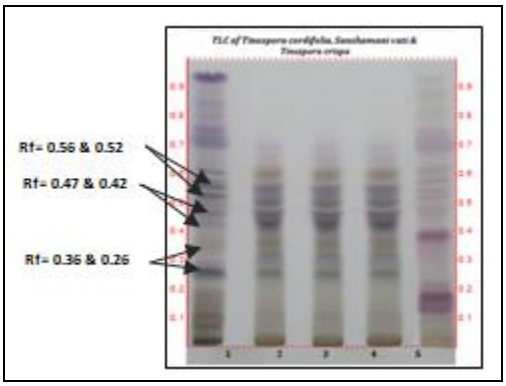

FIG. 1: HPTLC FINGERPRINT OF, TRACK 1 - *TINOSPORA CORDIFOLIA*, TRACK 2 TO 4 - SANSHAMANI VATI, TRACK 5 - *TINOSPORA CRISPA*

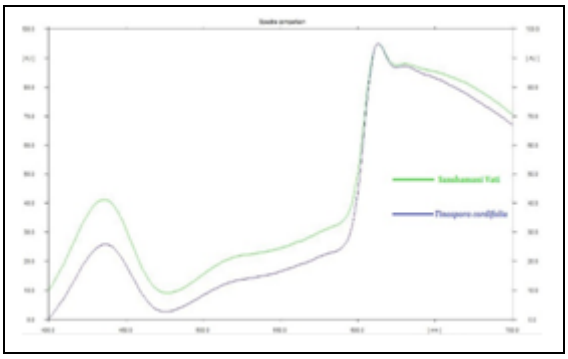

FIG. 2: DENSITOMETRIC UV SPECTRA OF SANSHAMANI VATI AND *TINOSPORA CORDIFOLIA*

FIG. 3: HPTLC DENSITOGRAMS OF *TINOSPORA CORDIFOLIA*, SANSHAMANI VATI AND *TINOSPORA CRISPA* AFTER DERIVATIZATION AT 540NM

Method Validation by HPTLC: For development of a successful method the first important step is to optimize the mobile phase. Trials with various solvent system combinations carried out mobile phase optimization. The chamber saturation time was optimized to 20 min at room temperature with

relative humidity 38 ± 2%. The chromatographic run was roughly 90 mm, and the distance between two tracks was 15 mm. The optimized chromatographic conditions are given in **Table 2.**

Specificity: It was observed that other phytochemical constituents present in Sanshamani vati & Mahalaxmivilas rasa did not interfere with the peaks of Berberine and Myristicin, respectively.

Thus the proposed method was proved to be specific. The spectra of standard Berberine and Myristicin corresponded with Sanshamani vati & Mahalaxmi vilas rasa are shown in **Fig. 4A & 4B.**

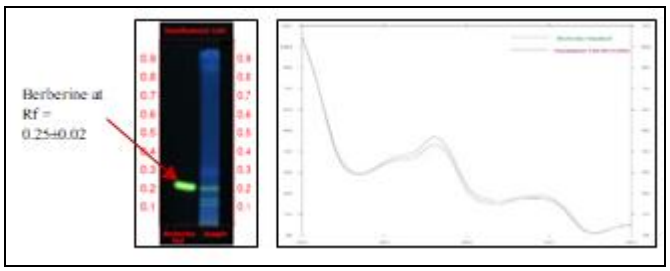

FIG. 4(A): CHROMATOGRAM AND SPECTRAL DISPLAY OF BERBERINE AT 366NM IN SANSHAMANI VATI

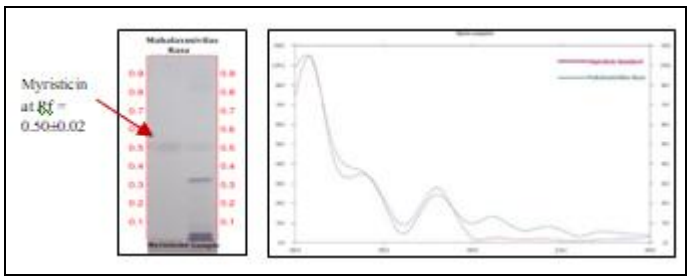

FIG. 4(B): CHROMATOGRAM AND SPECTRAL DISPLAY OF MYRISTICIN AT 212 NM IN MAHALAXMIVILAS RASA

Linearity: Different concentrations of standards were analyzed to get linearity. Under optimized chromatographic conditions peak areas for the corresponding standard were found proportional to the concentrations of Berberine and Myristicin **Fig. 6**. The statistical parameters of the linearity such as range, correlation coefficient, slope, intercept, SD of the intercept were presented in **Table 5**. The linearity graphs are presented in **Fig. 6**. The limit of detection (LOD) estimated for Myristicin was 21.95 pg/spot and limit of quantification (LOQ) 66.51 pg/spot. Similarly, the limit of detection (LOD) estimated for Berberine was 910.03 pg/spot and limit of quantification (LOQ) 2757.65 pg/spot.

TABLE 6: LINEARITY PARAMETERS OF BERBERINE & MYRISTICIN

Product Name	Sanshamani Vati	mahalaxmivilas rasa
Marker compound	Berberine	Myristicin
Linearity range (ng/spot)	2450-9800 pg	98 pg - 686 pg
Correlation coefficient ®	0.9994	0.9999
Slope	0.34	5.34
Intercept	234.60	237.76
LOD	910.03 pg/spot	21.95 pg/spot
LOQ	2757.65 pg/spot	66.51 pg/spot

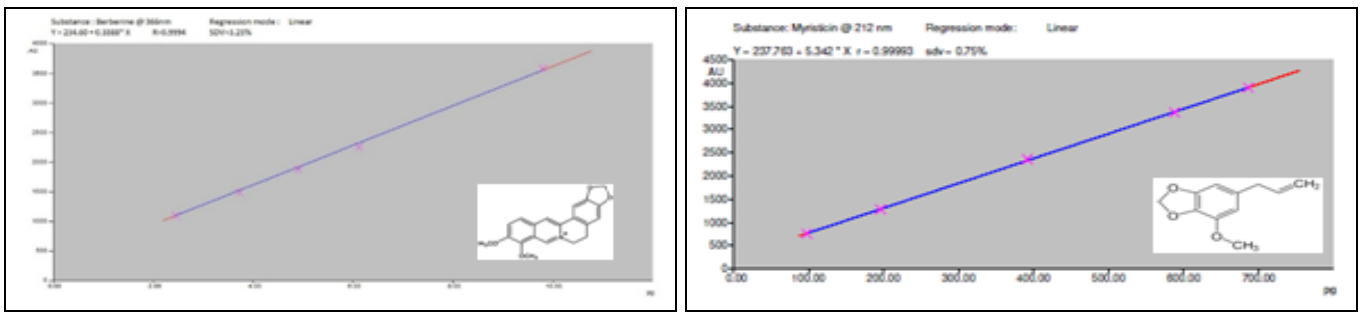

FIG. 6: LINEARITY GRAPHS OF BERBERINE & MYRISTICIN

Intermediate Precision (Reproducibility): Precision of the method was evaluated for interday analysis. For precision % RSD was found to be <5% for Berberine & Myristicin **Table 7A.**

TABLE 7(A): INTERDAY PRECISION BY HPTLC

Sanshamani Vati	**Sample 1**			**Sample 2**			**Sample 3**		
Levels	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Berberine - Peak area (pg/4ul)	2430	2410	2340	2470	2400	2380	2360	2330	2430
% RSD		1.97			1.96			2.16	
Mahalaxmivilas rasa	**Sample 1**			**Sample 2**			**Sample 3**		
Levels	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Myristicin - (pg/15ul)	158.35	150.21	160.08	164.76	170.59	164.27	165.63	173.29	161.88
% RSD		3.37			2.11			3.48	

Robustness: The robustness of the methods was determined by analysis of same sample with different analyst. % RSD was estimated to be < 5.00 **Table 7B**.

TABLE 7(B): DIFFERENT ANALYST PRECISION BY HPTLC

Sanshamani Vati	**Sample 1**			**Sample 2**			**Sample 3**		
Levels	Analyst 1	Analyst 2	Analyst 3	Analyst 1	Analyst 2	Analyst 3	Analyst 1	Analyst 2	Analyst 3
Berberine - Peak area (pg/4ul)	2720	2770	2840	2370	2300	2280	2330	2420	2400
% RSD		2.17			2.04			1.98	
Mahalaxmivilas rasa	**Sample 1**			**Sample 2**			**Sample 3**		
Levels	Analyst 1	Analyst 2	Analyst 3	Analyst 1	Analyst 2	Analyst 3	Analyst 1	Analyst 2	Analyst 3
Myristicin - (pg/15ul)	196.74	193.58	189.46	170.59	174.31	173.29	172.65	169.94	178.19
% RSD		1.89			1.11			2.42	

Method Precision (Repeatability): 10 Samples with same concentration were quantified under same experimental conditions and % RSD was <5.00 **Table 8**.

TABLE 8: REPEATABILITY BY HPTLC

Analyte	**Amount of Sample (n = 10)**	**Amount of drug detected (n = 10)**	**RSD (%)**
Berberine in Sanshamani Vati	2.8669 g	2331 pg/4 ul	4.90
Myristicin in Mahalaxmivilas rasa	1.6377 g	162.36 pg/15 ul	1.92

Accuracy: By adding known amount of standard analyte in the sample % recovery was measured which was found to be in range from 90 to 110 % **Table 9**. % RSD for all parameters were below 5% for Berberine in Sanshamani Vati and Myristicin in Mahalaxmivilas rasa, which shows the proposed methods have high level of precision.

TABLE 9: ACCURACY STUDIES OF BERBERINE & MYRISTICIN BY HPTLC

Analyte	**Amount of drug Analyzed (pg)**	**Amount of drug added (pg)**	**Theoretical concentration (pg)**	**Amount of drug found (pg)**	**% Recovery**
Berberine	382	305	687	638.33	92.92
	382	392	774	699.33	90.35
	382	457	839	755.33	90.03
Myristicin	9.03	7.19	16.22	15.46	95.31
	9.03	9.03	18.06	17.67	97.82
	9.03	10.78	19.81	18.95	95.66

Method Validation by ICP-OES: The Method validation for Quantification of Elemental Analysis (Au, Fe, Hg & Cu) in Mahalaxmi vilas Rasa by ICP-OES was performed as per standard ICH guidelines, which included linearity, precision, accuracy, LOD and LOQ.

Linearity: Different concentrations of samples were analyzed to get linearity for elemental assay methods under optimized conditions. The statistical analysis of the linearity graph such as linearity range, correlation coefficient, slope, intercept was presented in **Table 10**.

TABLE 10: LINEARITY PARAMETERS OF ELEMENTAL ASSAY BY ICP-OES

Product Name	mahalaxmivilas rasa			
Elemental Assay	**Gold (Au)**	**Iron (Fe)**	**Mercury (Hg)**	**Copper (Cu)**
Linearity range	50 – 250 mg	50 – 250 mg	50 – 250 mg	50 – 250 mg
Correlation coefficient ®	0.9998	0.9999	0.9991	0.9991
Slope	0.0121	0.0183	0.0136	0.0088
Intercept	0.076	0.007	0.05	-0.118
LOD	4.5 ppb	4.8 ppb	0.34 ppm	7.8 ppb
LOQ	15.0 ppb	16.0 ppb	1.14 ppm	26.0 ppb

The linearity graphs are presented in **Fig. 7**.

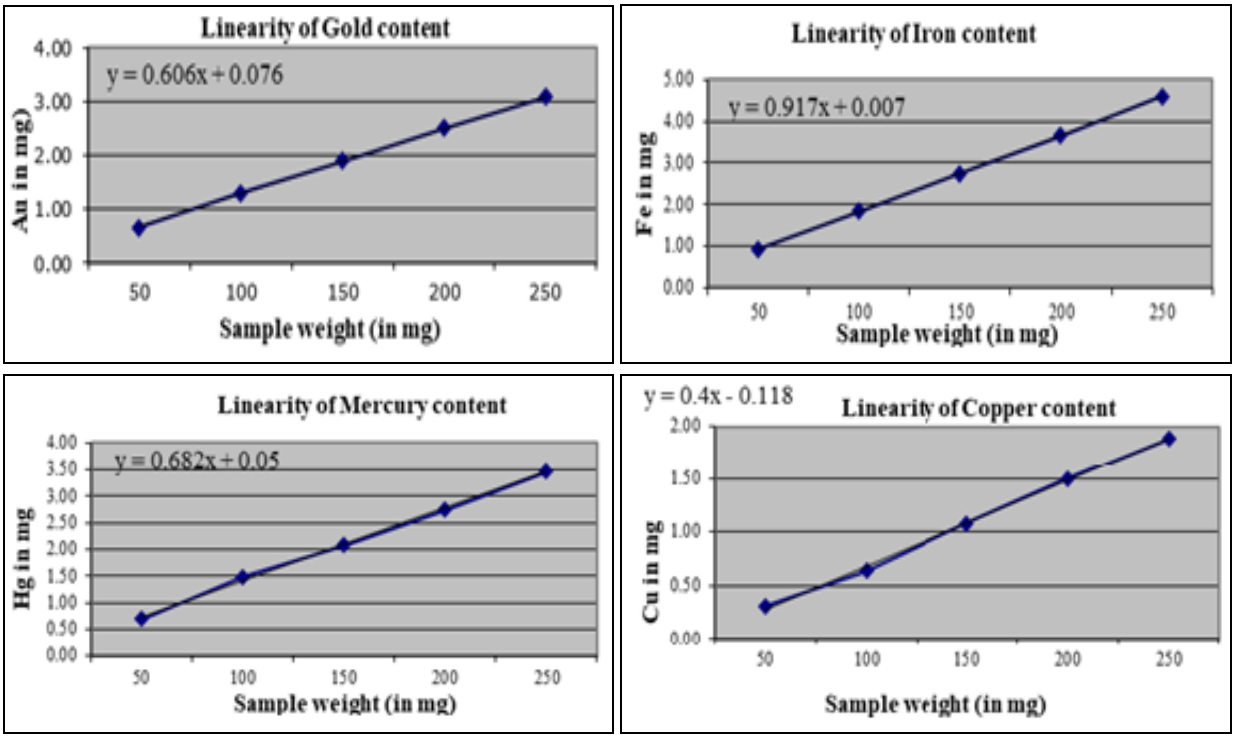

FIG. 7: LINEARITY GRAPHS OF ELEMENTAL ASSAY BY ICP-OES

Intermediate Precision (Reproducibility): Precision of the Elemental assay methods were evaluated for intraday and interday analysis. For intraday and Interday precision data summarized in **Table 11** and% RSD was found to be < 2.00.

TABLE 11(A): INTRADAY PRECISION OF ELEMENTAL ASSAY BY ICP-OES

Elemental assay in Mahalaxmivilas Rasa		Gold (Au)		Iron (Fe)		Mercury (Hg)		Copper (Cu)	
		% Conc.	% RSD	% Conc.	% RSD	% Conc.	% RSD	% Conc.	% RSD
Sample 1	Session 1	0.91	1.28	1.89	0.31	1.42	1.07	0.47	1.24
	Session 2	0.91		1.88		1.41		0.47	
	Session 3	0.89		1.88		1.44		0.46	
Sample 2	Session 1	0.93	1.67	1.88	0.53	1.44	0.81	0.47	1.25
	Session 2	0.92		1.89		1.42		0.46	
	Session 3	0.90		1.90		1.44		0.46	
Sample 3	Session 1	0.91	1.26	1.92	0.30	1.42	0.41	0.46	1.24
	Session 2	0.93		1.92		1.42		0.47	
	Session 3	0.91		1.91		1.41		0.47	

TABLE 11(B): INTERDAY PRECISION OF ELEMENTAL ASSAY BY ICP-OES

Elemental assay in Mahalaxmivilas Rasa		Gold (Au)		Iron (Fe)		Mercury (Hg)		Copper (Cu)	
		% Conc.	% RSD	% Conc.	% RSD	% Conc.	% RSD	% Conc.	% RSD
Sample 1	Day 1	0.91	0.63	1.89	0.53	1.42	0.41	0.46	1.25
	Day 2	0.92		1.87		1.42		0.47	
	Day 3	0.92		1.88		1.43		0.46	
Sample 2	Day 1	0.93	0.62	1.86	0.54	1.43	0.40	0.45	1.26
	Day 2	0.94		1.87		1.44		0.46	
	Day 3	0.93		1.85		1.43		0.46	
Sample 3	Day 1	0.90	1.27	1.84	1.12	1.43	0.40	0.45	1.27
	Day 2	0.92		1.88		1.42		0.45	
	Day 3	0.90		1.87		1.43		0.46	

Robustness: The robustness of the methods was determine by analysis of same sample with different analyst. % RSD was estimated to be < 2.00 **Table 11(C)**.

TABLE 11(C): DIFFERENT ANALYST PRECISION OF ELEMENTAL ASSAY BY ICP-OES

Elemental assay in Mahalaxmivilas Rasa		Gold (Au)		Iron (Fe)		Mercury (Hg)		Copper (Cu)	
		% Conc.	% RSD	% Conc.	% RSD	% Conc.	% RSD	% Conc.	% RSD
Sample 1	Analyst 1	0.93	1.88	1.88	0.53	1.43	0.40	0.46	1.27
	Analyst 2	0.90		1.86		1.44		0.45	
	Analyst 3	0.93		1.87		1.44		0.45	
Sample 2	Analyst 1	0.91	1.88	1.86	0.31	1.44	1.62	0.45	1.29
	Analyst 2	0.91		1.86		1.44		0.44	
	Analyst 3	0.94		1.87		1.40		0.45	
Sample 3	Analyst 1	0.92	1.09	1.86	0.53	1.44	1.07	0.46	1.26
	Analyst 2	0.91		1.87		1.43		0.46	
	Analyst 3	0.93		1.88		1.41		0.45	

Method Precision (Repeatability): 10 Samples with same concentration were quantified under same experimental conditions and % RSD was found to be < 2.00 **Table 12**.

TABLE 12: REPEATABILITY OF ELEMENTAL ASSAY BY ICP-OES

Sample	Elemental assay	Amount of Sample (mg) (n = 10)	Concentration of Element (% w/w) (n = 10)	RSD (%)
Mahalaxmivilas rasa	Gold (Au)	100	0.91	1.75
	Iron (Fe)	100	1.89	0.94
	Mercury (Hg)	100	1.43	1.66
	Copper (Cu)	100	0.47	1.76

Accuracy: By adding a known amount of standard analyte in the sample % recovery was measured, which was found to be in the range from 92 to 105 % **Table 13**.

TABLE 13: ACCURACY STUDIES OF ELEMENTAL ASSAY BY ICP-OES

Elemental assay	Amount of drug Analyzed (% w/w)	Amount of drug added (% w/w)	Theoretical concentration (% w/w)	Amount of drug found (% w/w)	% Recovery
Gold (Au)	0.92	0.63	1.55	1.44	92.63
	0.92	1.00	1.92	1.86	97.10
	0.92	1.40	2.32	2.15	92.64
Iron (Fe)	1.87	2.00	3.87	3.81	98.54
	1.87	3.50	5.37	5.26	97.89
	1.87	4.97	6.84	6.68	97.61
Mercury (Hg)	1.40	2.00	3.40	3.26	96.04
	1.40	3.00	4.40	4.60	104.54
	1.40	4.50	5.90	5.98	101.36
Copper (Cu)	0.46	2.06	2.52	2.50	99.13
	0.46	3.85	4.31	4.11	95.52
	0.46	5.49	5.95	5.64	94.79

X-ray Diffraction (XRD): The XRD profile of Mahalaxmivilas rasa (MLVR) confirms absence of free Mercury (Hg) and Arsenic (As) when compared with XRD spectras of Mercury (Hg) and Arsenic (As) in standard database ICDD PDF-2 2021 (International Center for Diffraction Data).

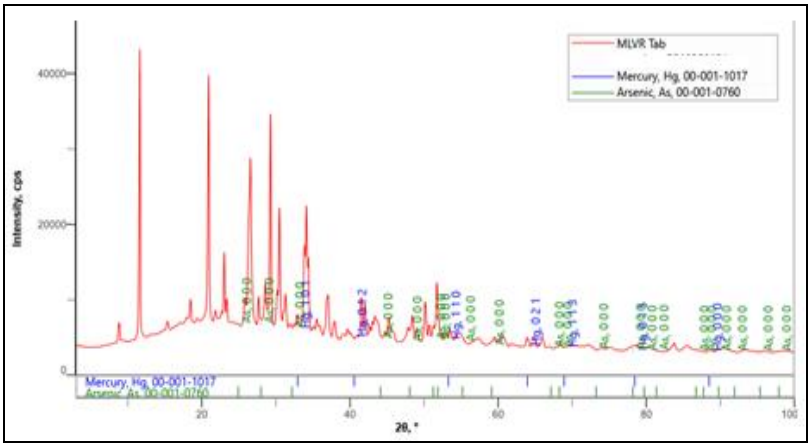

FIG. 8: XRD SPECTRA COMPARISON SHOWING THE ABSENCE OF Hg & As IN MLVR

CONCLUSION: To maintain the enormous trust in Ayurveda, it's necessary to ascertain the quality, efficacy & safety of Ayurvedic preparations on scientific lines using modern techniques. Present work attempts have been made to characterize and validate the formulations with modern techniques such as High-Performance Thin Layer Chromatography (HPTLC) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The present work will be helpful to understand therapeutic value with respect to the quality parameters of the formulations.

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