WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 7, 829-836. Review Article ISSN 2277– 7105

STANDARD MANUFACTURING PROCEDURE AND PHYSICO-CHEMICAL EVALUATION OF *PUNARNAVADI* EYE DROPS - AN AYURVEDIC EYE DROP FORMULATION

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Article Received on
02 April 2019,

Revised on 24 April 2019,
Accepted on 15 May 2019

DOI: 10.20959/wjpr20197-15098

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ABSTRACT

Punarnavadi Eye Drops (PED) - a clinically experienced formulation was selected for evaluating its efficacy in *Vataja Abhishyanda* (Allergic Conjunctivitis) containing *Punarnava Yavakuta churna, Mishri, Sphatika* and *Saindhava* were used to prepare the formulation. The present paper highlights preparation of eye drops, physico-chemical study of the different parameters and HPTLC of *Punarnavadi* eye drops.

KEYWORDS: HPTLC**,** *Punarnavadi* Eye Drops, *Vataja Abhishyanda.*

INTRODUCTION

Ashchyotana is the method of instilling liquid medicine drop by drop in opened eye at inner canthus (*kaninaka sandhi*) from two *angula* of height.[1] *Ashchyotana* is indicated in *Aamavastha* of *Netraroga* (acute inflammatory condition of eye) that is presented with pain, foreign body sensation, burning sensation, redness, itching, discharge, lacrimation, and swelling.[2] In this study, an experience based formulation, *Punarnavadi Eye Drops* is selected to evaluate its therapeutic efficacy in *Vataja Abhishyanda* (Allergic Conjunctivitis) containing *Punarnava Yavakuta Churna, Mishri, Sphatika* and *Saindhava* used to prepare the formulation. Experience based formulation named *Netra Bindu – Fullika Drava* has been chosen for its modification as *Punarnavadi* Eye Drops. In the book of Unjha Pharmacy named *Bhaishajya Sara Samgraha*[3], *Netra Bindu* is said therapeutically effective in *Netra raktata* (Congestion), *Paka* (Inflammation), *Ushnata* (hot sensation) etc. These are the

complaints of the disease *Abhishyanda*. To evaluate its efficacy in *Vataja Abhishyanda* the formulation was selected. The name *Punarnavadi* Eye Drops was given as the *Punarnava* is contributing as main drug.

Table. 1: Contents of *Punarnavadi* eye drops(*Anubhoot Yoga*).

No.o.	Drug Drug	Botanical Name Name	Part used	Proportion
1	*Punarnava*	*Boerhavia diffusa* Linn.	Root	1 Part
2	*Mishri*	Sugar	-	1/2 Part
3	*Sphatika*	Alum	-	1/2 Part
4	*Saindhava*	*Sodii chloridum*	-	1/2 Part

MATERIAL AND METHODS

Collection of the Drug: The *Yavakuta* was prepared at the Pharmacy of IPGT and RA, G.A.U, Jamnagar. Other drugs were purchased from local market and authenticated in the dept. of *Rasa shashtra* and *Bhaishajya kalpana*, IPGT and RA, G.A.U, Jamnagar. Distillation procedure was done at department of *Shalakya Tantra* of the institute. Sterile packing was also done in Surendranagar.

Preparation of the eye Drop (Plate 1)

The procedure was performed in following steps

1. Soaking: The preparation area was sterilized with formalin before 24 hours of starting the preparation. Quantity sufficient amount of coarse powder of *Punarnava* was soaked in RO water for 12 hours.

2. Distillation: Distillation process was started with simple distillation process. 50 ml of the beginning of first distillation is discarded. Total 1780 ml of distillation was obtained at the end of distillation process. From it 100 ml distillation was separated for analytical purpose in sterile conical flask capped with a plug.

3. Addition of Powders: Fine powders of *Mishri*, *Sphatika* and *Saindhava* were added to the distillate and kept for 12 hours to dissolve in UV sterilised glass bottles.

5. Filtration: The contents were filtered with the help of filter paper and glass funnel and stored in glass bottles.

6. Preservation: Then the distillation is shifted into conical flask and preservative (benzalkonium chloride) was added and thoroughly mixed. The final product was filled in the dropper bottles (each of 10 ml) and stored in sterile aseptic conditions.

7. Packing and Storage: The bottles were stored in refrigerated condition till use. Three batches were prepared following same procedure **(Table 2 and Table 3)**.

Table. 2: Preparation of *Punarnavadi* Eye Drops.

Batch	Punarnava root	Quantity of water taken for first distillation	Discarded distillate in the beginning of first distillation	Quantity of distillate
1.	150gm	1500 ml	50 ml	500 ml
2	150gm	1500 ml	50 ml	600 ml
3.	150gm	1500 ml	50 ml	680 ml

Table. 3: Preparation of *Punarnavadi* Eye Drops(continue).

Batch	Mishri powder	Sphatika Powder	Saindhava powder	Distillate after Adding powders	distillate after filteration
1.	12.5gm	12.5gm	12.5gm	520ml	470ml
2.	15gm	15gm	15gm	620ml	540ml
3.	17gm	17gm	17gm	700ml	630ml

Organoleptic Parameters: The organoleptic character of *Ayurvedic* drugs are very important and give the general idea regarding the genuineness of the sample. Besides quality control measures *Rupa* (colour), *Rasa* (taste), *Gandha* (odour) and *Sparsha* (texture) pertaining *to Panchajnanendriya Pariksha* are noted **(Table 4)**. These primary subtle parameters are important, the affirmation of which generates confidence in patient as well as in the physician.

Table. 4: Organoleptic characteristics of all the ingredients and final product.

Material	Color	Touch	Odor	Taste
Punarnava root (Boerhavia diffusa Linn.)	Brownish	Coarse	Characteristic	Bitter
Mishri (Saccharum officinarum Linn.)	White	Smooth	Characteristic	Sweet
Sphatika (Alum)	Greyish white	Smooth	None	Astringent, Sour
Saindhava (Sodii cloridum)	White	Smooth	Characteristic	Salty
Final product (PED)	transparent with slight whitish	Liquid	Characteristic	Salty-Astringent

Physico - chemical and HPTLC Study

The prepared *Arka* (drops) was analyzed for the physico-chemical parameters i.e. Specific gravity[4], Refractive index, pH and Total solid as well as HPTLC were carried out for ee drop as per pharmacopoeial standards.

(i) Physico-chemical Analysis

The samples were analyzed for the parameters like refractive index, specific gravity, pH and total solid.

(a) Specific gravity[5]

Definition: Specific gravity of a liquid is the weight of a given volume of the liquid at 25º (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air.

Significance: The presence of dissolved substances in *Arka* is expected to change its specific gravity. So it is considered to be an important parameter for analyzing.

Method: A clean and dry 25 ml capacity Pycnometer was taken and its weight was noted. It was filled with the sample, cleaned properly from outside and the weight was taken at 40°C. Then it was cleaned, rinsed and filled with distilled water, dried from outside and the weight was noted at 40°C. The weight of sample and distilled water was calculated. Then the Specific gravity was determined by dividing the weight of the sample by the weight of the water. The Specific gravity of the samples are shown in **Table 5**.

(b) Refractive Index[6]

Definition: The refractive index of a substance is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.

Significance: The consistency of the media and solutes present in the media brings the difference in the refractive index. So, it is an important parameter for differentiating the *arka*.

Method: Refractive index of a substance varies with temperature. Hence, temperature is to be noted while determining R.I. The R.I. of different samples was measured in Abbe's Refracto-meter at 40°C. The temperature was maintained at 40°C by circulating warm water. The data of the samples are shown in **Table 5**.

(c) Determination of pH value[7]: This test is carried out to determine the pH of the test drug with the help of pH meter (**Table 5)**.

(d) Total solid[8,9]

Procedure: 25 ml of the *PED* were taken in previously dried and weighed evaporating dish and was heated on hot water bath. After complete evaporation of the liquid, it was kept in hot air oven at 110□C till constant weight of the residue obtained. The total solid content was calculated with reference to volume taken and expressed in terms of % w /v **(Table 5)**.

Table. 5: Physico-chemical Parameters - *Punarnavadi Eye Drops*.

Sr. No.	Parameters	Results
1.	Specific Gravity	1.038
2.	Refractive Index	1.357
3.	pH	5
4.	Total Solid Content (%)	4.8%

High Performance Thin Layer Chromatography (HPTLC) (Plate-2)

Stationary Phase – Silica Gel GF 254

Mobile Phase – Toluene: Ethyl Acetate : Acetic Acid (7 : 2 : 1) V/V

Detection – Short UV (254 nm), Short UV(366 nm)

Rf values obtained by HPTLC

Sample	Visualize under short UV (254 nm)		Visualize under short UV (366 nm)	
	No. of spots	Rf value	No. of spots	Rf value
Punarnavadi eye drop	13	0.06, 0.10, 0.12, 0.15, 0.17, 0.24, 0.36, 0.39, 0.45, 0.81, 0.84, 0.87, 0.98	15	0.06, 0.10, 0.12, 0.15, 0.17, 0.24, 0.37, 0.40, 0.68, 0.70, 0.81, 0.83 0.87, 0.89, 0.98

***Punarnava* root-coarse powder**

Distillation process

Distillate

Mishri

Sphatika

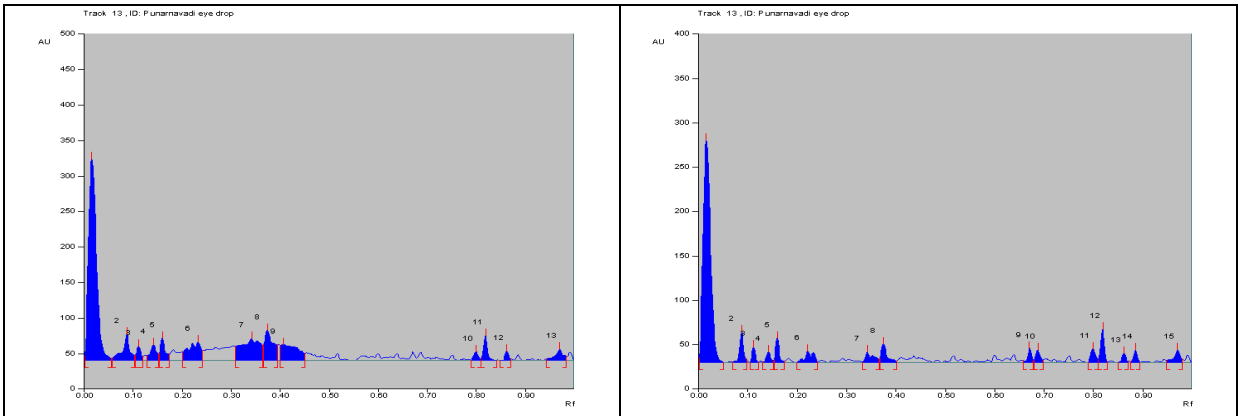
Saindhava

Distillate after adding powders

Distillate after filteration (*PED*)

Packed eye drops

Plate. 1: Preparation of *Punarnavadi* eye drops.



Densitogram at 254nm **Densitogram at 366nm**

Plate. 2: HPTLC plate of *Punarnavadi* eye drops.

CONCLUSION

Physico-chemical profile of *Punarnavadi* eye drops is an essential parameter for quality assurance; in present work the obtained results were found within prescribed limits. For the first time, pharmaceutical and analytical profile of *Punarnavadi* eye drops was established. On the basis of observations and experimental results, this study may be used as reference standard in the further quality control researches.

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