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Quality Control Evaluation of Tapyadi Loha Using Physicochemical and HPTLC Parameters

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Abstract

Background: Sickle Cell Anaemia (SCA) is a chronic inherited haemoglobin disorder that is characterized by haemolytic anaemia, oxidative damage, inflammation, and involvement of various organs. In Ayurvedic practice, such symptoms can be attributed to Pāṇḍu Roga, which is treated with Loha-based herbo-mineral formulations. Tāpyādi Loha is a well-established Ayurvedic formulation used in the treatment of haematological disorders, but it is necessary to standardize and characterize it. **Objectives:** Development of a quality profile for Tāpyādi Loha tablets prepared as per the classical reference through physicochemical, elemental, microbial, TLC, and HPTLC fingerprint analyses and to assess the inter-batch variability. **Results:** Three samples of Tāpyādi Loha were prepared using the same pharmaceutical method and were subjected to analysis for standardisation. All the tested parameters, such as loss on drying, hardness, friability, disintegration time, ash values, extractive values, pH, and elemental content (iron, silver, copper), were found to be within the prescribed limits. TLC and HPTLC studies showed similar and reproducible chromatographic patterns. The HPTLC fingerprint of the methanolic extract showed well-defined peaks at 254 nm and 366 nm, which indicated the presence of polyphenols, flavonoids, tannins, alkaloids, terpenoids, and fulvic acid-like fractions, which could be attributed to the herbal constituents of the formulation. Conclusion: The pharmaceutical standardization and characteristic HPTLC fingerprint of Tāpyādi Loha tablets have been established successfully in this study. The consistency in the results obtained for different batches of the formulation indicates the authenticity of the manufacturing process. This study provides a scientific rationale for the use of Tāpyādi Loha in the treatment of Pāṇḍu Roga and other chronic anaemic disorders, such as those similar to sickle cell anaemia.

Introduction

Sickle cell anaemia (SCA) is a hereditary hemoglobinopathy that presents chronic haemolytic anaemia, recurrent vaso-occlusive crises, and progressive multi-organ dysfunction. These manifestations occur due to a point mutation in the β-globin gene, resulting in the production of abnormal haemoglobin S (HbS) [1,2] The prevalence of the disease is high in sub-Saharan Africa, Saudi Arabia, India, Turkey, Greece, and Italy [3]. In India, SCA is predominantly found in tribal and rural areas, mainly in central India and in the states of Gujarat, Maharashtra, and Kerala, causing immense morbidity, mortality, and socioeconomic burden [4,5]. It is estimated that approximately 7% of the world population is a carrier of the abnormal haemoglobin gene, and every year, 300,000 to 500,000 children are born with hemoglobinopathies, thus underlining the extent of this neglected public health problem [6,7]. In a pathophysiological perspective, the erythrocytes in sickle cell disease become stiff with increased adhesive properties, leading to microvascular obstruction, ischemia, recurrent vaso-occlusive episodes, acute chest syndrome, increased susceptibility to infections, and multi-organ damage [8]. The current treatment modalities for SCA are mainly palliative and preventive, including red blood cell transfusions, hydroxyurea therapy, folic acid supplementation, prophylactic antibiotics, and comprehensive supportive care [9]. Curative therapies like hematopoietic stem cell transplantation are limited by patient age, availability of a matched donor, cost, and transplant-related risk, making it unavailable to most adult patients [10]. In the Ayurvedic perspective, the clinical presentation of SCA, such as pallor, weakness, reduced vitality, and features of tissue depletion, corresponds to Pāṇḍu Roga, which is a disease essentially characterized by Pitta derangement with impairment of Rasa and Rakta Dhātu formation [11]. This provides a classical perspective on haematological disorders that present with chronic anaemia and systemic weakness. Tāpyādi Loha is a traditional herbo-mineral drug used in Pāṇḍu Roga and other haematological and metabolic disorders [12,13]. Due to its iron-based composition and long therapeutic use, it is important to standardize the drug using physicochemical analysis and chromatographic characterization to ensure safety and efficacy [14]. Among various modern analytical tools, thin-layer chromatography (TLC) and high-

performance thin-layer chromatography (HPTLC) are established fingerprinting techniques for the identification of the drug and the characteristic phytoconstituents of herbal ingredients [15,16]. Taking into consideration the chronic nature of SCA and the requirement of continuous supportive therapy to improve haematological conditions, digestion, and tissue feeding, the scientific standardization of the classical compound Tāpyādi Loha is of utmost significance. HPTLC is a powerful analytical tool for developing phytochemical fingerprints, ensuring quality, and providing chemical rationality for therapeutic significance. Accordingly, this research work proposes to develop and interpret the HPTLC fingerprint spectrum of Tāpyādi Loha tablets to authenticate their quality and therapeutic significance in diseases like Pāṇḍu Roga.

Materials and Methods

Pharmaceutical preparation

Tāpyādi Loha was prepared in accordance with the classical formulation described in *Bharata Bhaiṣajya Ratnākara* [17]. For pharmaceutical standardization and assessment of reproducibility, three independent batches of the formulation were prepared following identical procedures. Purified Tapya (Svarṇamakṣika; copper–iron sulphide, chalcopyrite), Śuddha Śilājatu (purified mineral pitch), Rajata Bhasma (incinerated silver), and Māṇḍūra Bhasma (incinerated iron oxide) were taken in equal quantities of five pala (240 g) each and powdered separately to obtain a fine and uniform mineral blend. To this mixture, one pala (48 g) each of powdered Citraka (*Plumbago zeylanica* L.; leadwort), Harītakī (*Terminalia chebula* Retz.; chebulic myrobalan), Bibhitakī (*Terminalia bellirica* (Gaertn.) Roxb.; belleric myrobalan), Āmalakī (*Emblica officinalis* Gaertn. syn. *Phyllanthus emblica* L.; Indian gooseberry), Śuṇṭhī (*Zingiber officinale* Roscoe; dried ginger), Pippalī (*Piper longum* L.; long pepper), Marica (*Piper nigrum* L.; black pepper), and Vidaṅga (*Embelia ribes* Burm.f.; false black pepper) was added and mixed thoroughly to ensure homogeneity. Subsequently, eight pala (64 g) of Śarkarā (sugar) was incorporated as a binding and palatability-enhancing agent. The formulation mass was uniformly triturated and compressed into tablets of specified weight using standard pharmaceutical procedures.

Physicochemical analysis

The three batches of Tāpyādi Loha tablets were tested for their quality in terms of physicochemical, elemental, microbial, and chromatographic tests following standard pharmacopeial quality control procedures. Tests such as loss on drying,

hardness, friability, weight uniformity, disintegration time, ash value, extractive value, and pH were carried out. Elemental analysis for iron, silver, and copper was carried out to check compliance with the specified limits. Microbiological analysis included total plate count, yeast and Mold count, and absence of specified pathogens. Chromatographic analysis utilized thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) to obtain chromatographic fingerprints and to check the identity of the formulation.

HPTLC Fingerprint Analysis

The methanol extract of Tāpyādi Loha was analysed for high-performance thin-layer chromatography (HPTLC) fingerprint using solution volumes of 10.0 µL, 15.0 µL, 20.0 µL, and 25.0 µL, with detection at 254 nm (UV-absorbing compounds) and 366 nm (fluorescent compounds). The preparation of the test solution involved weighing 5 g of the sample in a beaker, adding 100 mL of methanol, and sonicating for 15 minutes. After cooling, the solution was filtered using standard filter papers. The test solution obtained was used for HPTLC fingerprint analysis. Chromatographic separation was performed using 10 × 10 cm thin-layer chromatography (TLC) plates pre-coated with a 0.2 mm layer of silica gel 60 F254 (Merck). The samples were applied to the TLC plates using a Linomat 5 sample applicator (CAMAG, Switzerland) as 6 mm wide bands. The chromatograms were developed to a height of 8.0 cm using the mobile phase toluene: ethyl acetate: formic acid (5:4:1 v/v/v) in a CAMAG twin-trough chamber saturated with the mobile phase vapor. After drying at room temperature for five minutes, the plates were analysed using a CAMAG TLC Scanner 3 with winCATS 4 software (CAMAG, Switzerland) at 254 nm.

Results

Three different batches of Tāpyādi Loha were prepared using the same pharmaceutical procedure, and all three batches had similar organoleptic properties and tablet qualities. The representative samples uniformity in physicochemical properties such as moisture content, hardness, friability, disintegration time, ash values, extractive values, and elemental composition. The TLC/HPTLC fingerprint analysis of the samples showed similar patterns, indicating similarity in phytochemical constituents. All the three samples of Tāpyādi Loha tablets met the required quality standards of physicochemical properties, elemental content, and microbial safety. Loss on drying was within acceptable standards, signifying stability, and low moisture

content. Tablet hardness, friability, thickness, diameter, and disintegration time showed sufficient mechanical strength and uniformity. Ash and acid-insoluble ash content indicated the presence of mineral parts within acceptable standards. Water- and alcohol-soluble extractive values supported sufficient extraction of polar and moderately non-polar phytoconstituents. Elemental analysis indicated the presence of iron, silver, and copper within acceptable standards, confirming the presence of Bhasma. Microbial testing confirmed the absence of pathogenic microorganisms, ensuring the formulation's safety. TLC analysis confirmed acceptable standards of chromatographic patterns. (Table 1)

Table 1: Physicochemical parameters of *Tapyadi Loha*;

Parameter	Batch 1	Batch 2	Batch 3	Specification
Loss on drying (% w/w)	4.79	4.98	4.65	NMT 7%
Friability (% w/w)	0.372	0.18	0.42	NMT 1%
Hardness (kg/cm²)	7.7	7.2	7.6	NLT 2
Disintegration time (min)	14.0	9.0	11	NMT 30
Ash value (% w/w)	46.88	44.83	45.12	NMT 55
Acid insoluble ash (% w/w)	23.28	20.19	21.3	NMT 25
Water soluble extractive (% w/w)	41.36	25.44	41.21	NLT 14.5
Alcohol soluble extractive (% w/w)	13.52	12.72	12.32	NLT 3.5
pH (1% aq. solution)	6.94	6.58	6.86	5.0–7.0
Iron (mg/tab)	15.81	12.71	13.5	9–16
Silver (mg/tab)	7.83	7.60	7.50	7–14
Copper (mg/tab)	1.34	2.54	2.42	NLT 1.2
TLC analysis	Complies	Complies	Complies	Comparable to standard
Microbial limits	Within limits	Within limits	Within limits	As per standards

The HPTLC fingerprint analysis of the methanolic extract of Tāpyādi Loha showed a clear and well-defined chromatographic pattern under both 254 nm and 366 nm illumination conditions. The presence of several distinct peaks over a wide Rf range of 0.24–1.00 indicated the presence of phytoconstituents with different polarities. At 254 nm, several prominent peaks with higher area percentage values were noticed in the middle Rf regions (approximately 0.59–0.77), indicating the predominance of UV-absorbing phenolic, glycosidic, and alkaloid compounds. The illumination at 366 nm also showed intense fluorescence in the same Rf regions, along with additional fluorescent bands, which indicated the

presence of flavonoids, tannins, and bioactive organic fractions. The similarity in Rf values and relative area percentages with increasing concentrations of the sample confirms the chemical stability and reproducibility of the formulation, thus establishing a distinct HPTLC fingerprint for Tāpyādi Loha. (Table 2, Table 3, Figure 1)

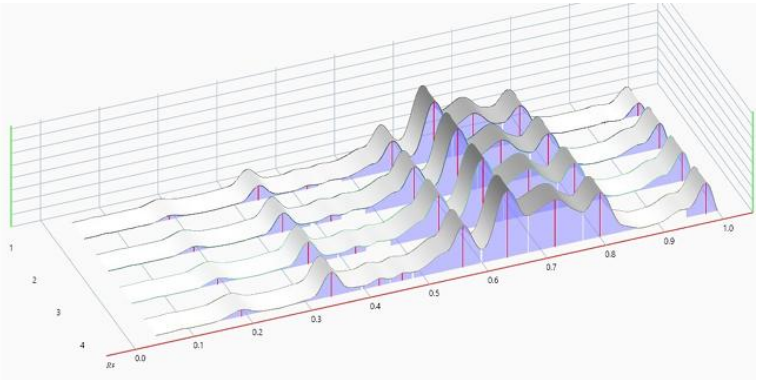
Table 2: Rf values obtained for Methanol extract of *Tapyadi Loha* Under 254 nm visualisation;

Spot No.	10.0 µL		15.0 µL		20.0 µL		25.0 µL	
	Rf value	Area%	Rf Value	Area%	Rf value	Area%	Rf value	Area%
1	0.250	1.73	0.253	1.73	0.256	1.80	0.254	2.09
2	0.393	5.71	0.389	6.08	0.396	6.78	0.396	7.03
3	0.444	1.40	0.442	1.80	0.444	1.94	0.443	1.78
4	0.599	15.76	0.597	16.45	0.599	16.64	0.482	1.71
5	0.671	27.71	0.674	25.54	0.676	24.45	0.599	16.60
6	0.754	26.67	0.762	25.26	0.768	25.25	0.678	23.87
7	0.863	17.71	0.871	15.07	0.874	14.59	0.768	25.53
8	0.951	0.28	1.000	8.06	1.000	8.54	0.874	14.83
9	1.000	3.03					1.000	6.56

Table 3: Rf values obtained for Methanol extract of *Tapyadi Loha* Under 366 nm visualisation;

Spot No.	10.0 µL		15.0 µL		20.0 µL		25.0 µL	
	Rf value	Area%	Rf Value	Area%	Rf value	Area%	Rf value	Area%
1	0.285	1.55	0.274	1.56	0.268	1.64	0.313	1.95
2	0.524	8.97	0.314	0.78	0.524	10.64	0.524	10.48
3	0.597	29.99	0.522	10.50	0.603	33.08	0.604	33.14
4	0.683	39.45	0.600	33.85	0.683	29.28	0.685	26.43
5	0.871	15.42	0.681	33.83	0.732	5.05	0.733	5.07
6	0.989	4.63	0.878	19.49	0.879	20.31	0.882	22.92

Figure 1: Comparative Densitograms of Tapyadi Loha at different concentrations and under different visualisations;



Discussion

The three batches of Tāpyādi Loha were very consistent in most quality checks, indicating the good reproducibility and reliability of the manufacturing process. Physicochemical properties and chromatographic fingerprints show close agreement from batch to batch, reflecting uniform inclusion of mineral and herbal ingredients. This uniformity is an important factor in obtaining predictable therapeutic results. From a pharmaceutical perspective, such batch-to-batch concurrence is particularly significant for herbo-mineral preparations, where minor variations in processing have the potential to alter bioavailability and safety. Overall, the findings confirm that the adopted preparation method can produce a standardized formulation and be at a very solid quality benchmark for the forthcoming pharmacological and clinical work. Analytical evaluation of Tāpyādi Loha tablets indicated a sound, reproducible pharmaceutical profile, matching adherence to standardized manufacturing and quality-control practices. The consistently low loss on drying across batches reflected a minimum amount of residual moisture, which helps avert hydrolytic degradation and microbial growth, thereby enhancing shelf stability. The tablets have presented favourable mechanical properties—sufficient hardness, low friability, uniform size, and acceptable disintegration time—which signals strong tablet integrity, dose uniformity, and predictable drug release upon administration. Ash and acid-insoluble ash values remained well within the prescribed limits, reflecting proper incineration and purification of Loha and related minerals, and also excluding excessive inorganic impurities or foreign matter. Water- and alcohol-soluble extractive values indicated a wide array of polar to moderately non-polar phytoconstituents from the herbal moiety. Elemental analysis confirmed the presence of iron, silver, and copper within therapeutically acceptable limits, justifying the classical indication for iron-based

formulations in disorders characterized by Dhātu Kṣaya and depressed haematopoiesis. These trace elements, from a modern pharmacological perspective, participate in erythropoiesis, enzyme activities, and cellular metabolic processes. The TLC fingerprint consistency within the batch and between batches acts as chemical validation for the consistency of the phytochemical composition within a batch and between different batches. When the chromatographic patterns appear similar, this points to uniform extraction and integration of the herbal ingredients during formulation, enhancing confidence in the production process.

HPTLC fingerprint analysis of Tāpyādi Loha was carried out to generate a characteristic chromatographic profile for formulation identity and quality control. The chromatographic pattern primarily reflects organic phytoconstituents derived from the herbal ingredients of the formulation. Analysis at four application volumes demonstrated reproducibility and proportionality of peak responses, supporting the reliability of the generated fingerprint [1,6,18]. Visualization at 254 nm revealed nine well-resolved UV-absorbing spots across different concentrations, indicating the presence of aromatic and conjugated phytoconstituents such as phenolics, tannins, alkaloids, and glycosides [15]. Consistent Rf values across increasing volumes confirmed chromatographic stability. Prominent peaks in the Rf range of 0.59–0.68 showed higher area percentages (approximately 16–27%), suggesting the predominance of polar to mid-polar compounds, while peaks between Rf 0.75–0.87 contributed significantly to the total area and were indicative of relatively less polar aromatic constituents. Minor peaks at lower Rf values (0.25–0.44) represented highly polar components. The overall area distribution reflected a balanced and reproducible phytochemical composition.

At 366 nm, six distinct fluorescent bands were observed, with major zones appearing between Rf 0.52–0.88 and exhibiting high area percentages. Peak areas increased proportionally with application volume, indicating a dose-dependent response. Dominant peaks around Rf 0.59–0.68 (29–39% area) suggested the presence of fluorescent phenolic compounds, while peaks near Rf 0.87–0.88 were indicative of alkaloidal constituents. A minor high-Rf fluorescent band at Rf ~0.98 suggested the presence of non-polar fluorescent compounds in small quantities. The combined HPTLC profile under both wavelengths demonstrated a complex yet reproducible chromatographic pattern, reflecting the polyherbo-mineral

nature of Tāpyādi Loha and the wide polarity range of methanol-extractable phytoconstituents. Prominent mid-Rf peaks are suggestive of polyphenols, flavonoids, glycosides, and alkaloids, largely attributable to Triphalā and Trikātu. These compounds are known to exert antioxidant, digestive stimulant (Deepana–Pācana), and bioavailability-enhancing effects, which are relevant for improving the therapeutic performance of mineral components such as Māṇḍūra and Svarṇamakṣika Bhasma [18,19,20,21,22,23]. Lower to mid-Rf bands (0.24–0.60) are characteristic of hydrolysable tannins and phenolic acids derived mainly from Harītakī, Bibhitakī, and Āmalakī, which are documented for antioxidant, hepatoprotective, and iron-modulating activities [19,20,21]. Fluorescent bands around Rf 0.38–0.53 correspond to flavonoids, particularly from Āmalakī and Bibhitakī, known for anti-inflammatory and free-radical scavenging properties [22,23]. Prominent peaks around Rf 0.67–0.69 are indicative of alkaloids such as piperine from Pippalī and Marica, recognized for their absorption-enhancing properties [24,25]. Higher mid-Rf bands (0.73–0.77) are plausibly attributed to terpenoids from Citraka and Vidaṅga, which contribute digestive and metabolic stimulant actions [26,27]. Minor fluorescent peaks near the solvent front (Rf 0.95–1.00) may correspond to fulvic acid–like fractions of Śuddha Śilājatu, supporting its role in mineral transport and adaptogenic activity [28,29]. Although individual marker compounds were not identified, the generated HPTLC fingerprint fulfils the objective of qualitative standardization and serves as a reliable reference chromatogram for quality control. Overall, the HPTLC profile provides a phytochemical rationale supporting the traditional use of Tāpyādi Loha in Pāṇḍu and related chronic anaemic and metabolic disorders.

Conclusion

This research work has been able to establish the pharmaceutical standardization and quality profile of Tāpyādi Loha, a classical herbo-mineral drug, on a scientific basis. The three different batches of the formulation have been prepared in a uniform manner, which is a positive aspect of the research work. The formulation meets the required standards of physicochemical properties, elemental composition, microbial safety limits, and chromatographic fingerprinting. The HPTLC/TLC analysis shows a uniform phytochemical pattern, which is a result of equal addition of bioactive herbal components along with processed mineral parts. The findings of this research work can provide scientific justification for the

traditional method of formulation preparation, which establishes the pharmaceutical authenticity of Tāpyādi Loha and makes it eligible for further pharmacological studies and use in the treatment of conditions classified under Pāṇḍu Roga.

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