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Original Article

A detail phyto-chemical evaluation of ayurvedic capsule formulation used for hair care

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ABSTRACT

Standardization describes all measures taken during manufacturing process and quality control leads to reproducible quality of particular product. Growing need for standardization and quality control of herbal medicines is recognized by WHO that specifies guidelines for the assessment of the safety and quality of herbal medicines as a prerequisite for global harmonization. The present study was focused on an exhaustive standardization of a Herbo-mineral capsule preparation namely "Trichup Capsule" which was carried out employing the basic organoleptic test, physico-chemical tests, and bio-assays by sophisticated instruments like HPLC and HPTLC. HPTLC fingerprinting, assays of marker compounds were carried out to confirm the quantitative presence of the raw materials in the finished product which in turn reflects the quality and potency of product. The study results revealed that the Capsule formulation was well standardized at various levels such as Physical consistency, Chemical profile, Microbial and Heavy metal limits.

Keywords: Standardization, Herbo-mineral Capsule Formulation, HPLC, HPTLC, Trichup Capsule

INTRODUCTION

According to pharmaceutical manufacturers association of U.S. "quality is the sum of all the factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product" In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. Standardization is an essential measurement for ensuring the quality control of the herbal drugs[1]. Standardization of herbal drugs is not an easy task as numerous factors influence to their bio-chemical profile. In order to obtain quality herbal products, a special care should be taken right from the proper identification of plants, season and area of collection, their extraction and purification process and rationalizing the combination in case of polyherbal drugs[2-4].

World Health Organization (WHO) encourages, recommends and promotes traditional /herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards[5-6].

Herbal product cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility at the manufacturing as well as at quality testing level. Hence standardization is a very important process for the authentication of the drug.

In the present study, the poly herbal Capsule preparation (Trichup Capsule) has been selected to establish its standardization status. The key ingredients used in this formulation are extracts of *Eclipta alba* (Bhringraj) Whole Plant[7], *Centella asiatica* (Mandukparni) Whole Plant[7], Rasayan

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Churna[7] (classical combination of Guduchi, Gokhshur and Amalaki), *Glycyrrhiza glabra* (Yashtimadhu) Root[7], *Hibiscus rosa-sinensis* (Japa) Flower[7] and powder of Classical formulations such as Saptamrit Loha[8], Narsinh Churna[9], Gandhak Rasayan[10], Muktashukti Bhasma[10] and the required excipients.

MATERIAL AND METHODS

Organoleptic parameters: Organoleptic parameter like appearance, colour and odour were used to confirm uniformity in visual identity of raw materials and finished product. The results are as tabulated in **Table 2**

Physicochemical parameters for extracts[11]: The physiochemical parameters include tests like Loss on drying, pH, Water soluble extractive, Alcohol soluble extractive, Determination of total ash of the relevant raw materials. The results are as tabulated in **Table 3 & 4**

Estimation of Actives[12]: Assay analysis includes estimation of Assay of Nor-wedalolactone, Triterpene, Glycyrrhizin, Calcium, Saponin Tannin and Bitter in respective ingredients. The results are as tabulated in **Table 5**

Evaluation of Standardization Parameters selected for Finished Product: The finished product was analyzed for parameters like Description, pH, Average Net Content per capsule and Disintegration time. The results are as tabulated in **Table 6**

Microbial Analysis[13]: Bio-burden analysis consists of parameters like Total Bacterial Count, Total Fungal Count, and presence of pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enterica*. The results are as given in **Table 7**

Heavy Metal Analysis[14]: Sample preparation for heavy metal analysis was done by MARS Express microwave digestive system. The standard solutions of Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) were prepared. Then samples were analyzed for the presence of the four heavy metals using atomic absorbance spectrophotometer AA 6300, SHIMADZU and HVG-1 by using a calibration curve of the standard. The results are as given in **Table 7**

HPLC analysis for estimation of active components:

Apparatus, equipment and reagents: HPLC system's pump was from Shimadzu LC 20ATVP, Japan with 20mL Rheodyne injector, Phenomenex (Torrance, CA) Luna C_{18} (250cm x 4.6mm id) column and SPD-20 AT UV-Visible and spinchrom/LC solution software was used. All the reagents were of the HPLC grade.

Estimation of Glycyrrhizin in *Glycyrrhiza glabra* (Yashtimadhu) Extract

Chromatographic Condition

Stationary phase: Phenomenex C_{18} column (250mm x 4.6mm i.d., 5µm particle size) was used at ambient temperature

Mobile Phase: Buffer: acetonitrile (60:40, v/v)

Flow rate: 1mL/min.

Injection volume: 20µL

Detection: At 254nm with UV detector

Preparation of solutions:

Preparation of standard solution: Dissolve 1mg of the standard Glycyrrhizin in 10mL of the Mobile phase solution. Filter the solution using a 0.22mm filter paper and use the filtrate as the standard solution

Preparation of sample solutions: Dissolve 50mg of Yashtimadhu Extract in 25mL of the Mobile phase solution. Filter the solution using a 0.22mm filter paper and use the filtrate as the sample solution

Estimation of HCA (Hydroxycitric Acid) in *Hibiscus rosa-sinensis* (Japa) Extract

Chromatographic Condition

Stationary phase: Phenomenex C_{18} column (250mm x 4.6mm i.d., 5µm particle size) was used at ambient temperature

Mobile Phase: 100% Potassium dihydrogen phosphate

Flow rate: 1mL/min.

Injection volume: 20µL

Detection: At 215nm with UV detector

Preparation of solutions

Standard Preparation: Dissolve 1mg of standard HCA in 10mL solvent mixture (1mL of 30% H_3PO_4 + 9mL water). Filter the solution using 0.22mm filter paper and use the filtrate as the standard solution.

Sample Preparation: Dissolve 10mg of *Hibiscus rosa-sinensis* extract in 10mL of solvent mixture (1mL of 30% H_3PO_4 + 9mL water). Filter the solution using a 0.22mm filter paper and use the filtrate as the sample solution.

HPTLC analysis for Trichup Capsule and its raw materials:

HPTLC is one of the most advanced separation techniques available today which gives better precision and accuracy with extreme flexibility for

various steps (stationary phase, mobile phase, development technique and detection). HPTLC analysis was carried out using a Hemilton 100µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-4, WINCAT integration software, aluminium sheet precoated with Silica Gel F254 (Merck) 0.2mm thickness.

Steps involved in HPTLC analysis:

Selection of plate and adsorbent: Precoated aluminum plates with Silica Gel F254 of 20 x 20cm and 0.2mm thickness, was used for detection. The plates were pre washed by methanol and activated at 60°C for 5 min prior to chromatography.

Sample solution:
Extract: Extract 1.0g of the sample raw material (Reference Standard / Test Drug) with 10mL of Methanol with constant shaking for 5minutes. Heat on a water bath at 90 to 100°C for 5minutes. Filter it through a Whatman filter paper No.41 and use the filtrate for HPTLC Profiling.

Preparation of solution for Finished Product: Extract 2.0g of Trichup Capsule powder with 20mL of Methanol & reflux it on water bath at 90 to 100°C for 15minutes. Filter and evaporate up to 5mL in a porcelain dish and use this solution for HPTLC Profiling.

Preparation of Spray reagent (Anisaldehyde sulphuric acid reagent): 0.5mL of Anisaldehyde EP is mixed with 10mL of Glacial acetic acid AR, followed by 85mL Methanol AR and 5mL Sulphuric acid 98% GR.
Track 1: 8µl/mL methanol extract of the reference standard of the raw material
Track 2: 8µl/mL methanol extract of test drug under observation
Track 3: 8µl/mL methanol extract of Trichup Capsule

RESULTS

HPTLC: During HPTLC analysis, the sample shows comparison of individual extract with the finished product. The visualization of TLC plates was carried out in all 3 different wavelengths i.e. 254nm, 366nm and 540nm. From this only the best visualization result was selected and included in our study along with its 3D image. The R_f values found during this study indicated the prominent presences of that particular raw material in the finished product which is used to establish its qualitative presence.
Tracks of HPTLC fingerprinting plates were spotted in following way:
Track 1: 8µl/mL methanol extract of the reference standard of the Extract
Track 2: 8µl/mL methanol extract of test drug under observation
Track 3: 8µl/mL methanol extract of Trichup Capsule.

DISCUSSION & CONCLUSION

Standardization solely refers to evaluating a drug by means of confirming its identity and determination of its quality, purity and nature of adulteration. Since herbal drugs are prepared from plant material, its standardization is a very complex task. There are multiple factors which influence its bio-chemical profile and so as its reproducible therapeutic effect. However, Standardization is nowadays considered an essential to ensure qualitative and quantitative profile of bio-actives in finish product which in turn confirms consistency in producing desired effect in patient.

In present study, an effort has been made to evaluate standardization status of Herbo-mineral Ayurvedic formulation with a perspective that it may serve as a guideline to many Ayurvedic medicine manufacturer to establish standardization parameters for their similar formulations.

Trichup Capsule is a herbo-mineral Ayurvedic propriety product manufactured and marketed by Vasu Healthcare Pvt. Ltd. As a part of standardization procedure, the finished product and the raw materials of three different batches were analyzed for various physicochemical parameters.
The testing method for each parameter was standardized and validated. The protocols for the same were adopted from standard reference books.
Organoleptic characters like physical appearance, colour, odour and taste of the raw materials and finish product were first evaluated for identification and batch to batch uniformity before any further tests are undertaken.

pH and moisture content play important role in reflecting quality of product. These parameters were found well within the limits during the analysis and thereby confirmed the consistency in quality of product.

Extractive Value determines the amount of active constituents in medicinal plant material when extracted with a solvent media such as Water. These values provide an indication of the extent of polar, medium polar and non-polar compounds present in the plant material. Thus from the above study it can be concluded that all extracts used in Trichup capsule have good solubility in water which is a polar solvent.

The total ash usually consists of carbonates, phosphates silicates and silica that include the physiological ash which is derived from the plant tissue itself and non physiological ash which is the residue of the adhering material to the plant material e.g. sand and soil. Total ash was performed to measure the total amount of material remaining after ignition. This test is important to control impurities in actives. Study results showed ash values much within the prescribed limits.

WHO has specified the limits for the presence of contaminants like four pathogenic micro-organisms viz *E.coli, Staphylococcus aureus, P.aeruginosa* & *Salmonella enterica* along with yeast-moulds and four heavy metals viz; Lead, Cadmium, Arsenic and Mercury as the consumption of which can lead to complications in one's routine life. Trichup Capsule was found in full compliance of the permissible microbial and heavy metal limits.

HPTLC study confirmed the qualitative as well as quantitative presence of the raw material in the finished product. Present standardization study revealed Trichup Capsule in full compliance with all the above discussed parameters hence it can be concluded that it is a well standardized product at essential physicochemical parameters.

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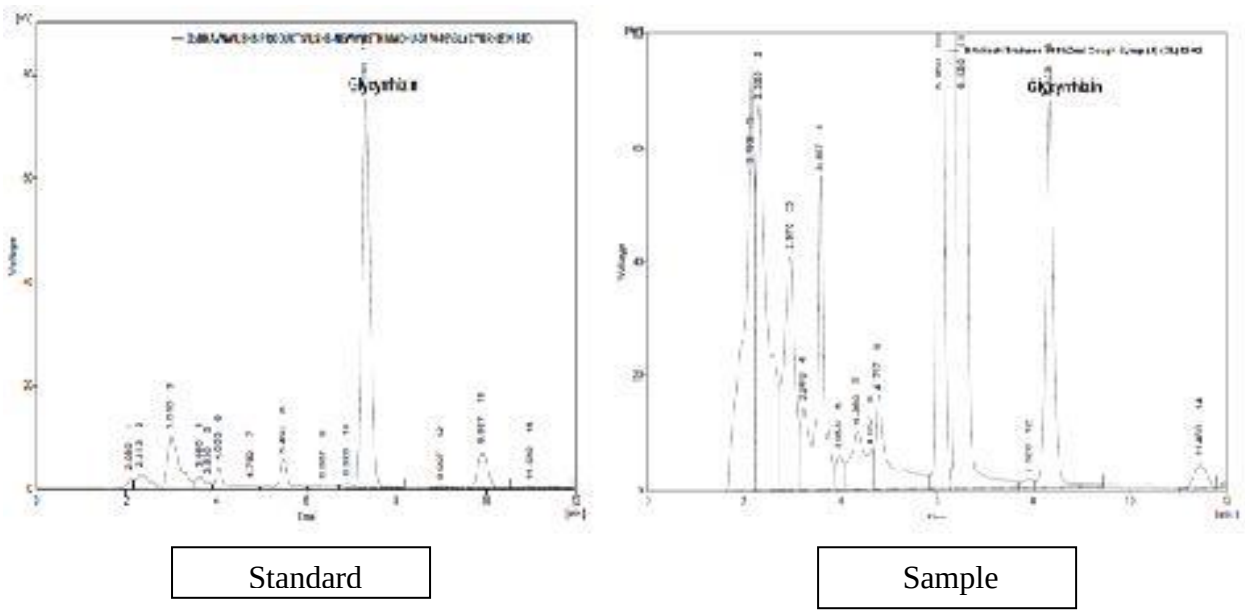


Figure 1: [A] HPLC chromatogram of standard glycyrrhizin & [**B**] *Glycyrrhiza glabra* extract. The result indicated 27.32% of glycyrrhizin in *Glycyrrhiza glabra* Root extract

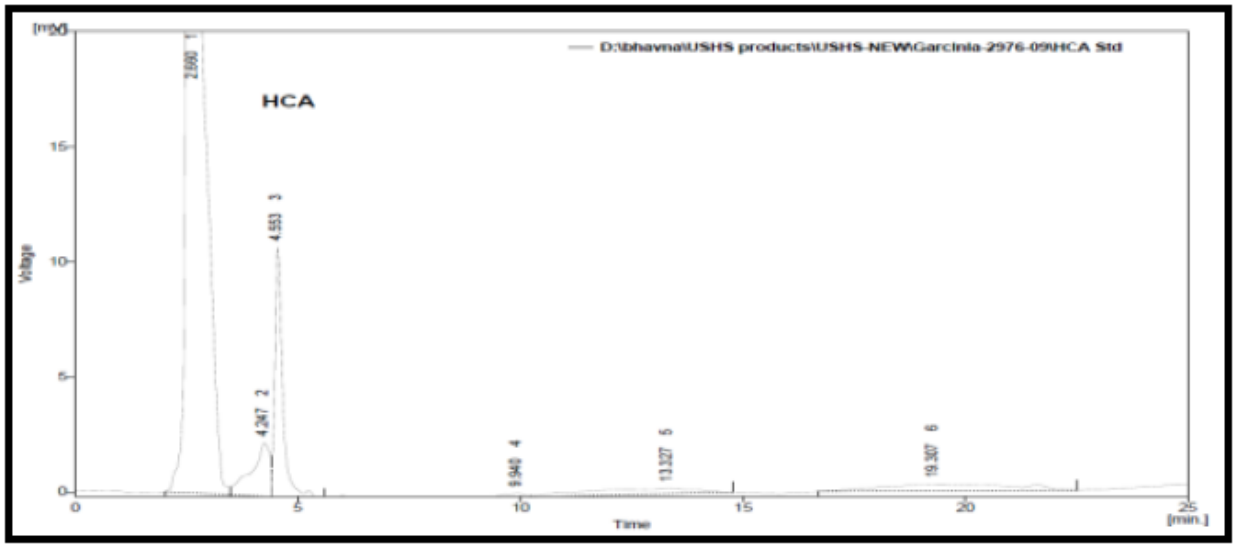


Figure 2: HPLC chromatogram of standard HCA

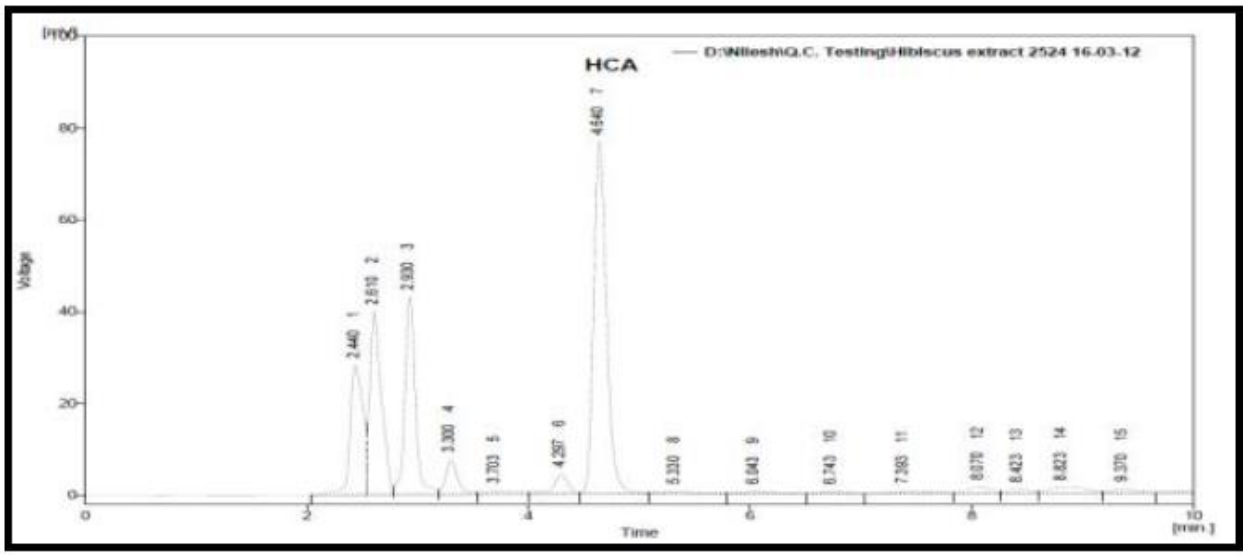


Figure 3: HPLC chromatogram of ***Hibiscus rosa-sinensis*** **Extract**

Table 1: Solvent System used for the Raw materials of Trichup Capsule for HPTLC Analysis

Ingredients	**Solvent System**
Eclipta alba (Bhringraj) Ext	Toluene: Acetone: Formic Acid (9:6:1)
Centella asiatica (Mandukparni) Ext	Chloroform: GAA: Methanol: Water (6:3.2:1.2:0.8)
Glycyrrhiza glabra (Yashtimadhu) Ext	Toluene: EA: GAA (12.5:7.5:0.5)
Hibiscus rosa-sinensis (Japa) Ext	Toluene: EA: Methanol (4.4:5:0.6)
Saptamrit Loha	Toluene: EA: GAA: Formic Acid (2:4.5:2:0.5)
Narsinh Churna	Toluene: EA: Formic Acid (10:3:1)

EA: Ethyl Acetate; **GAA:** Glacial Acetic Acid

Table 2*: *Organoleptic parameters and ingredient's part used

Ingredient	Parts used	Organoleptic characters		
		Colour	**Odour**	**Taste**
BH	Panchang	Dark Brown	Characteristic	Bitter
MP	Panchang	Brown	Characteristic	Slightly Bitter
RC	Extract	Brown	Characteristic	Slightly Bitter
YA	Root	Brown	Peculiar	Sweet
JP	Flower	Dark Brown	Characteristic	Sour
SL	Formulation	Brown	Characteristic	Bitter
NC	Formulation	Brown	Characteristic	Sweet
GR	Formulation	Light Greenish	Characteristic sulphorous	Bitter
MB	Formulation	White	Odourless	Characteristic (Chalky)

BH: Bhringraj Ext; **MP:** Mandukparni Ext; **RC**: Rasayan Churna; **YA:** Yashtimadhu Ext; **JP:** Japa Ext; **SL***:* Saptamrit Loha; **NC**: Narsinh Churna; **GR**: Gandhak Rasayan; **MB:** Muktashukti Bhasma

Table 3: Physico-chemical parameters

Ingredients	Physicochemical parameter	
	pH	**M/S (by LOD) %**
BH	6.58 ± 0.02	3.45 ± 0.12
MP	4.71 ± 0.15	2.57 ± 0.36
RC	4.36 ± 0.11	4.13 ± 0.22
YA	4.50 ± 0.14	2.87 ± 0.16
JP	4.36 ± 0.11	4.98 ± 0.15
SL	3.55 ± 0.18	3.81 ± 0.11
NC	5.63 ± 0.21	4.20 ± 0.21
GR	4.84 ± 0.23	0.29 ± 0.35
MB	11.41 ± 0.31	0.41 ± 0.21

BH: Bhringraj Ext; **MP:** Mandukparni Ext; **RC**: Rasayan Churna; **YA:** Yashtimadhu Ext; **JP:** Japa Ext; **SL***:* Saptamrit Loha; **NC**: Narsinh Churna; **GR**: Gandhak Rasayan; MB: Muktashukti Bhasma*;* **NA**: Not Applicable; **M/S:** Moisture; **LOD**: Loss on Drying

Table 4: Extractive values and Ash value of Ingredients of Trichup Capsule

Ingredients	WSE (%)	ASE (%)	TA (%)
BH	95.68 ± 0.12	21.60 ± 0.25	13.83 ± 0.41
MP	91.60 ± 0.23	07.70 ± 0.32	6.52 ± 0.36
RC	85.77 ± 0.28	25.53 ± 0.15	---
YA	92.48 ± 0.21	26.20 ± 0.11	3.92 ± 0.21
JP	76.33 ± 0.15	34.66 ± 0.01	6.50 ± 0.14
SL	31.92 ± 0.18	30.84 ± 0.21	18.72 ± 0.11
NC	25.80 ± 0.22	26.80 ± 0.11	4.66 ± 0.18
GR	53.52 ± 0.24	14.88 ± 0.23	0.49 ± 0.21
MB	NA	NA	NA

BH: Bhringraj Ext; **MP:** Mandukparni Ext; **RC**: Rasayan Churna; **YA:** Yashtimadhu Ext; **JP:** Japa Ext; **SL***:* Saptamrit Loha; **NC**: Narsinh Churna; **GR**: Gandhak Rasayan; **MB**: Muktashukti Bhasma**; NA**: Not Applicable; **WSE**: Water Soluble Extractive; **TA**: Total Ash; **ASE**: Alcohol Soluble Extractive

Table 5: Assay estimation in extract raw material of Trichup Capsule

Sr No.	Name of the Ingredient	Assay of:	Results
1	Bhringraj Ext	Norwedalactone	03.68 ± 0.02
2	Mandukparni Ext	Triterpene	33.17 ± 0.11
		Saponin	12.88 ± 0.15
3	Rasayan Churna	Tannin	06.17 ± 0.21
		Bitter	00.89 ± 0.32
5	Yashtimadhu Ext	Glycyrrhizin	35.67 ± 0.01
6	Japa Ext	Triterpene	33.17 ± 0.12
7	Saptamrit Loha	Tannin	29.91±0.26
8	Narsinh Churna	Saponin	04.27±0.25
9	Muktashukti Bhasma	Calcium	44.23±0.19

Table 6: Standardization parameters for the finished product Trichup Capsule

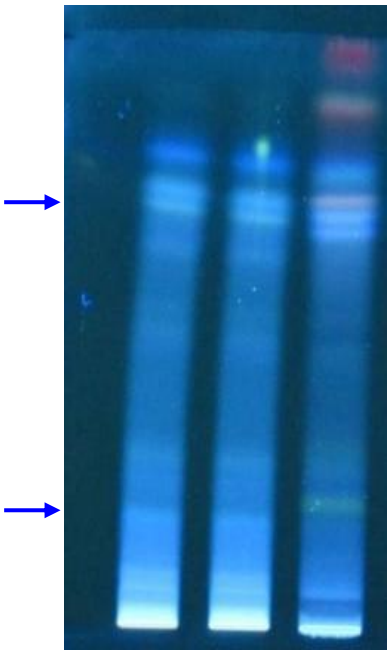
Parameter	**Limits**	**Results**		
		Batch 1	**Batch 2**	**Batch 3**
BULK RELEASE				
Description	Brown to Creamish brown coloured powder	Brown coloured powder	Brown coloured powder	Brown coloured powder
Moisture (KF)	NMT 3.2 %	2.43	2.51	2.58
Bulk Density	0.500 to 0.750g/mL	0.570g/mL	0.580g/mL	0.585g/mL
Tap density	0.650 to 0.850g/mL	0.690g/mL	0.700g/mL	0.700g/mL
FINISHED PRODUCT				
Description	Hard Gelatin Capsule containing brown to Creamish brown coloured powder	Hard Gelatin capsule containing brown coloured powder.	Hard Gelatin capsule containing brown coloured powder.	Hard Gelatin capsule containing brown coloured powder.
Colour of capsule	Green cap with Vasu in white ink & Green body with Vasu in white ink	Green cap with Vasu in white & Green body with Vasu in white ink.	Green cap with Vasu in white & Green body with Vasu in white ink.	Green cap with Vasu in white & Green body with Vasu in white ink.
Size of capsule	"Zero"	Zero	Zero	Zero
pH	5.00 -7.00	5.26	5.31	5.28
Average Net Content Per capsule	475 ± 15 mg	476.3 mg	479.2mg	481.6 mg
Disintegration time	NMT 15 min	09 min 50 sec	09 min 54 sec	10 min 12 sec

Table 7: Results of Heavy metal content and Bio-burden in raw material of Trichup Capsule

Ingre-dients	Heavy metal content				Bio-burden					
	Pb 10ppm	**Cd** 0.3ppm	**As** 3.0ppm	**Hg** 1.0ppm	**TBC** NMT 10^7 cfu/g	**TFC** NMT 10^5 cfu/g	***E. coli*** Ab	***P.a*** Ab	***S.e*** Ab	***S.a*** Ab
BH	1.021	0.114	0.251	ND	4×10^2	Absent	Absent	Absent	Absent	Absent
MP	0.321	0.154	1.254	ND	1×10^2	Absent	Absent	Absent	Absent	Absent
RC	0.521	0.012	0.352	ND	6×10^2	Absent	Absent	Absent	Absent	Absent
YA	1.254	0.214	1.265	ND	3×10^2	Absent	Absent	Absent	Absent	Absent
JP	2.012	0.145	0.251	ND	5×10^2	Absent	Absent	Absent	Absent	Absent
SL	2.325	0.254	0.045	ND	NA	NA	NA	NA	NA	NA
NC	0.154	0.114	0.234	ND	NA	NA	NA	NA	NA	NA
GR	2.154	0.023	0.214	ND	NA	NA	NA	NA	NA	NA
MB	2.014	0.041	0.251	ND	NA	NA	NA	NA	NA	NA
TC	1.245	0.052	0.025	ND	11×10^2	Absent	Absent	Absent	Absent	Absent

BH: Bhringraj Ext; **MP:** Mandukparni Ext; **RC**: Rasayan Churna; **YA:** Yashtimadhu Ext; **JP:** Japa Ext; **SL***:* Saptamrit Loha; **NC**: Narsinh Churna; **GR**: Gandhak Rasayan; **MB**: Muktashukti Bhasma**; TC**: Trichup Capsule; **NA**: Not Applicable; **ppm**: parts per million, **cfu/g**- colony forming unit per gram, **Pb**: Lead, **Cd**: Cadmium, **As**: Arsenic, **Hg**: Mercury, **ND**: Not Detected; **TBC**: Total bacterial count, **TFC**: Total fungal count, ***E. coil***: *Escherichia coli*, ***P.a***.: *Pseudomonas aeruginosa*, ***S.e***: *Salmonella enterica.*, ***S.a***: *Staphylococcus aureus;* **Ab**: Absent

Eclipta alba (Bhringraj) Whole plant Extract (BH)



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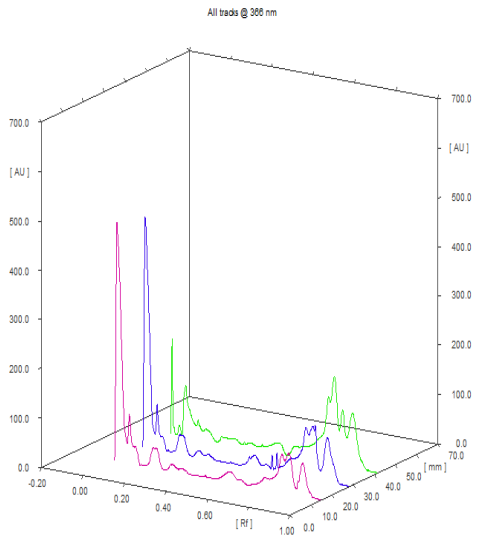


B

Figure 4: It shows the HPTLC Chromatogram of BH**.**

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Capsule

A: HPTLC Plate of BH at 366nm under UV.

B: 3D image of the Fingerprinting of BH and finished product (366nm). The results indicated that HPTLC Chromatogram of BH and finished product has the similar R_f value of 0.20 and 0.73 at 366nm.

***Centella asiatica* (Mandukparni) Whole plant Extract (MP)**

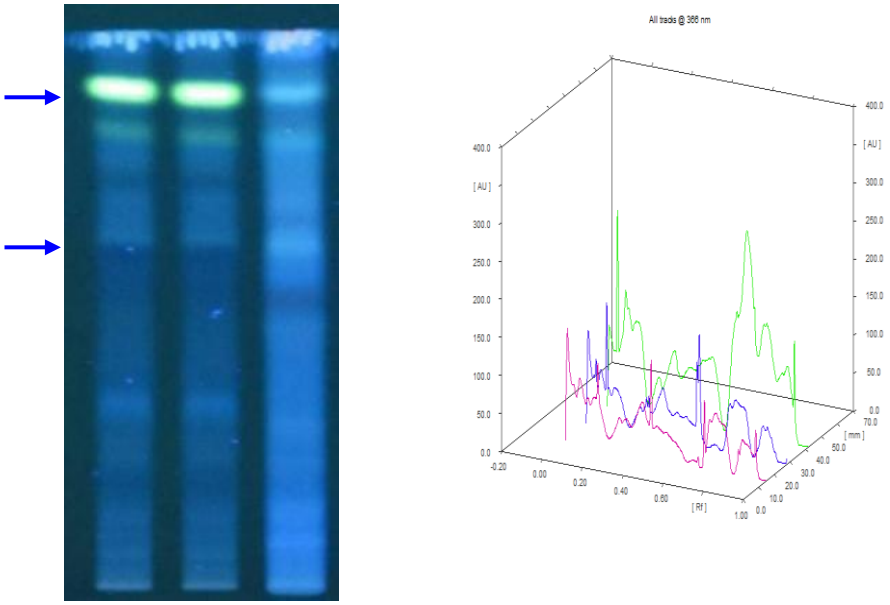


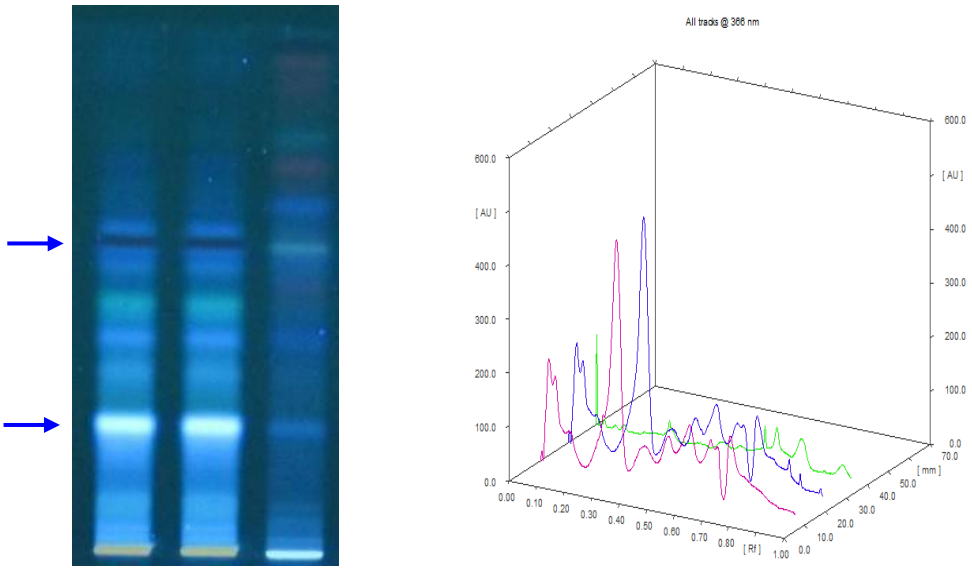
Figure 5: It shows the HPTLC Chromatogram of MP**.**

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Capsule

A: HPTLC Plate of MP at 366nm under UV.

B: 3D image of the Fingerprinting of MP and finished product (366nm). The results indicated that HPTLC Chromatogram of MP and finished product has the similar R_f value of 0.62 and 0.87 at 366nm

***Glycyrrhiza glabra* (Yashtimadhu) Root Extract (YA)**



A **B**

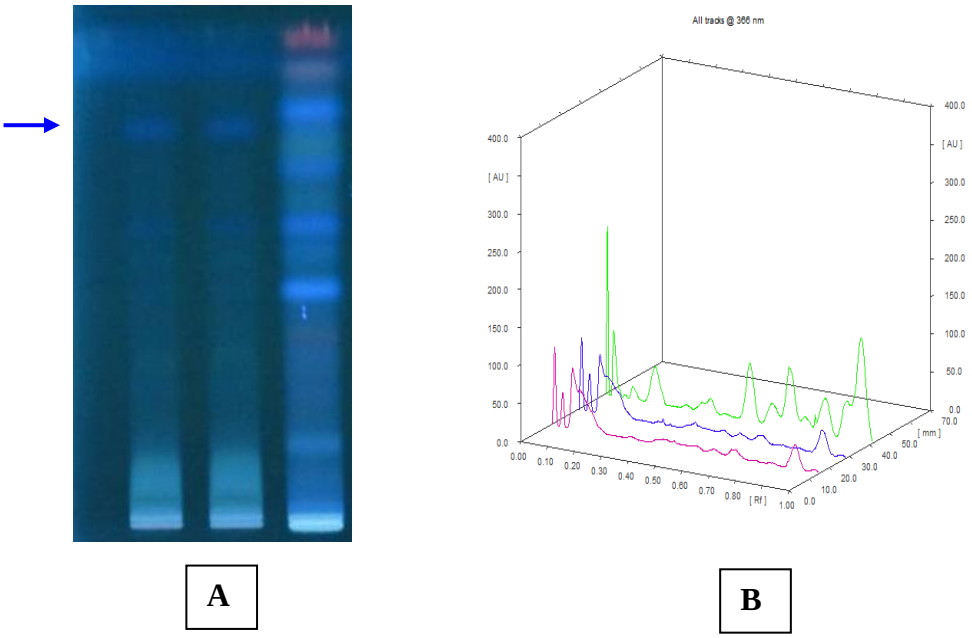
Figure 6: It shows the HPTLC Chromatogram of YA**.**

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Capsule

A: HPTLC Plate of YA at 366nm under UV.

B: 3D image of the Fingerprinting of YA and finished product (366nm). The results indicated that HPTLC Chromatogram of YA and finished product has the similar R_f value of 0.33 and 0.60 at 366nm

***Hibiscus rosa-sinensis* (Japa) Flower Extract (JP)**



A **B**

Figure 7: It shows the HPTLC Chromatogram of JP**.**

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Capsule

A: HPTLC Plate of JP at 366nm under UV.

B: 3D image of the Fingerprinting of JP and finished product (366nm). The results indicated that HPTLC Chromatogram of JP and finished product has the similar R_f value of 0.78 at 366nm.

Saptamrit Loha

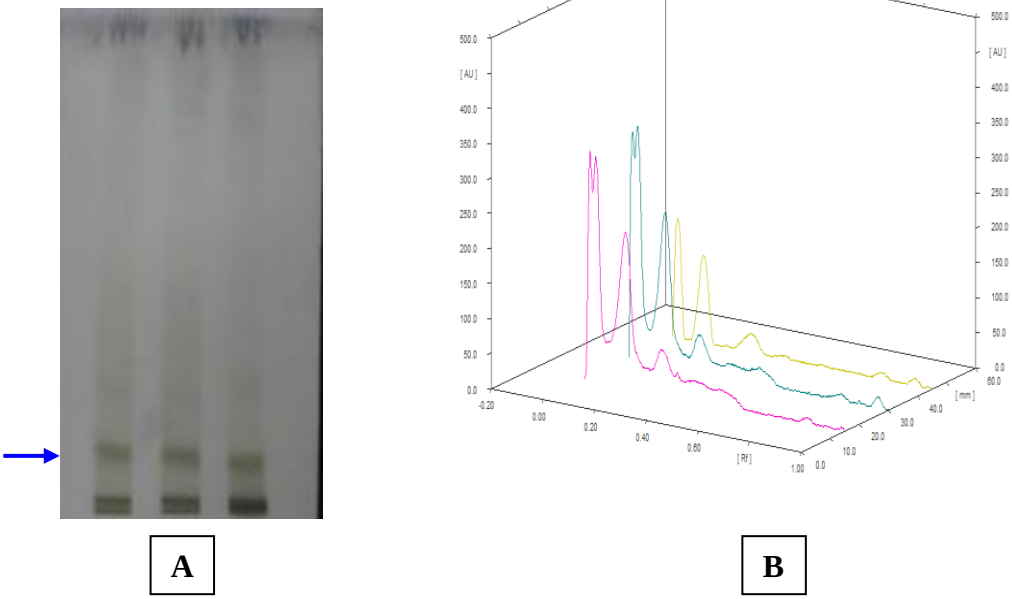


Figure 8: It shows the HPTLC Chromatogram of SL**.**

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Capsule

A: HPTLC Plate of JP at 366nm under UV.

B: 3D image of the Fingerprinting of JP and finished product (366nm). The results indicated that HPTLC Chromatogram of JP and finished product has the similar R_f value of 0.10 at Visible.

Narsinh Churna

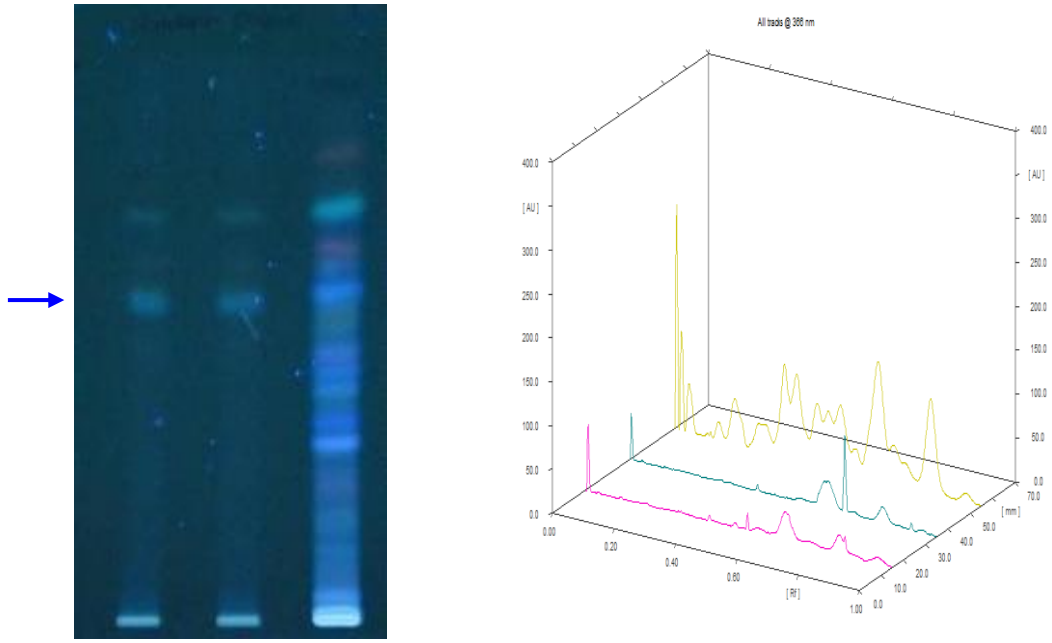


Figure 9: It shows the HPTLC Chromatogram of NC**.**

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Capsule

A: HPTLC Plate of JP at 366nm under UV.

B: 3D image of the Fingerprinting of JP and finished product (366nm). The results indicated that HPTLC Chromatogram of JP and finished product has the similar R_f value of 0.56 at 366nm

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