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PHYSIOCHEMICAL EVALUATION AND STANDARDIZATION OF VIBHITAKADI EYE OINTMENT

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ABSTRACT

Drug standardization is essential to ensure the quality, efficacy, and authenticity of pharmaceutical preparations. *Vibhitakadi Ghrita*, described in *Yogaratnakara*, has been found to be effective in the management of *Shushkakshipaka*. The formulation comprises *Vibhitaki*, *Haritaki*, *Amalaki*, *Nimba*, *Patola*, *Vasa*, and *Go Ghrita*. In the present study, this GhIrita-based formulation was modified into an ointment form to enhance bioavailability and improve ocular absorption The present analytical study was undertaken to standardize *Vibhitakadi* eye ointment and to establish its organoleptic, physicochemical, chromatographic, and microbial quality parameters. The results revealed that the formulation possesses suitable physicochemical characteristics, including neutral pH, low moisture content, satisfactory spreadability, and overall stability. Microbial evaluation further confirmed the complete absence of bacterial and fungal contamination, indicating the formulation's safety for ocular use. Collectively, these findings support the successful standardization of Vibhitakadi eye ointment and affirm its suitability as an ophthalmic preparation for the management of *Shushkakshipaka* (Digital Eye Strain).

KEYWORDS: *Vibhitakadi* Eye Ointment, Standardization, HPTLC.

INTRODUCTION

Shushkakshipaka is a *Sadhya Vyadhi* under *Sarvagata Netra Rogas*, caused by vitiation of *Vata* and *Pitta*. Its clinical features such as *Gharsha*, *Toda*, *Bheda*, *Upadeha*, *Krichronmeelana* and *Rooksha-Daruna Vartma* closely resemble the symptoms of Digital Eye Strain, including irritation, fluctuating blurred vision, and photophobia. Digital Eye Strain significantly affects quality of life and may lead to serious complications if untreated.

Modern treatments like tear substitutes provide only short-term symptomatic relief, highlighting the need for safer, economical, and more effective therapies. Ayurveda recommends *Snehapana*, *Nasya*, *Aschyotana*, *Anjana*, and other *Vatahara* measures. Vibhitakadi Ghrita, mentioned in *Yogaratnakara*, is indicated for Shushkakshipaka due to its *Vata-Pittahara*, *Chakshushya*, and *Ropana* properties.

Since drug retention in *Aschyotana* is limited, the *Ghrita* was modified into an ointment form using bee wax (1:6 ratio) to enhance ocular retention, improve bioavailability, and increase patient compliance. Thus, Vibhitakadi eye ointment is proposed as a novel, patient-friendly, and effective therapeutic option for managing *Shushkakshipaka* (Digital Eye Strain).

MATERIALS AND METHODS

1. Preparation of Vibhitakadi Eye Ointment

1 part of Bee wax was melted on hot water bath and to it added 6 parts of *Vibhitakadi Ghrita* and mix well. Then it was allowed to cool till it get solidified.

2. Physicochemical Evaluation

a. Refractive Index

The refractive index of Vibhitaki ointment was found to be **1.46671**, which reflects the optical consistency and uniformity of the formulation. A stable refractive index

suggests the absence of major impurities or phase separation, supporting the formulation's homogeneity.

b. pH

The pH of the ointment was observed to be 7.0, indicating a neutral nature. This is particularly significant for ophthalmic preparations, as the eye is highly sensitive to acidic or alkaline substances. A neutral pH enhances ocular comfort and reduces the risk of irritation, making the formulation suitable for therapeutic use in Shushkakshipaka (Digital Eye Strain).

c. Loss on Drying

The loss on drying value was recorded as 0.53%, suggesting minimal moisture content in the formulation. Low moisture levels are desirable in eye ointments, as they reduce the possibility of microbial contamination and improve shelf stability.

d. Spreadability

The spreadability of Vibhitaki ointment was found to be 11.89 g·cm/sec, indicating good application characteristics. Adequate spreadability is crucial for ocular ointments, as it ensures uniform distribution over the ocular surface and enhances patient compliance.

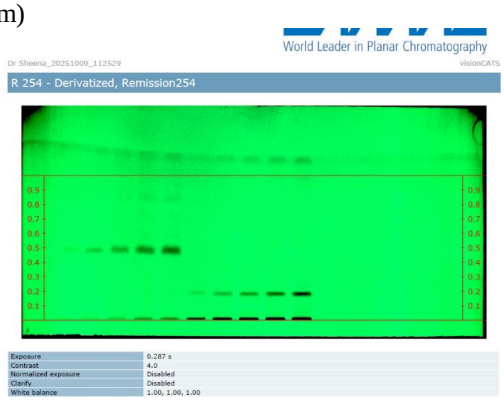
e. Unsaponifiable Matter

The unsaponifiable matter content was measured as 0.21%, indicating the presence of minor bioactive lipid components. These constituents may contribute to the therapeutic efficacy of the formulation by providing lubricating and protective effects on the ocular surface, which is beneficial in dry eye conditions associated with digital eye strain.

f. Chromatographic Analysis (HPTLC)

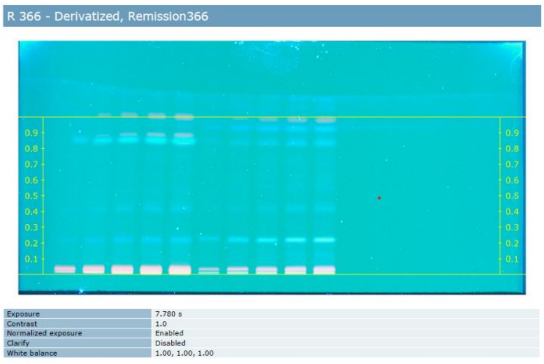
The HPTLC analysis of Vibhitakadi formulations was carried out using vision CATS software (version 3.2.23095.1) with standardized instrumentation. Sample application was performed using an Automatic TLC Sampler (ATS 4), while densitometric scanning was done with a TLC Scanner. Visualization of the developed plates under UV light was achieved using a TLC Visualizer. Chromatographic separation was carried out on Merck HPTLC silica gel 60 F_{254} plates (200 × 100 mm). Samples were applied in the form of bands at a Y-position of 8.0 mm with a band length of 8.0 mm. The first track was positioned at 20.0 mm from the left edge, maintaining a track distance of 11.4 mm between successive applications. The mobile phase was allowed to migrate up to a solvent front distance of 70 mm, ensuring adequate separation of phytoconstituents and generation of a reproducible chromatographic fingerprint for standardization.

HPTLC Interpretation (R 254 – Derivatized, Remission 254 nm)



The HPTLC analysis of Vibhitakadi eye ointment under Remission at 254 nm after derivatization revealed a well-defined chromatographic fingerprint with multiple distinct bands at different Rf values. Prominent spots were observed in the mid-Rf region (around 0.45–0.55) along with additional bands in the lower Rf region (around 0.15–0.25), indicating the presence of both moderately polar and polar phytoconstituents. The consistent appearance of these bands across the tracks confirms the uniformity and reproducibility of the formulation. Derivatization enhanced the detection of UV-active secondary metabolites such as phenolics, tannins, and other bioactive compounds, which may contribute to the therapeutic potential of the ointment. Overall, the developed HPTLC fingerprint serves as a reliable tool for the standardization, quality control, and authentication of Vibhitakadi eye ointment intended for the management of Shushkakshipaka (Digital Eye Strain).

HPTLC Interpretation (R 366 – Derivatized, Remission 366 nm)



The HPTLC analysis of Vibhitakadi eye ointment observed under Remission at 366 nm after derivatization exhibited a characteristic chromatographic fingerprint with several fluorescent bands at distinct Rf values. Prominent spots were noted in the higher Rf region (around 0.80–0.90) along with additional bands in the lower Rf region (around 0.15–0.30), indicating the presence of a wide range of phytoconstituents with varying polarity. The fluorescent nature of the bands at

366 nm suggests the presence of bioactive compounds such as flavonoids, phenolic derivatives, and other secondary metabolites. The uniformity and reproducibility of band patterns across the tracks confirm the consistency of the formulation. Overall, the developed HPTLC profile at 366 nm serves as a reliable chromatographic marker for the standardization, authentication, and quality control of Vibhitakadi eye ointment intended for the management of Shushkakshipaka (Digital Eye Strain).

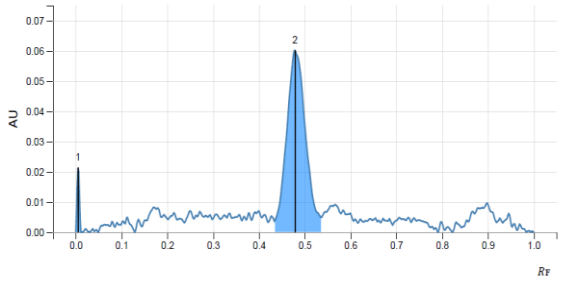
Track-wise Application

Samples were applied using an Automatic TLC Sampler (ATS 4) with nitrogen gas, using methanol as the sample and rinsing solvent. Chromatographic development was carried out in a twin-trough chamber (20×10 cm) using Toluene: Ethyl acetate (7:3) as the mobile phase, with a saturation time of 20 minutes. The developed plate was dried for 5 minutes at room temperature. Derivatization was performed by spraying with anisaldehyde–sulphuric acid reagent (prepared using anisaldehyde, glacial acetic acid, methanol, and concentrated sulphuric acid) without heating, to enhance visualization of phytoconstituents.

Tracks

- Track 1 – 2 µL
- Track 2 – 4 µL
- Track 3 – 6 µL
- Track 4 – 8 µL
- Track 5 – 10 µL

Track 1

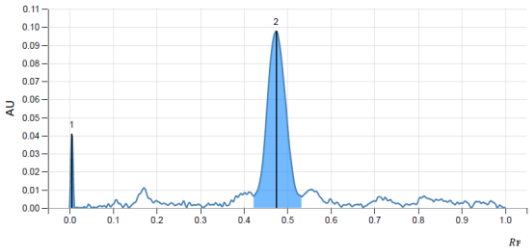


Peak #	Start R_F	Start H	Max R_F	Max H	Max %	End R_F	End H	Area A	Area %
1	0.000	0.0000	0.005	0.0212	26.12	0.011	0.0000	0.00013	4.36
2	0.434	0.0036	0.479	0.0601	73.88	0.535	0.0048	0.00290	95.64

HPTLC densitometric analysis of Vibhitakadi Eye Ointment at 254 nm showed two peaks within the Rf range of 0.000–1.000. Peak 1 near the origin (Rf ~0.005) contributed 4.36% area, possibly representing highly polar constituents or excipients. Peak 2, the major peak at Rf ~0.479, accounted for 95.64% area, indicating the predominance of UV-absorbing phytoconstituents.

The formulation ingredients, including Triphala, Nimba, Patola, and Vasa, are rich in tannins, phenolics, flavonoids, and alkaloids, which likely contribute to the dominant peak. The presence of a single major peak suggests good chemical uniformity of the formulation. The HPTLC profile can serve as a preliminary fingerprint for quality control and standardization, with further confirmation recommended using reference standards.

Track 2

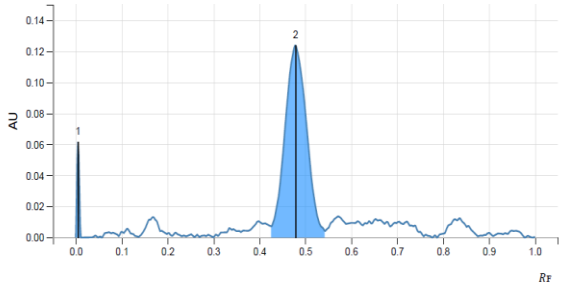


Peak #	Start R_F	Start H	Max R_F	Max H	Max %	End R_F	End H	Area A	Area %
1	0.000	0.0000	0.005	0.0407	29.44	0.011	0.0000	0.00025	4.79
2	0.423	0.0064	0.474	0.0977	70.56	0.532	0.0062	0.00500	95.21

HPTLC densitometric analysis of Vibhitakadi Eye Ointment at 254 nm for Track 2 (4.0 µL) showed two peaks within the Rf range of 0.000–1.000. Peak 1 near the origin (Rf ~0.005) contributed 4.79% area, indicating minor polar constituents, while Peak 2 at Rf ~0.474 accounted for 95.21% area, representing the major UV-absorbing phytochemical fraction.

The dominant peak is likely due to phenolics and tannins from Triphala, with additional contributions from flavonoids and alkaloids of Nimba, Patola, and Vasa. The consistent Rf values confirm good reproducibility, and the profile serves as a reliable fingerprint for quality control and standardization of the formulation.

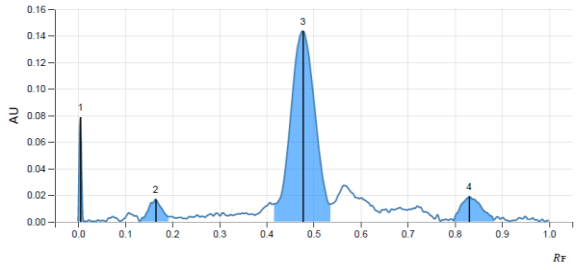
Track 3



HPTLC densitometric analysis of Vibhitakadi Eye Ointment at 254 nm for Track 3 (6.0 µL) showed two peaks within the Rf range of 0.000–1.000. Peak 1 near the origin (Rf ~0.005) contributed 5.15% area, indicating minor polar constituents, while Peak 2 at Rf ~0.479 accounted for 94.85% area, representing the major UV-absorbing phytoconstituents.

The dominant peak is mainly due to phenolics and tannins from Triphala, with contributions from flavonoids and alkaloids of Nimba, Patola, and Vasa. The consistent Rf values and proportional response confirm reproducibility, supporting HPTLC as a reliable tool for standardization and quality control of the formulation.

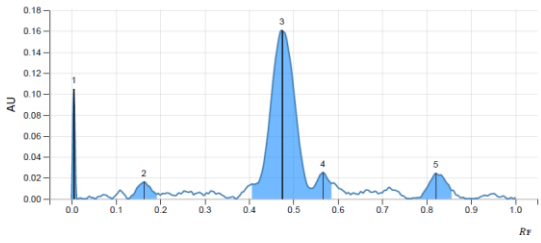
Track 4



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.005	0.0783	30.47	0.011	0.0000	0.00045	4.25
2	0.131	0.0000	0.165	0.0167	6.48	0.197	0.0035	0.00055	5.23
3	0.413	0.0128	0.477	0.1433	55.80	0.535	0.0128	0.00866	81.67
4	0.797	0.0007	0.831	0.0186	7.24	0.884	0.0014	0.00094	8.84

HPTLC densitometric analysis of Vibhitakadi Eye Ointment at 254 nm (Track 4, 8 µL) showed four peaks (RF 0.005–0.816). Peak 3 (RF ~0.477) was dominant (81.67%), corresponding to phenolic and tannin-rich compounds from Triphala. Minor peaks—Peak 1 (4.25%), Peak 2 (5.23%), and Peak 4 (8.44%)—likely represent polar constituents, flavonoids, and alkaloids from Nimba, Patola, and Vasa. Higher sample volume enhanced detection of secondary constituents without affecting the major peak. This profile confirms the ointment's chemical complexity and supports HPTLC fingerprinting for quality control.

Track 5



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.005	0.1047	31.66	0.011	0.0000	0.00063	4.67
2	0.127	0.0000	0.163	0.0160	4.85	0.192	0.0042	0.00058	4.35
3	0.403	0.0133	0.474	0.1606	48.58	0.539	0.0097	0.01024	76.26
4	0.539	0.0097	0.566	0.0250	7.55	0.587	0.0144	0.00086	6.44
5	0.771	0.0007	0.821	0.0243	7.35	0.856	0.0079	0.00111	8.28

HPTLC analysis of Vibhitakadi Eye Ointment at 254 nm (Track 5, 10 µL) showed five peaks (RF 0.005–0.821). The major peak (RF ~0.466, 76.26%) corresponds to phenolic and tannin-rich Triphala compounds, while minor peaks (RF 0.005–0.821) likely represent flavonoids and alkaloids from Nimba, Patola, and Vasa. Higher sample volume enhanced detection of secondary constituents without shifting the major peak. This profile confirms the ointment's chemical complexity and supports HPTLC fingerprinting for quality control.

g. Microbial Contamination Test

Total Fungal Count Analysis

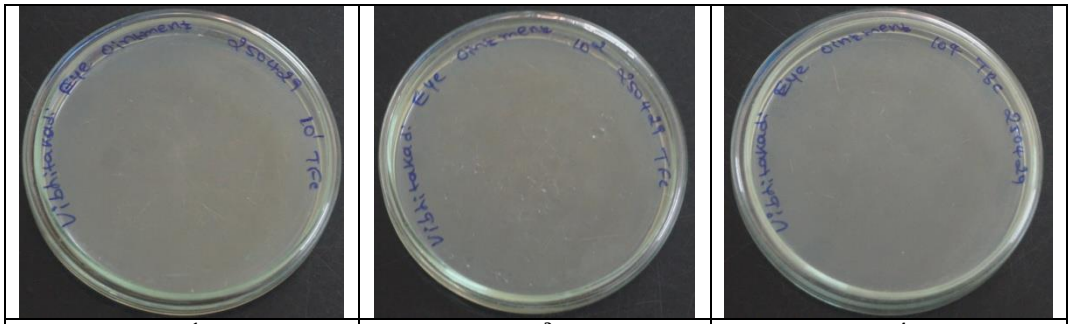
The total fungal count of Vibhitakadi Eye Ointment was evaluated using the serial dilution technique and culture on Sabouraud Dextrose Agar medium. The sample was subjected to dilutions of 10^1, 10^2, and 10^4 to assess the presence of fungal contamination.

The results showed no fungal colony growth in any of the tested dilutions, with the colony-forming units (CFU/ml) recorded as zero throughout.

This outcome indicates that the Vibhitakadi Eye Ointment is microbiologically safe and free from fungal contamination. The absence of fungal growth confirms the formulation's hygienic preparation and supports its suitability for ophthalmic application, contributing to its quality and safety as per standard microbiological requirements.

Table: Total Fungal Count with dilution of Vibhitakadi Eye ointment.

Sl. No.	Dilutions	Number of Colonies (NOC)		CFU/ml
1	1/10(10^1)	0	0	0
2	1/100(10^2)	0	0	0
3	1/10000(10^4)	0	0	0



10^1	10^2	10^4

Figure: Total Fungal Count with dilution of Vibhitakadi Eye ointment.

Total Bacterial Count Analysis

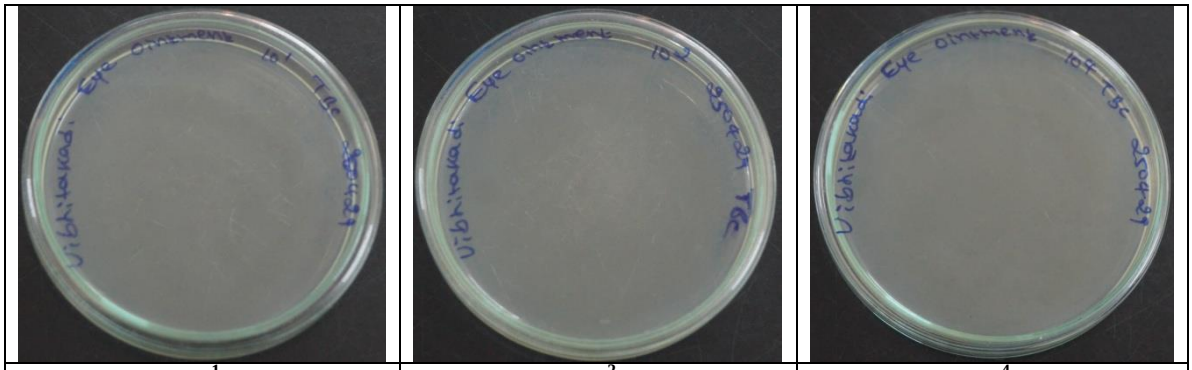
The total bacterial count of Vibhitakadi Eye Ointment was determined using the serial dilution method and culture on Casein Soya Bean Digest Agar Medium (CSDAM), which is commonly employed for the detection of bacterial contamination. The sample was tested at dilutions of 10^1, 10^2, and 10^4 to evaluate the presence of viable bacterial colonies.

The results revealed no bacterial colony growth in any of the plated dilutions, with the colony-forming units (CFU/ml) recorded as zero throughout.

This finding indicates that Vibhitakadi Eye Ointment is free from bacterial contamination and meets the required microbiological quality standards. The absence of microbial colonies reflects appropriate hygienic preparation and supports the formulation's safety and suitability for ophthalmic use.

Table: Total bacterial count with dilution of Vibhitakadi Eye ointment.

Sl. No.	Dilutions	Number of Colonies (NOC)		CFU/ml
1	1/10(10^1)	0	0	0
2	1/100(10^2)	0	0	0
3	1/10000(10^4)	0	0	0



10^1 **10^2** **10^4**

Figure: Total bacterial count with dilution of Vibhitakadi Eye ointment.

Microbial Load Analysis (Direct Method)

The microbial load of Vibhitakadi Eye Ointment was evaluated using the direct plate count method on Casein Soya Bean Digest Agar Medium (CSDAM), a standard medium for the detection of viable bacterial contamination. The sample was directly inoculated without dilution to assess the overall microbial burden.

The results showed no colony growth, with the number of colonies recorded as zero and the microbial count expressed as 0 CFU/ml.

This indicates that Vibhitakadi Eye Ointment is free from microbial contamination. The absence of microbial colonies confirms the microbiological safety, hygienic preparation, and suitability of the formulation for ophthalmic application, supporting its quality and compliance with standard microbial limits

DISCUSSION

Standardization of Ayurvedic ophthalmic formulations is essential to ensure their quality, safety, and therapeutic reliability. *Vibhitakadi Ghrita Yoga*, mentioned in *Yogaratnakara* for the management of *Shushkakshipaka*, was modified into an ointment form using beeswax to improve ocular retention, bioavailability, and patient compliance. The present analytical study was undertaken to establish the physicochemical, chromatographic, and microbiological quality parameters of *Vibhitakadi* eye ointment.

Physicochemical evaluation revealed that the formulation possesses desirable characteristics for ophthalmic use. The ointment showed a neutral pH (7.0), which is ideal for ocular comfort and reduces the risk of irritation. Low moisture content (loss on drying 0.53%) supports better stability and minimizes microbial susceptibility. Good spreadability (11.89 g·cm/sec) indicates uniform application over the ocular surface, enhancing therapeutic acceptability. The refractive index and unsaponifiable matter further confirmed the homogeneity and presence of beneficial lipid constituents.

HPTLC fingerprinting demonstrated a consistent chromatographic profile with a dominant peak around Rf 0.46–0.48, mainly attributed to phenolic and tannin-rich compounds from Triphala. Additional minor peaks at higher sample volumes indicated the presence of secondary phytoconstituents such as flavonoids and alkaloids from Nimba, Patola, and Vasa. The reproducibility of band patterns confirms the chemical consistency of the formulation and supports HPTLC as a useful standardization tool.

Microbiological analysis showed complete absence of bacterial and fungal contamination (0 CFU/ml),

confirming the formulation's microbiological safety for ophthalmic application.

Overall, the findings validate the successful standardization of *Vibhitakadi* eye ointment and support its suitability as a safe and stable Ayurvedic ophthalmic preparation for the management of *Shushkakshipaka* (Digital Eye Strain).

CONCLUSION

The present analytical study successfully established the standardization parameters of Vibhitakadi eye ointment through physicochemical, chromatographic, and microbiological evaluation. The formulation exhibited suitable stability, neutral pH, good spreadability, and a consistent HPTLC fingerprint profile. Microbial testing confirmed the complete absence of bacterial and fungal contamination, ensuring safety for ocular use. Thus, Vibhitakadi eye ointment can be considered a standardized and promising Ayurvedic preparation for the management of *Shushkakshipaka* (Digital Eye Strain.

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