Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

PHYSICO-CHEMICAL STANDARDISATION AND DEVELOPMENT OF HPTLC METHOD FOR THE DETERMINATION OF ANDROGRAPHONIN IN KALMEGH NAVAYAS LOHA - AN AYURVEDIC FORMULATION

Pawar R.K.[1*], Sharma Shivani[2], Singh K.C.[2] and Sharma Rajeev Kr.[1]
[1]Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad, India
[2]Department of chemistry, R.S.S. (P.G.) College, Pilkhuwa, Ghaziabad, India

Received on: 02/01/2011 Revised on: 29/01/2011 Accepted on: 11/02/2011

ABSTRACT
A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Andrographonin in Andrographis paniculata (Whole Plant) and its formulation-Kalmegh Navaya Loha. The alcoholic extract of Andrographis paniculata (Whole Plant) and its ayurvedic formulation-Kalmegh Navayas Loha samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF_{254} and developed using Toluene : Ethyl acetate : Formic acid (5:4:1) v/v as a mobile phase. The plate was sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110^0C for 10 minutes and detection and quantification were carried out densitometrically using an UV detector at wavelength of 235 nm. Content of marker compound- Andrographonin found in the Andrographis paniculata (Whole Plant) and its formulation-Kalmegh Navaya Loha were 0. 0.5194% w/w and 0.04966 % w/w respectively.
KEYWORDS: Andrographonin, Andrographis paniculata (Whole Plant), Kalmegh, Bhunimba, Chiretta, King of Bitter, HPTLC, Kalmegh Navayas Loha, Ayurvedic Formulation.

***Corresponding Address**
R.K. Pawar, Qtr. No. 211, Type-III, Kamla Nehru Nagar, Ghaziabad-201 002 U.P., India
Email- pawarplim@Gmail.com

INTRODUCTION

Kalmegh is vernacularly known as Kirata, Chrayetah, Kiryat, Nila vembu, Karyatu, Kreata, Nelaberu, Mahatita, Chiretta, Creat, King of Bitter etc. It is chiefly found in the plains throughout India from Himachal to Assam and Mizoram, West Bengal and all over south India.

Kalmegh is used mainly for liver disorders and jaundice. A decoction or infusion of leaves is used in general debility and dyspepsia and a tincture of the root as a tonic, stimulant and aperients. The macerated leaves and juice, together with carminative spices such as cardamom, clove and cinnamon, may be made into pills and prescribed for gripe and other stomach ailments in infants. The leaves and roots also find use as an adjunct in the treatment of diabetes, malaria, cholera dysentery, enteritis, gastritis, pneumonia, pyelonephritis, diarrhea and even rabies.[1-6] The plant, especially the leaves, has been used to treat dhatoora (datura) poisoning, maggots in wounds, worms in the eyes and abdomen, liver fluke, glossitis, holes in the hard palate, constipation, tuberculosis, leeches in the nostrils, contagious abortion, retention of placenta, tetanus and scabies[7]. The plant contains bitter glucosides: andrographolide, neoandrographolide, panaculoside, flavnoids, andrographonin, panicalin, apigenin 7-4-dimethyl ether[4]; diterpenoids- 14-deoxy-11-oxo- andrographolide; 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, neo- androgra-pholide and andrographolide. The roots gave flavones-apigenin-7,4'-di-O-methyl ether, 5-hydroxy-7,8,2'3'-tetramethoxyflavone, andrographonin and panicolin and α-sitosterol. Leaves contain homoandrographolide, andrographosterol and andrographone.[2-12]

Literature survey reveals that the TLC and HPTLC methods are not reported yet for the determination of Andrographonin in Andrographis paniculata (whole plant). This method can be used for phytochemical profiling of Andrographis paniculata whole plant and quantification of Andrographonin.

With increasing demand for herbal products in medicines and cosmetics there is an urgent need for standardization.

So the aim of the work is to develop a simple, rapid, selective and cost effective HPTLC method for the quality evaluation of herbal products containing Andrographis paniculata (Whole plant) in market samples.

MATERIAL AND METHOD

Kalmegh whole plant was locally collected sample from Ghaziabad. It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad.

An herbal product **Kalmegh Navayas Loha,** B.No. Nil containing Andrographis paniculata (Whole plant) was procured from the Local Market, Ghaziabad.

Label claimed

Each pills contains: Badi Haran (Terminalia chebula), Bahera (Terminalia belerica), Kalimirch (Piper nigrum), Pippali (Piper longum), Sonth (Zingiber officinale), Amla (Embelica officinalis), Chitrak (Plumbago zeylanica), Baybidang (Embelica ripes), Nagarmotha (Cyprus rotundus) each equal parts; Kalmegh Kwath (Andrographis paniculata) Q.S. and Loha Bhasma(Farrum-calcined) – 9 parts.

Determination of Physico-Chemical Constants

The following Physico-chemical constants has been analyzed and result given in **Table 1**.

HPTLC (High Performance Thin Layer Chromatography)

Equipment

A Cammag (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm^2) with SS lid, TLC Scanner III, Reprostar III and Wincats an integrated Software 4.02 (Switzerland), Rotavapour.

Chemical & Reagents

Analytical grade; Alcohol, Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Anisaldehyde, Sulphuric acid and n-Hexane were used; obtained from S.D. Fine Chem. Ltd. (Mumbai, India). TLC Aluminium pre coated plate with Silica gel 60 GF_{254} (20x10 cm^2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard- Andrographonin procured from Natural Remedies Pvt. Ltd., Bangalore (CAS No.82209-74-3).

Sample & Standard preparation

Sample preparation

(1) 1g of coarsely powdered drug sample (Kalmegh Navayas Loha) was extracted with 10 ml absolute alcohol for 24 hours by cold extraction method. The extract was filtered by Whatmann filter paper and make up to 10 ml in a volumetric flask. Filtrate was concentrated to 2 ml and used for T.L.C.

(2) 1g of coarsely powdered drug sample (Kalmegh, whole plant) was extracted with 10 ml absolute alcohol for 24 hours by cold extraction method. The extract was filtered by Whatmann filter paper and make up to 10 ml in a volumetric flask.

Standard Preparation

5mg of standard Andrographonin dissolved in 5ml of absolute alcohol and made up to 5ml in standard volumetric flask.

Chromatography

TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20x10 cm^2; 0.2 mm thick) was used with Toluene : Ethyl acetate : Formic acid (5:4:1) V/V as mobile phase. absolute alcoholic extract of samples and Andrographonin standard solution applied on plate by using Linomat V applicator. Cammag Twin Trough Glass Chamber (20x10 cm^2) with SS lid was used for development of TLC plate. The Twin Trough Glass Chamber was saturated with mobile phase for 30 minutes. TLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature. This plate was sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110^0C for 10 minutes and HPTLC finger print profile was snapped by Cammag Reprostar III, before deivatization under UV 254 nm, 366 nm and after derivatization (Fig. 1). The plate was scanned after derivatization using Camag TLC Scanner III at wavelength 559nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data.

Method Validation and Recovery Study

To study the accuracy and precision of the proposed method, recovery experiment was carried out. To a fixed amount of absolute alcoholic extract of samples, the standard solution of Andrographonin was added (ratio 9:1 v/v) and total amount of standard Andrographonin were determined. Percent recovery was calculated from the amount of Andrographonin found via graph (**Table 3**).

Linearity of Detector Response, Assay and Recovery

In order to establish linearity, standard solution of Andrographonin (1mg/ml) applied on TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20X10 cm^2; 0.2 mm thick), 2µl, 4µl, 6µl on Track No. S1, S2 & S3 respectively and for assay, absolute alcoholic extract of both samples applied on Track No. T1 & T2 and for recovery study, the absolute alcoholic extract of both samples were spiked with standard Andrographonin solution (ratio 9:1v/v) and applied on Track No. T3 & T4 on the same plate. TLC plates were developed to 8 cm

distance above the position of the sample application and removed from the chamber and air dried at room temperature. This HPTLC finger print profile was snapped by Cammag Reprostar III, before derivatization under UV Light 254 nm, 366 nm and after derivatization (**Fig. 1**). The plate was scanned immediately after derivatization using Camag TLC Scanner III at wavelength 559nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that Andrographonin appeared at R_f. 0.57 (dark violet colour). The peaks, graph and spectra obtained were given in Fig. 2 and 3 and R_f. values, colour of bands (**Table 2**), quantity of Andrographonin linearity, standard deviation & regression coefficient found via graph (**Table 3**) and calculated quantity of Andrographonin & % recovery were given in **Table4**.

RESULT AND DISCUSSION

Of the various mobile phases tried, the mobile phase containing Toluene : Ethyl acetate : Formic acid (5:4:1) v/v and the active principle Andrographonin resolved as a dark violet colour band at R_f. 0.57 very efficiently from the other components in alcoholic extract of Andrographis paniculata Linn. (Whole plant) and Kalmegh Navayas Loha (**Fig. 1**). Sharp peaks of Andrographonin (Standard and samples) were obtained when the plate was scanned at wavelength 559 nm (Fig. 2). Quantity of Andrographonin found in samples were obtained automatically (**Table 3**) via graph (Fig. 3) and % Andrographonin found in samples and % recovery were calculated (**Table 4**). Quantity of Andrographonin found in Locally Collected Sample, Ghaziabad (U.P.) is 7.746mg in 1g drug sample (0.7746% w/w) and quantity of Andrographonin found in Kalmegh Navayas Loha is 1.155mg in 1g drug sample (0.1155%w/w). The % recovery of Andrographonin in Locally Collected Sample, Ghaziabad (U.P.) is 99.82% w/w and 99.60%w/w in Kalmegh Navayas Loha. The mean % recovery was 99.71%.

The accuracy and reproducibility of the method was established by means of recovery experiment. The mean recovery was close to 100% which indicates the accuracy of the method.

The robustness of the method was studied, during method development, by determining the effect of small variation, of mobile phase composition (±2%), chamber saturation period, development distance, derivatization time, and scanning time (10% variation of each). No significant change of R_f. or response to Andrographonin was observed, indicating the robustness of the method.

CONCLUSION

The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of Kalmegh Navayas Loha powder and quantitative determination of Andrographonin in Kalmegh Navayas Loha.

REFERENCES

1. Dhiman AK. Ayurvedic Drug Plants, Daya Publishing House, Delhi, 2006, p-188.
2. Husain A *et. al*., Dictionary of Indian Medicinal Plant, CIMAP, Lucknow, 1992, p- 32.
3. Anonymus: The Wealth of India, Raw Materials, Vol. I, NISCAIR, CSIR, New Delhi, 1992, p-162.
4. Prajapati/Purohit *et. al*., A Handbook of Medicinal Plants, A Complete Source Book, Agrobio, India, 2004, p-45
5. Thakur RS, Puri HS, Husain A. Major Medicinal Plant of India, CIMAP, Lucknow, 1989, p- 61.
6. Sharma RK, Govil JN, Singh VK. Recent progress in Medicinal plants, Vol. p-33.
7. Elizabeth M. Williamson, Major Herbs of Ayurveda, 2002, p-41.
8. Rastogi RP, Mehrotra BN. Compandium of Indian Medicinal Plants, CDRI, PID, New Delhi, 1993, Vol. 2, p- 44
9. Rastogi RP, Mehrotra BN. Compandium of Indian Medicinal Plants, CDRI, PID, New Delhi, Vol. 3, p-41.
10. Rastogi Ram P, Mehrotra BN. Compandium of Indian Medicinal Plants, CDRI, PID, New Delhi,Vol. 4, p-48.
11. Rastogi Ram P, Mehrotra BN. Compandium of Indian Medicinal Plants, CDRI, PID, New Delhi,Vol. 5, p-52.
12. Jha MK, Folk veterinary medicine of Bihar- a research project NDDB, Anand, Gujarat, 1992

Table 1: Physico-chemical constants of Kalmegh navayas loha and Kalmegh (whole plant)

Name of Physico-chemical constants	KALMEGH NAVAYAS LOHA	KALMEGH (Whole Plant)
Moisture content	6.86% w/w	6.03% w/w
pH (of 5% aq. Solution)	5.23	9.37
Total ash	27.04% w/w	14.33% w/w
Acid in-soluble ash	3.23% w/w	1.78% w/w
Water soluble ash	17.05% w/w	0.67% w/w
Water soluble extractives	19.78% w/w	12.42% w/w
Ethanol soluble extractives	6.34% w/w	5.44% w/w
Chloroform soluble extractives	2.97% w/w	3.01% w/w
Hexane soluble extractives	0.656% w/w	1.57% w/w
Disintegration Time	5 minutes	-
Average weight of 20 pills	0.1334 gm	-
Weight variation with respect to average weight of 20 pills	Minimum variation - 0.75% w/w Maximum variation - 12.29% w/w	-

Table 2: HPTLC details of alcoholic extract of Kalmegh Navayas Loha

Detection/ visualization	Kalmegh whole plant (Track No. T1 and T3)		Standard- Andrographonin (Track No. S1, S2 and S3)		Kalmegh Navayas Loha (Track No. T2 and T4)	
	R_f. values	Colour of band	R_f. values	Colour of band	R_f. values	Colour of band
Under UV 254 nm	0.06 0.19 0.25 0.59 0.67 0.74 0.96	dark grey grey grey grey grey grey grey	-	No significant bands appear	0.23 0.31 0.43 0.51 0.59 0.62 0.67 0.74 0.82 0.95	dark grey dark grey dark grey dark grey dark grey dark grey dark grey dark grey dark grey dark grey
Under UV 366 nm	0.10 0.45 0.50 0.59 0.67 0.74 0.78 0.82 0.87 0.92	green bright sky blue light blue blue red red red red red red	-	No significant bands appear	0.23 0.31 0.38 0.43 0.46 0.59 0.74 0.82 0.86 0.91	blue green blue blue red bright sky blue red bright sky blue red blue
After derivatiz-ation	0.06 0.19 0.25 0.57 0.67 0.74 0.96	dark violet dark blue light violet dark violet violet light violet light violet	0.57	Dark violet	0.06 0.23 0.44 0.48 0.57 0.74 0.82 0.91 0.96	dark violet violet light violet light blue dark violet light violet green brown light violet

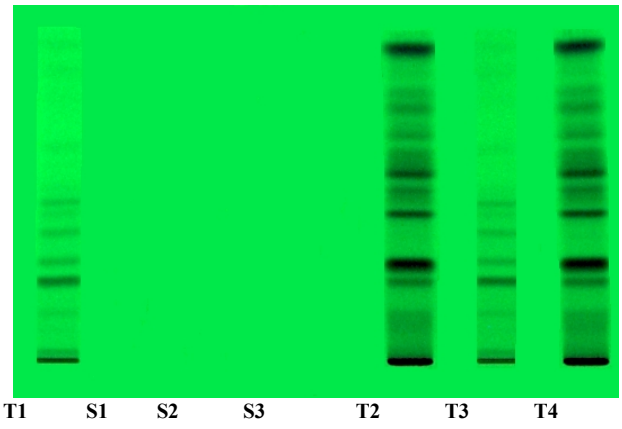
Table 3: Quantity applied on plate and values found via graph

Volume applied on plate	Quantity applied on plate	Quantity of Andrographonin via graph	Linearity & Regression Coefficient and Standard deviation via graph
9µl	900µg	4.679µg	Y = 810.592 + 1839.004 * X r = 0.99888 sdv = 3.02%
2µl	2µg	2.000µg	
4.0µl	4µg	4.000µg	
6.0µl	6µg	6.000µg	
9µl	4500µg	2.239µg	
10µl	900µg +1µg	5.671µg -1µg = 4.671µg	
10µl	4500µg +1µg	3.230µg -1µg = 2.230µg	

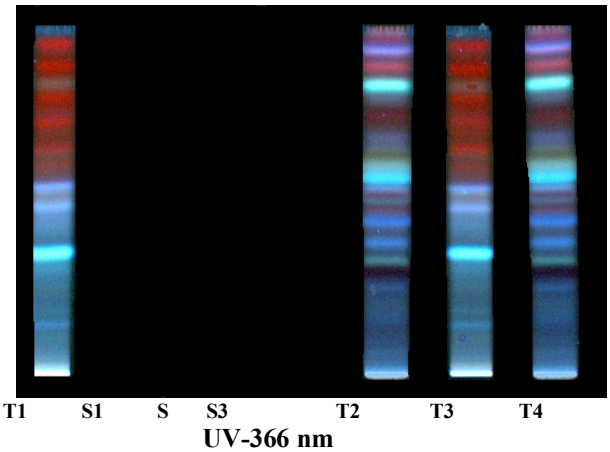
T1- Alcoholic extract of Kalmegh whole plant, Locally collected from Ghaziabad
S1- Andrographonin standard solution
S2- Andrographonin standard solution
S3- Andrographonin standard solution
T2- Alcoholic extract of Kalmegh Navayas Loha, Local Market Sample, Ghaziabad
T3- Alcoholic extract of Kalmegh whole plant, Locally collected from Ghaziabad
T4- Alcoholic extract of Kalmegh Navayas Loha, Local Market Sample, Ghaziabad

Table 4: Summary of results of Kalmegh Navayas Loha and Kalmegh Whole Plant

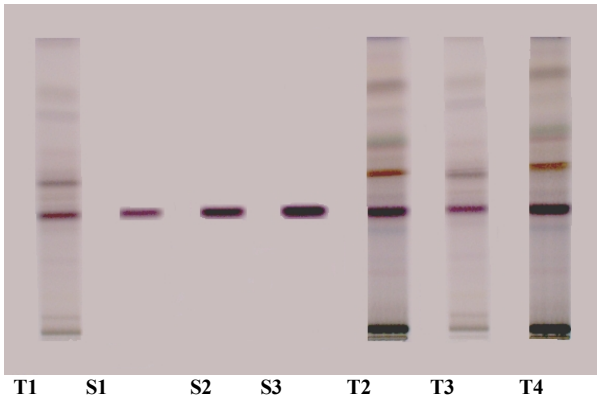
Sample	Kalmegh Whole Plant	Kalmegh Navayas Loha
Quantity of Andrographonin in 1gm	5.194mg	0.4966 mg
% Andrographonin	0.5194% w/w	0.04966 % w/w
% Recovery	99.82% w/w	99.60% w/w



UV-254 nm

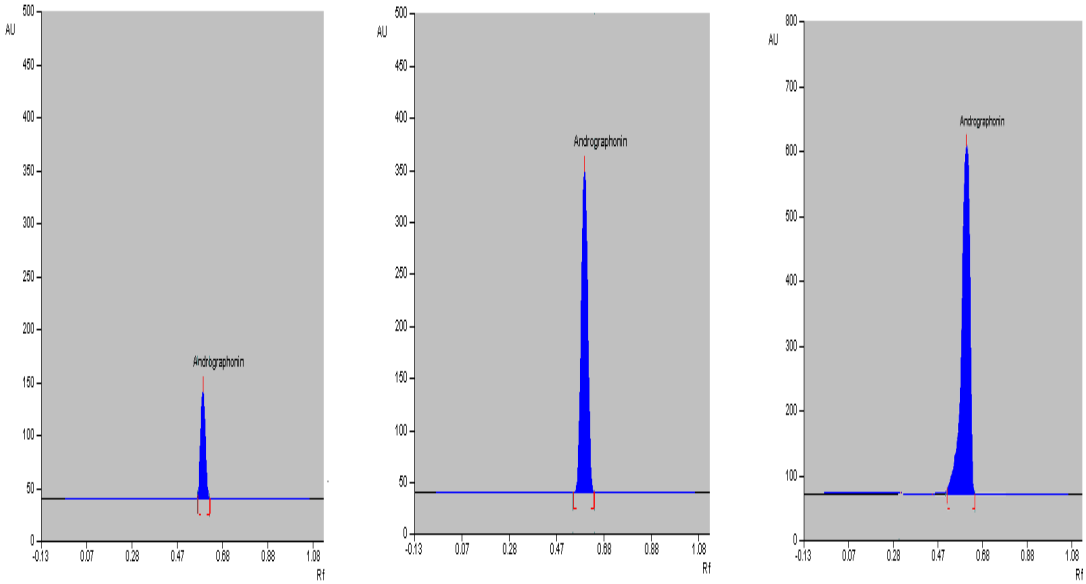


UV-366 nm



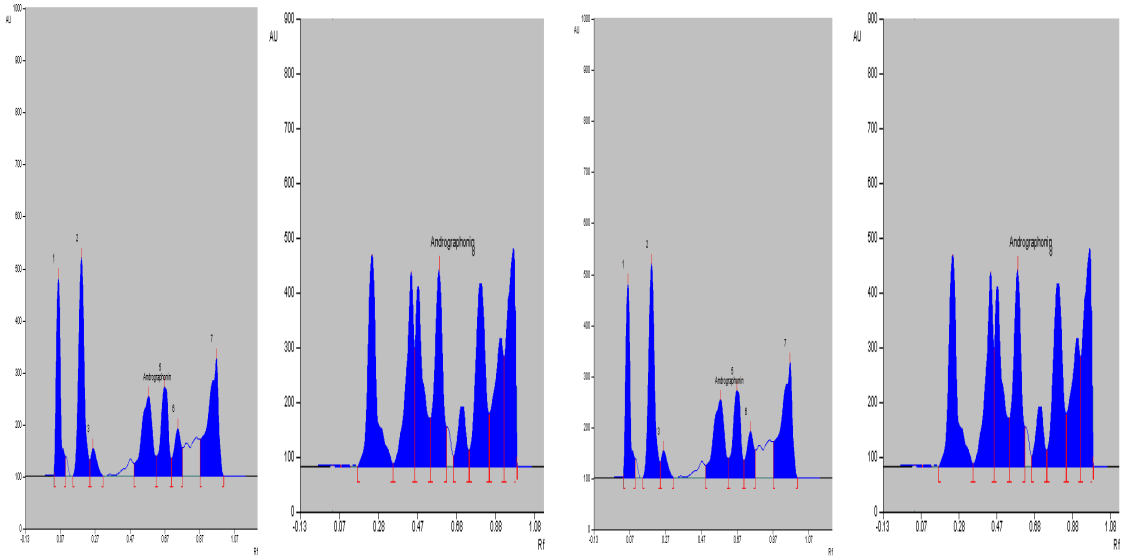
After derivatization

Fig 1: HPTLC Finger print of KALMEGH NAVAYAS LOHA



(Track S1) **(Track S2)** **(Track S3)**

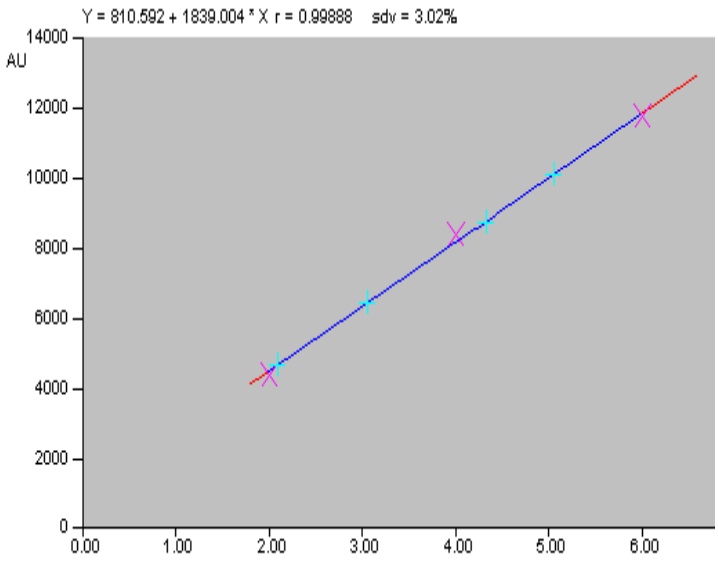
Peaks of Andrographonin (@ 559nm



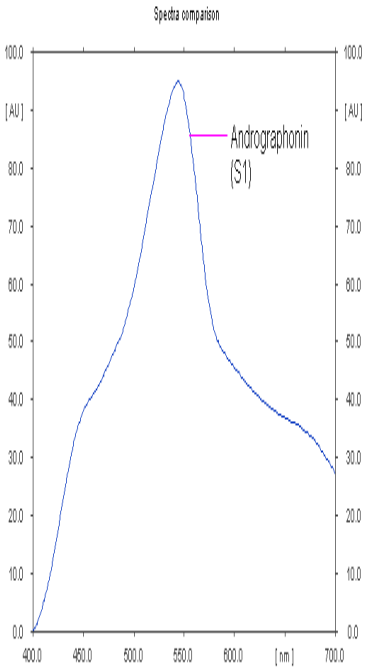
(Track T1) **(Track T2)** **(Track T3)** **(Track T4)**

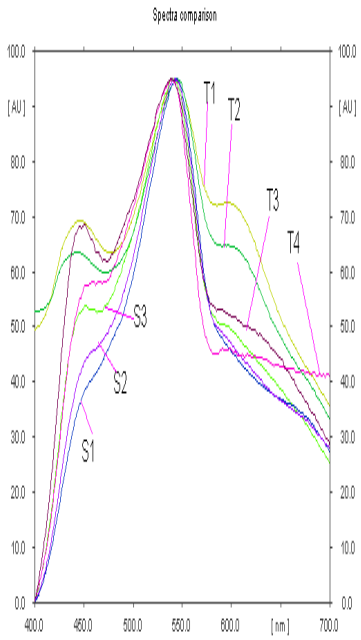
Peaks of Kalmegh (W.P.) extract @ 559nm (Track T1& T3)
Peak of Kalmegh Navayas Loha extract @ 559nm (Track T2& T4)

Fig 2: Peaks of KALMEGH NAVAYAS LOHA in all Tracks



Graph Area vs AU





Spectra of Andrographonin @ 559 nm **Spectra of Andrographonin in all tracks**

Fig 3: Graph and Spectrra of KALMEGH NAVAYAS LOHA

Source of support: Nil, Conflict of interest: None Declared