

ORA- Analytical Study

Preliminary Physicochemical, GC-MS Characterization, Dosage and Stability assessment of *Nayanasukha Rasakriya Anjana*: An Exploratory Analytical Study

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ABSTRACT:

Background: Among *Netra kriyakalpas*, *Anjana karma* is one of the targeted therapies with wide variety of formulations indicated for different kinds of ocular pathologies. *Nayanasukha Rasakriya Anjana* is a modified preparation based on the classical *Nayanasukha Varti Anjana*. **Objective:** To report preliminary physicochemical, phytochemical, chromatographic, microbial-limit and accelerated-stability observations, together with exploratory dosage calculations. **Methodology:** A prepared *Nayanasukha Rasakriya Anjana* sample was subjected to the tests of organoleptic characters, pH, specific gravity, phytochemical parameters, dosage calculation, stability, chemical profiling and microbial analysis. **Results:** Reported measurements included pH 6.05, specific gravity 1.33, viscosity 45.65 cSt, refractive index 1.453, total phenols 0.13 mg/g and terpenoids 1.04 mg/g. TAMC and TYMC were each reported below 10 CFU/mL. GC-MS chromatogram revealed twenty-one peaks corresponding to various organic compounds. A 60-day accelerated-storage comparison was reported. **Conclusion:** The findings constitute preliminary analytical observations of *NRA*. Replication, validated sterility assurance, confirmatory chemical identification, real-time stability and ocular safety investigations are required before ophthalmic clinical use can be considered.

KEYWORDS: *Anjana*, Dosage, GC-MS, *Nayanasukha Rasakriya Anjana (NRA)*, Physicochemical Analysis.

RECEIVED ON:
06-09-2026

REVISED ON:
15-10-2026

ACCEPTED ON:
22-10-2026

Access This Article Online:

Quick Response Code:



Website Link:

<https://jahm.co.in>

DOI Link:

<https://doi.org/10.70066/jahm.v14i9.3043>

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CITE THIS ARTICLE AS

Nithin V, Veerayya R Hiremat, Shashikala Kadabadi, Anita Kulkarni, Gururaj Natikar. Preliminary Physicochemical, GC-MS Characterization, Dosage and Stability assessment of *Nayanasukha Rasakriya Anjana*: An Exploratory Analytical Study. Journal of Ayurveda and Holistic Medicine (JAHM) 2026; 14(9):57-67.

1. INTRODUCTION

Netra Kriyakalpas are the localized therapeutic procedures designed to deliver active phytoconstituents directly to the ocular structures. Among them *Anjana karma* (collyrium) [1] is a targeted therapy with wide variety of formulations indicated for various ocular pathologies. This procedure involves applying medicinal pastes, liquids or powders to inner margin of lower lid from the *kaneenika sandhi* (inner canthus) to the *apanga sandhi* (outer canthus) or vice versa with *anjana shalaka* (applicator). [2]

Anjanas are divided into 3 types according to their forms of preparation. They are: *Gutika anjana* (pills), *Rasakriya anjana* (thick liquid), *Churna anjana* (powder). [3] *Nayanasukha varti anjana* (elongated pills) a classical formulation mentioned in *Bhaishajya Ratnavali* has classical indications in *Timira* (refractive errors), *Kacha* (vision impairments), *Arma* (pterygium) and *Asrupata* (lacrimal pathologies). It is prepared by triturating 1 part- *pippali* and 2 parts – *haritaki* with water and rolled into form of pills. [4] As the *anjana* has minimum ingredients with both *Lekhana* (scraping) and *Ropana* (healing) properties it becomes one of the ideal *anjana* to be used in post-operative care of *Arma Chedana* (pterygium excision) to prevent the recurrence of *arma*. While *Nayanasukha Varti* is effective but rubbing a solid *Varti* prior to each use results in variable dosing and risks microbial contamination in a surgically compromised post-*Arma Chedana* ocular surface, creating a critical need to reformulate it into a sterile drop-wise installable *Rasakriya*. Thus the form of *Nayanasukha varti* is modified to *Nayanasukha rasakriya anjana* (hereafter referred to as *NRA*) which shall be instilled as drops due to its liquid consistency.

It is important to ensure safety and quality of the drug when administered topically to the eye. For this novelty, evaluation of organoleptic characters, pH, specific gravity, phytochemical parameters, dosage calculation, stability, chemical profiling and microbial analysis is necessary. [5] Thus, this exploratory study reports the preparation procedure and preliminary analytical observations; it does

not assess recurrence prevention, ocular tolerability or therapeutic efficacy. Its proposed relevance to recurrence of *Arma* after *Arma Chedana* remains a future clinical research question.

2. MATERIALS AND METHODS:

Drug authentication and Preparation: The raw drugs (Table 1), including the honey, were procured from standard raw drug suppliers. They were authenticated by the botanist of the Quality Check Department of the GMP-certified Pharmacy K.S. Varier's Astanga Ayurvedics (P) Ltd., Trichy, Tamil Nadu, with GMP Certificate No. U24233TN1999PTC041899. Quality specifications were verified prior to formulation (QCD Certificate – KSVAA/QCD/2026/026-028). Preparation was carried out in the same pharmacy by following the standard technique mentioned in the Ayurvedic Formulary of India [6] and as per the reference of *Bhaishajya Ratnavali: Netrarogadikara*.

Table 1: Details of ingredients in NRA

Sl. No.	Drug	Botanical Name	Parts used	Ratio
1.	<i>Pippali</i>	<i>Piper longum</i> L.	Fruit	1 part
2.	<i>Haritaki</i>	<i>Terminalia chebula</i> Retz.	Fruit	2 part

Preparation of NRA: 50 gms of *Pippali* and 100 gms of *Haritaki* were cleaned, dried and coarsely powdered using a mechanical pulveriser. 1500 mL of water was added, boiled over a mild fire with continuous stirring until reduced to one-fourth of the initial volume (375 mL). A reported filtration loss of 25 mL yielded 350 mL decoction. After allowing the decoction to cool to room temperature, 260 mL of filtered *Madhu* (honey) (approximately ¾th part of the obtained *Kashaya*) was added. The mixture was triturated homogeneously to achieve a semi-liquid *Rasakriya* consistency. The theoretical combined volume was 610 mL and the reported final volume 600 mL after a further 10 mL processing loss (total volumetric yield 98.36%). The finished NRA was transferred into airtight 10 mL ophthalmic dropper bottles under controlled aseptic conditions of the pharmacy. Complete process log,

manufacturing environment, sterilisation/aseptic validation and container-closure integrity were supervised and certified by Quality check department chemist of the same pharmacy.

Table 2: Rasapanchaka of NRA

<i>Rasa</i>	<i>Kashaya, Katu, Madhura, Amla, Tikta</i>
<i>Guna</i>	<i>Laghu, Ruksha, Tikshna</i>
<i>Veerya</i>	<i>Ushna</i>
<i>Vipaka</i>	<i>Madhura</i>
<i>Karma</i>	<i>Lekhana, Rasayana</i>
<i>Doshaghnata</i>	<i>Tridoshashamaka</i>

Method of Analysis of NRA: In the current study, Analytical parameters including organoleptic characters, uniformity of content, volatile matter, refractive index, viscosity, total acid value, GC-MS, [7-8] Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC), Phytochemical quantitative analysis and stability study [9] were carried out at Amrith Labs, NABL Accredited and AYUSH Approved lab, Shimogga. Measurement of specific gravity and pH were analysed at Avishkar Central Research laboratory, SJGAMC, Koppal. Reported values are validated by Analytical scientist and Lab Analyst of the respective laboratories.

All the analytical tests were conducted in accordance with the methodologies prescribed by the Central Council for Research in Ayurvedic Sciences, Ayurvedic Pharmacopoeia of India (API) [8] and WHO guidelines. [9] But specific numerical acceptance limits for *Nayanasukha* in its modified *Rasakriya* form do not currently exist in the pharmacopoeia. Therefore, the physicochemical and phytochemical values reported in this study establish the preliminary baseline standardization parameters.

GC-MS Instrumentation and Methodology: The analysis was conducted using a Shimadzu GC–MS QP 2010 SE instrument with helium as the carrier gas (total flow 34.5 mL/min, column flow 1.50 mL/min). The injection mode

was set to split (ratio 20.0) with an injection temperature of 280.0°C. The column oven temperature program was initiated at 80.0°C (held for 4.00 min), ramped at 15.0°C/min to 160.0°C (held for 15.00 min), and finally ramped at 15.0°C/min to 280.0°C (held for 15.00 min). The mass spectrometer was operated with an ion source temperature of 200.0°C, an interface temperature of 300.0°C, and a mass scan range of m/z 35.00 to 500.00 (scan speed 1666). The analysis was performed as a single replicate injection.

3. RESULTS

The *NRA* sample has been baseline standardized as per standard testing protocol. The results of baseline parameters are given in respective [tables 3-5](#).

Table 3: Results of baseline parameters of NRA

Parameters	Results
Colour	Dark brownish
Odour	Slightly pungent
Texture	Dense oily
Uniformity of Content	Uniform
Loss on drying at 105°C	30.89 %
Rancidity	Not Rancid
Refractive Index	1.453
Viscosity	45.65 cSt
Acid Value	7.58
pH Value	6.05
Specific gravity	1.33
TAMC	Less than 10 CFU/mL
TYMC	Less than 10 CFU/mL

Table 4: Quantitative Phytochemical Analysis of NRA

Sl. No.	Parameter	Unit	Results
1	Total Alkaloids	mg/g	Absent
2	Total Flavonoids	mg/g	Absent
3	Tannin	mg/g	Absent
4	Total Phenol	mg/g	0.13
5	Saponins	mg/g	Absent
6	Terpenoids	mg/g	1.04

Table 5: 60-day accelerated-storage comparison; reference stored at –20°C and accelerated sample at 40°C ± 2 °C and 75% ± 5% RH

Sl.No.	Parameters	Reference Sample (- 20°C)	Accelerated Sample (40/75)
1.	Colour	Dark brownish	Dark brownish
2.	Odour	Slightly pungent	Slightly pungent
3.	Texture	Dense oily	Dense oily
4.	Uniformity of Content	Uniform	Uniform
5.	Loss on drying @ 105°C	30.32 %	30.57 %
6.	Rancidity	Not Rancid	Not Rancid
7.	Refractive Index	1.453	1.454
8.	Viscosity	46.78 cSt	45.31 cSt
9.	Acid Value	7.02	7.88
10.	TAMC	Less than 10 CFU/ml	Less than 10 CFU/ml
11.	TYMC	Less than 10 CFU/ml	Less than 10 CFU/ml

GC-MS:

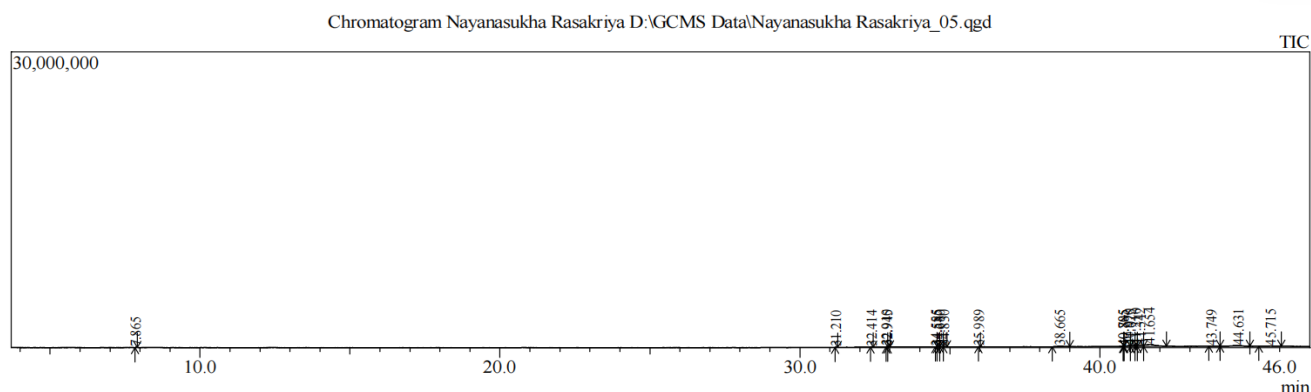


Figure 1: GC-MS chromatogram of NRA

Table 6: Phytochemical compounds identified in the chloroform extract of NRA by GC–MS

Peak	R. Time (min)	Identified Compound Name	Molecular Formula	Peak Area	Area (%)	Similarity Index (SI)	Retention Index (RI)	Spectral Library
1	7.865	1-Hexanol, 2-ethyl-	C ₈ H ₁₈ O	76,804	0.59	96	995	NIST20R.lib
2	31.21	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	100,047	0.77	96	1878	NIST20R.lib
3	32.414	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	71,075	0.55	90	2085	NIST20R.lib
4	32.91	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	88,165	0.68	81	1717	NIST20M1.lib
5	32.945	10-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	151,355	1.17	87	2085	NIST20M1.lib
6	34.555	Cyclopenta[d][1,3]dioxin-4-one, perhydro-4a-methyl-	C ₈ H ₁₂ O ₃	41,281	0.32	44	2004	NIST20M1.lib
7	34.585	(±)-α-Tocopherol acetate	C ₃₁ H ₅₂ O ₃	112,203	0.86	43	3308	NIST20R.lib
8	34.68	Eicosanoic acid, 2-hydroxyethyl ester	C ₂₂ H ₄₄ O ₃	112,302	0.86	39	2618	NIST20M1.lib
9	34.83	α-Tocopheryl acetate	C ₃₁ H ₅₂ O ₃	100,518	0.77	84	3308	NIST20R.lib
10	35.989	Lupeol	C ₃₀ H ₅₀ O	81,323	0.63	58	2848	NIST20R.lib
11	38.665	24-Norursa-3,12-diene	C ₂₉ H ₄₆	767,733	5.91	91	2622	NIST20M1.lib
12	40.785	Cyclohexanecarboxylic acid, 4-methyl-,	C ₉ H ₁₆ O ₂	32,981	0.25	36	1108	NIST20M1.lib

methyl ester									
13	40.805	Androstan-11-one, 3-hydroxy-, (3 β ,5 α)-	C ₁₉ H ₃₀ O ₂	15,913	0.12	45	2128	NIST20M1.lib	
14	40.97	Succinic acid, di(dodec-9-yn-1-yl) ester	C ₂₈ H ₄₆ O ₄	809,609	6.24	68	3174	NIST20M2.lib	
15	41.025	Glutaric acid, dec-2-yl dec-4-enyl ester	C ₂₅ H ₄₆ O ₄	885,201	6.82	44	2785	NIST20M2.lib	
16	41.21	Tricyclo[4.2.2.0(1,5)]decan-2-ol	C ₁₀ H ₁₆ O	584,628	4.5	61	1081	NIST20M1.lib	
17	41.342	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	C ₃₀ H ₅₂ O ₂	1,540,138	11.86	73	3350	NIST20M2.lib	
18	41.654	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	C ₃₀ H ₅₂ O ₂	4,567,524	35.18	91	3350	NIST20M2.lib	
19	43.749	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	C ₃₀ H ₅₂ O ₂	252,855	1.95	74	3350	NIST20M2.lib	
20	44.631	($\pm$)- α -Tocopherol acetate	C ₃₁ H ₅₂ O ₃	2,065,231	15.91	93	3308	NIST20R.lib	
21	45.715	Tridecanedial	C ₁₃ H ₂₄ O ₂	527,055	4.06	82	1690	NIST20M1.lib	
Total				12,983,941	100				

The GC–MS chromatogram revealed 21 peaks corresponding to various organic compounds with retention times ranging from 7.865 to 45.715 minutes, with a total peak area of approximately 12.98 million (12,983,941), indicating the total constituents detected in the sample. The major compound identified was Tricyclo[20.8.0.0(7,16)] triacontane derivatives detected at three different retention times of 41.342, 41.654, 43.749 minutes, contributing 11.86%, 35.18% and 1.95% of the peak area respectively, with the combined peak area of 48.99%. This was followed by α -Tocopherol acetate at two different retention times of 34.585 and 44.631 minutes with 0.86% and 15.91% peak area respectively. Glutaric acid dec-2-yl dec-4-enyl ester was detected at 41.025 minutes with a 6.82% peak area. Other notable compounds identified include Succinic acid di(dodec-9-yn-1-yl) ester, 24-Norursa-3,12-diene, Tricyclo[4.2.2.0(1,5)]decan-2-ol, Tridecanedial with >4% peak area. Minor constituents present in trace amounts (<2%) included 9, 10-Octadecenoic acid; methyl ester; Eicosanoic acid; 2-hydroxyethyl ester; alpha-Tocopheryl acetate; Hexadecanoic acid; 1,8,11-Heptadecatriene, (Z,Z); 1-Hexanol; Cyclopenta[d][1,3]dioxin-4-one; Lupeol;

Cyclohexanecarboxylic acid and Androstan-11-one, 3-hydroxy.

It is important to note that the reported Area (%) values represent relative peak area percentages within the specific chromatographic run. Without appropriate calibration curves using internal standards, these values reflect qualitative proportions rather than absolute chemical concentrations.

4. DISCUSSION

NRA is a widely used *lekhana anjana* for the management of *Arma* in the clinical practice. (Table 1) When *Arma* encroaches the cornea, it is indicated for *chedana* (excision). Post-excision *Lekhana anjana* is indicated to scrape off the residual 1/3rd of *arma*, [10] which also helps to prevent recurrence of *Arma*. *Varti / Gutika anjanas* should be rubbed with water or any liquid base and should be applied with *shalaka* or fingertip, which holds a major risk of cross contamination and infection. Hence with the same ingredients the *anjana* is converted into *rasakriya* form which helps the patient to apply in the hygienic method of drops due to its liquid consistency. Table 2 describes the traditional *Rasapanchaka* attributes.

According to Acharya Sushruta, [3] Sarangadhara [11] and Bhavaprakasha, [12] *Gutika anjana* is said to be superior to

Rasakriya anjana and *Rasakriya anjana* is more potent than *Churna anjana*. This statement needs analytical evaluation; hence such analytical studies are essential to ensure quality, safety and efficacy of *anjanas*. Therefore, the present study was undertaken to establish analytical reference parameters for *NRA* based on classical principles of *rasakriya kalpana* along with contemporary analytical techniques. In the present study, *NRA* was prepared according to *Rasakriya* preparation API Guidelines in which honey is used as base, which facilitates dosage fixation, improved stability and acts as natural preservative to improve shelf-life.

Previous studies of *NRA* converted to ophthalmic eye ointment had reported their analytical efficiency and utility of the same in clinical studies. [13] However, comprehensive analytical profiling of *anjana* is still limited. Therefore, the aim of the present study is to evaluate the physicochemical parameters and chromatographic profile of *NRA*.

The organoleptic characters analysis suggests that the formulation obtained was obeying all the standards as per API and the homogeneity is proven by uniformity of contents. Absence of Rancidity and growth of less than 10 CFU/mL in TYMC and TAMC, suggests that the samples possess microbiological purity and comply with standard microbial limits. (Table 3)



Figure 2: Measurement of NRA pH using a digital pH meter.

The pH of *NRA* is 6.05, which falls within the mildly acidic range. (Figure 2) This is due to the acidic nature of honey (3.4 to 4.5) which is neutralized during the *rasakriya* preparation. However the pH of eye drops above 4 does not affect the structural integrity of ocular surface. Many eye

drops ranging pH from 4 to 8 are used routinely in ophthalmic practice. [14]

The viscosity of *NRA* was found to be 45.65 cSt, and its specific gravity was 1.33 (Table 3). In ophthalmic drug delivery, a viscosity of 45.65 cSt (substantially higher than aqueous tears) prolongs residence time on tear film, reduces rapid nasolacrimal drainage and lubricating film over the ocular surface. Similarly, a specific gravity of 1.33 reflects the dense, dissolved solute and honey matrix of the *Rasakriya*, facilitating retention in the conjunctiva fornix.

The refractive index of the formulation is 1.453, which resembles the standard refractive index of the honey. This infers that the preparation matches the standard refractive index of oily/ lipid based eye drops ranging from 1.44 to 1.50 and also confirms the purity and authenticity of the honey used in the preparation. The Loss on Drying (moisture/volatile matter) in the formulation is 30.89% typically represents the evaporation of water (moisture) and any other volatile substances present in the preparation. (Table 3)

Accelerated Stability Assessment: Accelerated stability testing was conducted for 60 days at 40°C ± 2°C / 75% RH ± 5% in accordance with ICH Q1A (R2) guidelines and CCRAS stability protocols to rapidly assess formulation integrity under thermal and humidity stress for 60 days. Negligible variations were noted in viscosity (46.78 cSt vs. 45.31 cSt), refractive index (1.453 vs. 1.454), loss on drying (30.32% vs. 30.57%), and acid value (7.02 vs. 7.88), while microbial counts remained < 10 CFU/mL. (Table 5) Based on Arrhenius equation for Climatic Zone IV (where 1 month of accelerated stability at 40°C ± 2/ 75% RH ± 5% corresponds to approximately 3.33 to 4 months at ambient room temperature) [15], 60 days of stability under accelerated conditions confirms maintained physicochemical stability of approximately 6.6 to 8 months at room temperature.

Dosage calculation of *NRA*: As per *Acharya Sushruta* and the commentary of *Dalhana*, the classical dosage of *Rasakriya Anjana* is 1 *Harenu Matra* for *Lekhana Anjana*, 1 ½ *Harenu Matra* for *Prasadana Anjana*, and 2 *Harenu*

Matra for Ropana Anjana. [16] One Harenu (equivalent to the size of a green pea) corresponds to an average mass of 250 mg. Using the experimentally determined specific gravity of NRA (1.33), (Table 3) the mass-to-volume conversion was calculated as follows:

Density of NRA = Specific gravity X Density of Water = 1.33 X 1000 mg/mL = 1,330 mg/mL

Volume (mL) = mass (mg) / Density (mg/mL)

Table 7: Topical ophthalmic dosage calculation of NRA according to therapeutic action

Action	Classical dosage	Mass Equivalent	Dosage in mL (Total Daily Dose)	Nominal drop equivalent (1ml = 14-16 drops)
Lekhana	1 Harenu	250 mg	0.19 mL	2-3 drops per day
Prasadana	1 ½ Harenu	375 mg	0.28 mL	4-5 drops per day
Ropana	2 Harenu	500 mg	0.38 mL	6-7 drops per day

Thus NRA usage in post-operative care of *arma chedana* should be used based on the desired action accordingly.

Phytochemical Analysis: (Table 4)

- Total Phenols: a concentration of 0.13 mg/g primarily acts as a topical antioxidant and anti-inflammatory shield. This helps counteract oxidative stress and modulate pathways involved in ocular pathologies, assisting the conventional therapeutic strategies. [17]
- Terpenoids: A concentration of 1.04 mg/g of Terpenoids protects and heals eye tissues by reducing oxidative stress, controlling inflammation, and preventing abnormal cell death. [18]

The absence of detectable alkaloids, flavonoids, tannins and saponins in the final formulation—despite *Terminalia chebula* being rich in hydrolysable tannins and *Piper longum* containing piperidine alkaloids—is hypothesized to result from these pharmaceutical factors: (a) Solvent polarity: Water-only extraction (*Kashaya*) poorly extracts lipophilic alkaloids such as piperine; (b) Thermal and matrix precipitation: Prolonged boiling during *Kashaya* reduction (10:1 reduced to 1/4th) promotes thermal hydrolysis of gallotannins into simpler phenolic acids, while high-molecular-weight tannins complex with honey proteins and precipitate out during filtration. However, these

Therefore the Dosage of NRA (mL) = 250 mg / 1330 mg/mL = 0.1879 mL

Due to its higher viscosity when administered in the form of drops, 1 mL yields up to 14–16 drops when dispensed manually. The drop volume varies depending on the nozzle opening of the dropper. In this study, utilizing a standard nozzle opening of 0.2 mm to 0.45 mm, the average drop volume was determined to be 0.06 mL ± 0.01

degradation and precipitation pathways require future experimental validation.

Detailed Interpretation of Chemical Profile of GCMS analysis:

a. Tricyclo[20.8.0.0(7,16)]triacontane derivatives (48.99%)

Three peaks assigned to this structure account for nearly 49% of the chromatogram.

Possible significance:

- High molecular weight triterpenoid/steroid-like constituents
- Strong lipophilic nature
- May contribute to formulation viscosity
- Often associated with protective and membrane-stabilizing properties [19]

b. α-Tocopherol acetate (Vitamin E acetate)

Detected twice (0.86% and 15.91%) accounting for a relative peak area of 16.8%. Biological significance

- Potent antioxidant [20]
- Protects lipids from oxidation
- Stabilizes oils
- Helps preserve herbal formulations
- Contribute to anti-inflammatory activity

This is one of the most pharmacologically relevant constituents detected.

c. Long-chain Esters

Detected esters include

- Glutaric acid ester (6.82%)
- Succinic acid ester (6.24%)
- Hydroxyethyl eicosanoate (0.86%)

These compounds are generally associated with

- Lipophilic character
- Improved formulation stability
- Controlled release
- Lubricating properties

They likely arise from the lipid matrix or during processing.

d. 24-Norursa-3,12-diene (5.91%)

This is an ursane-type triterpenoid. Possible activities include

- Anti-inflammatory
- Antioxidant
- Wound healing
- Antimicrobial

Its presence supports the traditional therapeutic value of Rasakriya preparations.

e. Tridecanedial (4.06%)

Long-chain aldehydes are reported to possess

- Antimicrobial activity
- Antifungal activity
- Aroma contribution
- Oxidative intermediates

Its moderate abundance suggests it contributes to the formulation's overall chemical profile.

f. Fatty Acid Methyl Esters (FAMES)

Detected compounds include

- Hexadecanoic acid methyl ester (Palmitic acid methyl ester)
- Oleic acid methyl ester
- Octadecenoic acid methyl ester

These indicate the presence of saturated and unsaturated fatty acids.

Biological significance

Palmitic acid methyl ester

- Emollient
- Membrane component
- Mild antimicrobial activity

Oleic acid methyl ester

- Skin penetration enhancer
- Anti-inflammatory
- Improves absorption of herbal constituents

Unsaturated fatty acid esters

- Increase formulation stability
- Improve bioavailability
- Enhance lipid transport

g. Lupeol (0.63%)

Lupeol is a well-known pentacyclic triterpene. Reported pharmacological activities include

- Anti-inflammatory [21]
- Anti-cancer
- Antioxidant
- Anti-arthritic
- Wound healing
- Antimicrobial

Although present in low abundance, it is considered a biologically important phytochemical.

Table 8: Phytochemical Classification

Phytochemical Class	Relative Area (%)	Peak	Tentatively Identified Contributing Compounds (Peak Numbers)	Approximate abundance
Triterpenoids & Complex Compounds	60.15%	Cyclic	Tricyclo[20.8.0.0(7,16)]triacontane derivatives (17, 18, 19), 24-Norursa-3,12-diene (11), Tricyclo[4.2.2.0(1,5)]decan-2-ol (16), Lupeol (10), Androstan-11-one derivative (13)	Very High
Vitamin E derivatives	17.54%		α-Tocopherol acetate / α-Tocopheryl acetate (7, 9, 20)	High
Long-chain esters	13.92%		Glutaric acid dec-2-yl dec-4-enyl ester (15), Succinic acid di(dodec-9-yn-1-yl) ester (14), Eicosanoic acid 2-hydroxyethyl ester (8)	Moderate

Long-chain aldehydes	4.06%	Tridecanedial (21)	Moderate
Fatty acid esters (FAMES)	2.74%	Hexadecanoic acid methyl ester (2), 9-Octadecenoic acid methyl ester (3), 10-Octadecenoic acid methyl ester (5), Cyclohexanecarboxylic acid methyl ester (12)	Moderate
Fatty alcohols	0.59%	1-Hexanol, 2-ethyl- (1)	Low
Other (Alkenes/Misc)	1.00%	1,8,11-Heptadecatriene (4), Cyclopenta[d][1,3]dioxin-4-one derivative (6)	Low
Total	100%		

Pharmacological Interpretation: The GC–MS profile suggests that *NRA* contains compounds associated with:

- Strong antioxidant potential (α -tocopherol acetate) [20]
- Anti-inflammatory activity (lupeol, triterpenoids, oleic acid derivatives) [19]
- Membrane-protective effects (Vitamin E derivatives)
- Wound-healing properties (lupeol, triterpenoids) [21]
- Antimicrobial activity (fatty acids, aldehydes)
- Enhanced stability through lipid-rich constituents

Thus from the above interpretation, *NRA* will yield *Lekhana*, *prasadana* and *ropana* properties accordingly.

Limitations and future scope:

- Single-batch evaluation without analytical replicates ($n \geq 3$) limits statistical robustness.
- Sterility tested general limits (TAMC/TYMC) only; mandatory pathogen-specific screening (*P. aeruginosa*, *S. aureus*) was not conducted.
- GC-MS Analysis, absence of procedural/solvent blanks and replicate injections means trace analytical artefacts cannot be definitively excluded.
- Phytochemistry assays lacked full validation metrics (calibration curves, reference standards, LOD/LOQ); hypothesized degradation mechanisms require experimental confirmation.

5. CONCLUSION

The present analytical study establishes the baseline physicochemical, quantitative phytochemical, chromatographic, dosage, and stability standards for *Nayanasukha Rasakriya Anjana (NRA)*. The physicochemical parameters demonstrate that *NRA* possesses an ocular-

compatible pH (6.05), optimal viscosity (45.65 cSt), specific gravity (1.33), refractive index (1.453), microbial counts below the reported detection threshold (<10 CFU/mL), and 60-day accelerated stability corresponding to an estimated 8-month maintained physicochemical stability at room temperature, enabling safe and reproducible drop-wise ophthalmic dosing (0.19 mL - 0.38 mL). Furthermore, quantitative phytochemical analysis confirmed the presence of terpenoids (1.04 mg/g), phenols (0.13 mg/g). The GC–MS characterization showed 21 lipophilic compounds—most notably Tricyclo[20.8.0.0(7,16)]triacontane derivatives, α -tocopherol acetate, 24-norursa-3,12-diene, lupeol, tridecanedial, and fatty acid esters. While the physicochemical quality and stability of *NRA* are experimentally validated by this study, the inferred antioxidant, wound healing, anti-inflammatory, *Lekhana*, *Prasadana* and *Ropana* therapeutic activities suggested by its phytochemical constituents require confirmation through controlled preclinical and clinical trials in post-operative *Arma Chedana* patients.

Abbreviations:

NRA- *Nayanasukha Rasakriya Anjana*

GC–MS- Gas Chromatography- Mass Spectrometry

pH- potential of Hydrogen

GMP- Good Manufacturing Practice

TAMC- Total Aerobic Microbial Count

TYMC- Total Yeast and Mold Count

μ L- microliter

mL- milliliter

cSt- centistokes

CFU/mL- Colony Forming Units per milliliter

mg/g- milligrams per gram

mg/mL – milligrams per milliliters

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Acknowledgements – I extend my sincere gratitude to Mr. Abhinav and the team at Amrith Lab (an NABL Accredited and AYUSH Approved facility in Shimoga), alongside the staff at Avishkar Central Research Laboratory (Shree Jagadguru Gavisiddeshwara Ayurvedic Medical College and Hospital, Koppal), for their invaluable support in furnishing the analytical report. Furthermore, I am deeply thankful to Mr. Senthil Kumar and the team at the GMP-certified K.S. Varier's Astanga Ayurvedics (P) Ltd. in Trichy, Tamil Nadu, for preparing the Nayanasukha rasakriya anjana.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement: This study did not involve human participants or experimental animals; therefore, Institutional Ethics Committee approval and informed consent were not applicable.

Declaration of Generative AI: The authors declare this manuscript was written without the use of generative artificial intelligence tools. All the content, including text generation, data analysis and references was developed and reviewed by the author without assistance from AI technologies.

Conflict of Interest – The authors declare no conflicts of interest.

Source of Support – The authors declare no source of support.

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