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FORMULATION DEVELOPMENT AND EVALUATION OF *ANDROGRAPHIS PANICULATA* CREAM: HPLC AND HPTLC ANALYSIS OF ANDROGRAPHOLIDE FROM EXTRACT AND CREAM FORMULATION

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ABSTRACT

Andrographis paniculata is used for its numerous beneficial effects primarily for liver disorders. Currently, its only one semi-solid dosage forms are available in the market. It has been found to have antiallergic and anti-inflammatory effects. Hence, a cream formulation of Andrographolide leaves extract was developed. HPLC and HPTLC method was successfully developed and validated for determination of Andrographolide from extract and cream formulation. HPLC was performed using a mixture of Potassium dihydrogen orthophosphate: and acetonitrile as a mobile phase while for HPTLC a mixture of Tolune, ethyl acetate, methanol, and formic acid was used. Both methods were found to be robust, accurate and precise.

Keywords: *Andrographis paniculata,* cream formulation, HPLC, HPTLC

1. INTRODUCTION

Medicinal plants are crucial for the prevention and treatment of diseases as well as for human survival. Since, they contain active ingredients known as phytochemicals or secondary metabolites, which are responsible for the pharmacological effects, medicinal plants also serve as a source for the development of novel medications. Secondary metabolites are non-nutritive compounds found in plants that have disease-preventive and protective qualities. Saponins, flavanoids, alkaloids, terpenoids, tannins, and other phytochemicals are examples **[1]**. Herbs are quite bitter and many times fail to gain the patients acceptability, hence there is a need to formulate them as simple dosage forms for patient acceptability **[2]**.

Andrographis paniculata (Burm. f., AP) Wall.ex Nees, commonly known as "King of bitters", traditionally known as 'Kalmegh', green chirayta is an annual herb widely used in tropical Asia. It is found in the Indian states of Madhya Pradesh, Chhattisgarh, Orissa, Jharkhand, Maharashtra, Assam, Bihar, West Bengal, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka, and Kerala. It also has a crucial place in the Indian Pharmacopoeia. In Asia and Europe, whole plant leaves and roots are utilised as a folk cure for many illnesses. A wide range of pharmacological actions of AP have been described, including anticancer, antidiarrheal, anti-HIV, antihyperglycemic, anti-inflammatory, antimicrobial, antimalarial, antioxidant, cardiovascular, and immunostimulatory **[3]**. It contains lactones, flavonoids, diterpenoids, and flavonoid glycosides.

AP has been phytochemically investigated for a number of bioactive compounds including andrographolide, neoandrographolide, panaculoside, flavonoids, andrographonin, panicalin, apigenin-7 and 4'-di-O-methyl ether. The plant contains diterpenoids like 14-deoxy-11-oxoandrographolide; 14-deoxy-11, 12-dehydroandrographolide;14-deoxyandrographolide; neoandrographolide and andrographolide **[4]**. The major bioactive constituent 'Andrographolide' constitutes a group of diterpene lactones mainly found in leaves whereas stems contain the compound in traces **[5]**. AP is available in number of dosage forms. But there are limited papers describing its cream formulation **[6, 7]**. Examples of currently existing semi-solid dosage forms of AP is Bianca Rosa (Andrographis 3% Cream). AP has been found to have antiallergic effect **[8, 9]**. The developed cream formulations could find potential use as antiallergic and anti-inflammatory remedy.

In accordance with bare minimum standard of acceptability specified in pharmacopoeia, herbal industries prefer to receive Kalmegh (aerial parts) with not less than 40% leaves and total Andrographolides content not less than 1.8% w/w. For leaves, the industry expects not less than 2.8% w/w of total Andrographolides which would contain at least 2.5% w/w of pure Andrographolide **[10]**. Commercial formulations of this plant's extract have recently been employed in some nations. To improve their efficacy, the preparations must be standardised. There are some HPLC **[11-15]** and HPTLC **[16-20]** methods developed for quantitation of

Andrographolide from extract or formulation. This paper describes new methods of preparation and standardization of developed AP cream by HPLC and HPTLC.

2. MATERIALS AND METHODS

2.1 Standardization of extract

Selection of the raw material and its part was done on the basis of its activities described in the ancient Ayurvedic text and research papers. AP leaves extract was used in this study. The plant extract was procured from Kisalaya Herbal Pvt. Ltd., India (Batch No AP/0090815, 9.15%w/w Andrographolide). The extract was analysed for moisture content (Karl Fisher titration), pH, water and alcohol soluble extractives, total ash value, acid-insoluble ash and total saponins.

2.2 Formulation of cream containing AP extract

Selection of quantity of extract was done on the basis of dose of extract in crude dosage form described in ancient Ayurvedic texts. The formula of developed cream is given in **Table 1**.

Table 1: Formula of developed cream formulation

Sr. No	Ingredient	Quantity % (w/w)	Use
1.	AP extract	5	Active
2.	Acrypol 934	1	Gelling agent
3.	Tween 20	1.4	Emulsifying agents
4.	Sodium Benzoate	0.5	Chelating agent
5.	Phenoxyethanol	1.5	Stabilizer
6.	Cetosteryl Alcohol	1.6	Thickening agent
7.	Dimethicone	1.2	Emollients
8.	Butylated hydroxytoluene	0.1	Antioxidant
9.	EDTA	0.1	Chelating agent
10.	Butylated hydroxyanisole	0.2	Antioxidant
11.	Stearic Acid	2.0	Thickening agent
12.	Propylene Glycol	6.0	Humectant
13.	Light Liquid Parafin	0.6	Oil Phase material
14.	KOH	0.3	pH adjuster
15.	Glycerine	8.5	Humectant
16.	Purified water	65	Vehicle
	Total	100 gm	

Initially, the aqueous phase was prepared. The (50%) water was heated to about 70°C. To it, extract and ingredients of aqueous phase like propylene glycol, sodium benzoate, phenoxyethanol, glycerine, and tween 20 were dissolved. Similarly, oil phase was prepared. Stearic acid, BHT, cetostearylalcohol, glyceryl monostearate, light liquid paraffin, BHA were heated in a vessel at about 70°C to get clear oily solution. Both phases were heated separately to attain the temperature of 80°C. Oil phase was added in to aqueous phase and homogenized at 50°C to form emulsion. Similarly, gel phase was prepared**.** Acrypol 934 was soaked overnight in water. Aqueous disodium EDTA was added to it next day and the volume was made with remaining water. This mixture was stirred continuously until transparent gel was formed. Cream was prepared by transfer of emulsion into gel phase and mixed well in stirrer. In the last step, dimethicone and aqueous KOH was added to maintain pH of cream formulation.

2.3 Evaluation of cream

The cream formulation was studied for visual appearance, homogeneity and pH. Physical stability of the cream was also determined. Ease of removal of cream was also assessed. A portion of cream was applied on the skin and examined by washing with the tap water. The ability of cream to maintain its consistency was determined by keeping it at 25^0 C for 30 days.

2.4 HPLC method development for determination of Andrographolide from *Andrographis paniculata* extract and cream

Preparation of the standard solution

About 2.5 mg of andrographolide was accurately weighed. It was then transferred to 25.0 ml volumetric flask containing methanol. It was sonicated and dissolved in the methanol. It was further diluted with methanol to get concentration of 100 µg/ml.

Selection of analytical wavelength

With the mobile phase serving as a blank, a standard solution containing 100 µg/ml andrographolide was scanned using UV VIS-detector within the wavelength range of 400 to 200nm. The andrographolide exhibited maximum absorption at 223nm. This wavelength was selected for analysis.

HPLC method development

The andrographolide content in extract and cream was determined by HPLC with Phenomenex Luna C_{18} column (5µ, 4.6x250 mm) column. The elution was carried out with solvent system of potassium dihydrogen orthophosphate: acetonitrile at a flow rate of 1.5 mL/min. A mobile phase containing Potassium dihydrogen orthophosphate and acetonitrile was used. A gradient program as indicated in **Table 2** is used for analysis. Methanol was used as a diluent. The sample injection volume was 20 µL, and the analytes were monitored with the PDA detector at 223nm.The system was run for 30 minutes. The optimized chromatographic conditions are given in **Table 3**.

Table 2: Gradient program used for standardization

Time (Min)	Solvent A (V/V)	Solvent B (V/V)
0	95	5
8	55	45
25	20	80
30	95	5

Table 3: Optimized Chromatographic Conditions for HPLC

Column	Phenomenex Luna C_{18} (5µ ,4.6 X 250 mm)
Solvents	Potassium dihydrogen orthophosphate: acetonitrile
Flow rate	1.5 mL/min
Column temp	30°C ± 5°C
Sample temp	25° C ± 5°C
Injection volume	20 µl
Detector	PDA at 223 nm
Run time	30 minutes
Retention time	11.36 for andrographolide

Method Validation

The proposed method was validated by studying several parameters such as linearity, specificity, accuracy, precision and robustness.

Linearity and Range

The standard stock solution of Andrographolide was diluted to six different concentrations in the range of 100-1000µg/ml. The calibration graph was plotted by using the concentration versus average peak area at 223nm.The linearity of the detector response for the standards was determined by means of linear regression. The correlation coefficients, y-intercepts and slopes of the regression lines were calculated.

Specificity

The specificity of the method was demonstrated by injecting methanol diluent as a blank, standard solution and test solution. The interference at the retention time of andrographolide was assessed. Also, peak purity of andrographolide was determined to ensure absence of interference.

Precision

The % Relative standard deviation (RSD) of the peak area of test solutions was used to indicate the method precision. About 5 gm of cream formulation was weighed in 25mL volumetric flask. This was mixed with 15 mL of methanol, sonicated for 30 minutes, and then diluted with methanol. It was filtered using Whatman's no. 41 filter paper. The first few millilitres were discarded, and filtered using a 0.45µ syringe filter. This procedure was repeated six times. Each solution was analysed twice. Intermediate precision was performed by doing the analysis on two different HPLC systems and percent assay was calculated.

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The temperature of column was changed to ±5 ºC similarly detection wavelength was changed ±5nm.The sample solution of extract and cream was prepared as described under precision the effect of those changes on signal was measured.

Accuracy

The accuracy was determined from recovery studies. A known but varying amount of andrographolide was spiked into pre-analysed extract test solution at 80% (4.2mg for extract, 9.1mg for cream), 100% (5.3mg for extract, 11.4mg for cream) and 120% (6.4mg for extract, 13.7mg for cream) recovery levels of working standard in triplicate. The spiked test solution was analyzed according to the proposed procedure.

Assay

Preparation of the test solution for Extract

In a 25 mL volumetric flask with 58.2 mg of extract was weighed, 20 mL of methanol was added, and the mixture was then sonicated for 15 minutes. The mixture was allowed to cool before being diluted with methanol. Whatman's filter paper no. 41 was used to filter the solution. The same solution was used as test solution.

Preparation of test solution for Cream formulation

About 2.5 gm of cream formulation was weighed in 20 mL volumetric flask. This was mixed with 15 mL of methanol, sonicated for 30 minutes, and then diluted with methanol. It was filtered using Whatman's no. 41 filter paper. The first few millilitres were discarded, and filtered using a 0.45µ syringe filter.

2.5 HPTLC method for standardization of AP extract and cream formulation using Andrographolide as biomarker

A HPTLC method was developed for ensuring the quality of extract and cream formulation in terms of Andrographolide content. The samples were applied on a precoated silica gel 60F$_{254}$ aluminium plates (20 cm x 10 cm, 0.2 mm thick), in the shape of a band with a width of 6 mm using a Linomat 5 applicator by CAMAG (Switzerland). It was equipped with a CAMAG 100 µL syringe. The ascending development was conducted in a CAMAG twin trough chamber (20 x 10 cm) that had been previously saturated with mobile phase for 20 minutes. The mobile phase Toluene: Ethyl acetate: Methanol: Formic acid (5:5:0.5:0.5 v/v/v/v) was used. Plates were dried by a drier after development. The densitometric scanning was carried out with a CAMAG TLC scanner-3 running Win CATS software V. Peak area was used for the evaluation. The optimum chromatographic conditions are given in **Table 4**.

Table 4: Optimum HPTLC chromatographic conditions

Parameters	Specifications
Stationary phase	Aluminum plate coated with silica gel 60 F 254 (Merck)
Mobile phase	Tolune: ethyl acetate: methanol: formic acid (5:5:0.5:0.5, v/v/v/v)
Plate size	5 cm × 10 cm
Mode of application	Band
Band size	6mm (Distance between two bands: 14mm)
Sample volume	5 µl
Development chamber	Twin- trough glass chamber, 10 cm × 10 cm with stainless steel lid.

Saturation time	**20 minutes**
Separation technique	**Ascending**
Migration distance	**≈ 80 mm**
Temperature	**25 ± 5 ^{0} C**
Scanning mode	**Absorbance/Reflectance**
Slit dimensions	**5 × 0.45 mm**
Scanning speed	**20 mm per second**
Scanning wavelength	**232 nm**

Preparation of Standard solution

About 2.5 mg of Andrographolide was accurately weighed and transferred to 25mL volumetric flask. It was dissolved and sonicated in the methanol. The solution was made up to volume with methanol to obtain stock solution of concentration 100 µg/ml.

Linearity and range

To prepare the calibration curve, the standard solutions of 1µl, 2.5µl, 5µl, 7.5µl and 10µl (100-1000ng/band) were applied in duplicate on the HPTLC plate using the LINOMAT-V automatic sample applicator and a micro syringe. Utilizing the concentrations versus average peak area at 232 nm, the calibration graph was plotted. By using linear regression, the linearity of the detector response for the standards was ascertained.

Specificity

Specificity of the method was determined by determining peak purity data of standard and test formulations.

Precision

The method precision was determined by analysing test solutions six times. Precision was determined in terms of % RSD of peak area.

Robustness

The method parameters were deliberately varied to determine its impact on response. The composition of mobile phase was changed. The ratio of Toluene: Ethyl acetate: Methanol: formic acid was 5:5:0.5:0.4 and 5:5:0.5:0.6. The amount of formic acid was varied. Also chamber saturation time (18 min and 22 min) and plate development distance (7.5 and 8.5cm) were changed. Its impact on peak area was determined**.**

Accuracy (Recovery)

According to ICH recommendations, the recovery investigations were conducted at 80%, 100%, and 120% of the test concentration. For testing extract, about 4, 5 and 6 mg of Andrographolide standard was added to 58.2mg extract in separate volumetric flasks in triplicate and the procedure as described for assay was carried out with dilution being made to 50ml. For cream formulation, about 8, 10 and 12 mg of Andrographolide standard were added to cream formulation. The procedure described under assay of cream formulation was executed with dilution being made to 100ml.

Assay of extract

In a 25 mL volumetric flask with 58.2 mg of extract, 20mL of methanol was added, and the mixture was then sonicated for 15 minutes. The liquid was allowed to cool before being diluted with methanol. Whatman's filter paper no. 41 was used to filter the solution. About 2µL of the solution was applied for HPTLC analysis.

Assay of Cream Formulation

A 50mL volumetric flask containing 2.5 g of cream formulation was weighed. Then, 15 mL of methanol was added, sonicated for 30 minutes, and diluted with diluent to a maximum of 50 mL. The solution was filtered via Whatman's no. 41 filter paper after being thoroughly mixed. The first few millilitres were discarded before filtering through a 0.45 syringe filter. About 2µL of the solution was applied for HPTLC analysis.

3. RESULT AND DISCUSSION

3.1 standardization of extract

The results of physicochemical parameters of AP extract are given in Table 5 the values are found within specified limits.

The values of evaluation parameter of the cream are given in **Table 6**.

3.2HPLC method development for standardization of AP extract and cream formulation using Andrographolide as biomarker

Since HPLC has strong separation capabilities, it is possible to separate the complicated chemical components of extract into a large number of relatively easy sub fractions. In addition, more contemporary methods of using hyphenated chromatography and spectrometry, including HPLC-PDA detection, offers spectral information. HPLC method was developed with an aim to standardize extract and cream formulation in terms of Andrographolide. The biomarker was successfully separated from other components using a mixture of 50M Potassium dihydrogen phosphate and acetonitrile as a solvent system in a gradient mode. The retention time for Andrographolide was 11.36 minute. The system suitability parameters for developed method are given in **Table 7**.

3.2.1 Linearity: The method was found to be linear in the concentration range of 100 to 1000 µg/ml with correlation coefficient of 0.993 **(Table 8 and, 9 Figure 1)**. The chromatograms of blank, Andrographolide, extract and cream formulation are given in **Figure 2**.

3.2.2. Specificity

Specificity was assessed in terms of peak purity. The value of purity angle was found to be less than purity threshold indicating absence of coelution of any other matrix component with Andrographolide **(Table 10)**. Additionally, chromatogram of blank did not show any peak at the retention time of marker.

3.2.3. Precision: The findings demonstrated that Andrographolide's system, method, and intermediate variation of results all fell within acceptable ranges (% RSD less than 2%).

3.2.4. Robustness

There was no significant change in the % assay after changing column temperature and detection wavelength indicating method robustness **(Table 14)**.

3.2.5. Accuracy

Through recovery studies, the accuracy was determined. It was found to be in the range of 90-110% w/w **(Table 15)**.

Table 16 shows values of assay of extract and cream formulations.

3.3. HPTLC method for standardization of AP extract and cream formulation using Andrographolide as biomarker

The herbal extract and formulation can be standardized on the basis of active principle or major compound. A finished product specification should be defined to ensure consistent quality of the product. For the standardization of extract and cream formulation, an HPTLC method was also developed and validated. Andrographolide was used as the standard. The most effective solvent system was used in the HPTLC procedures to achieve the greatest possible separation of compounds. The composition of the solvent system was optimized to Toluene: Ethyl acetate: Methanol: Formic acid (5:5:0.5:0.5, v/v/v/v). The detection wavelength selected was 232nm. The retardation factor of Androgropholide was found to be 0.49. The linearity data is given in **Table 17**. The calibration curve and chromatogram are shown in **Figure 3 and Figure 4**. respectively.

3.3.1. Precision

The % RSD for system and method precision was found to be 1.61 % and 0.45% respectively.

The method was also found to be specific. The comparative 2D chromatogram of standard Andrographolide, AP extract and cream formulation is given in **Figure 5**. The principal band of Andrographolide was observed at Rf 0.49. There was no interference for Androgropholide. The peak purity was analysed. The peak purity of Andrographolide was accessed by comparing the spectra at peak start, peak apex and peak end position of the band in standard and test formulation. The r s, m and r m, e values were found to be more than 0.995. It was observed that other constituent's presents in the formulation did not interfere with andrographolide peak. The recovery of the proposed method was found to be 96.19-97.80 %w/w as indicated in **Table 20**.

Table 5: Results for Physicochemical parameters of AP extract

Sr. No	Name of parameter	Specification	Result
1.	Water soluble extractive value	NLT 60%w/w	91.41
2.	Alcohol soluble extractive value	NLT 50%w/w	81.96
3.	Total ash	NMT 20%w/w	1.52
4.	Acid insoluble ash	NMT 10%w/w	1.2
5.	pH	4.0-7.0	6.035
6.	Loss on drying	NMT 8.0%w/w	5.869
7.	Total bitter content	NMT 5%w/w	6.3

Table 7: System Suitability Parameters for HPLC

Sr.no.	Parameter	Andrographolide
1.	Tailing factor (T)	1.59
2.	No. of theoretical plates (N)	7260

Table 8: Standard calibration curve data for Andrographolide

Concentration (µg/ml)	Peak Area (mAu)
100	567362
250	1130061
500	1716376
750	2301049
1000	2884014
1200	3450452

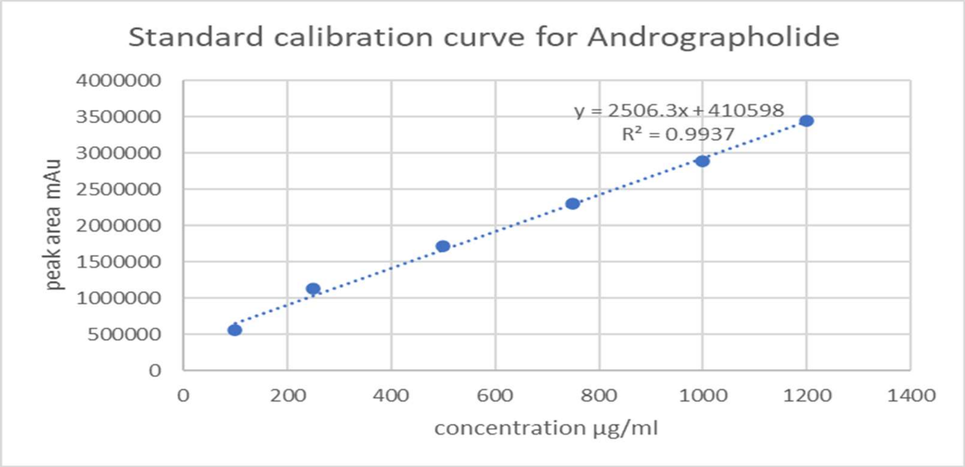
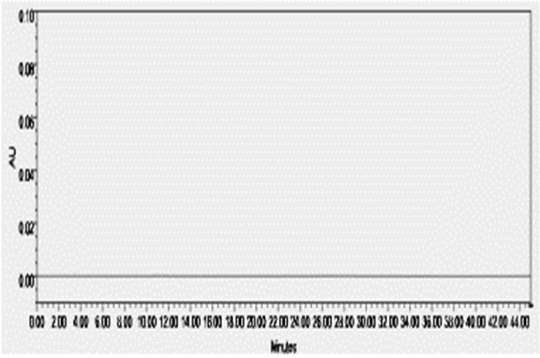
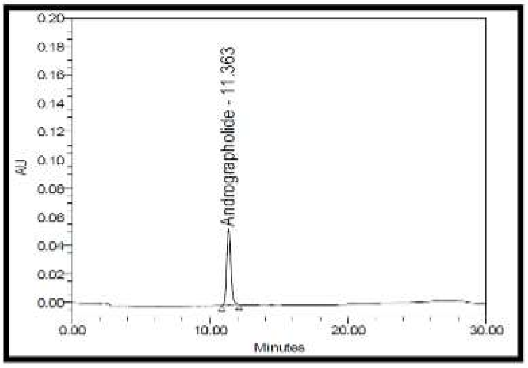


Figure 1: Linearity graph for Andrographolide by HPLC



(a)



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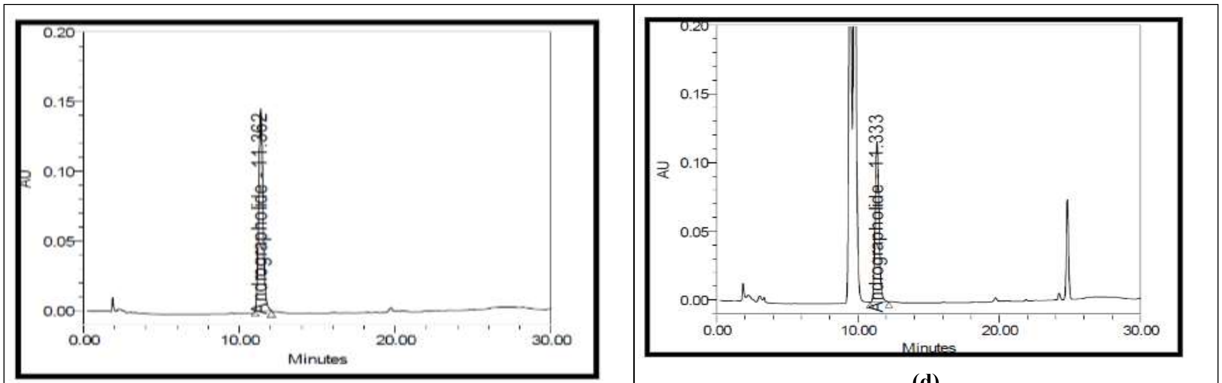


(c)

(d)

Figure 2: HPLC chromatograms of a) Blank solution b) Standard Andrographolide (Rt = 11.363 min) c) AP extract (Rt = 11.362min) d) Anti-allergic cream (Rt=11.333 min)

Table 9: Summary of linear regression of Andrographolide by HPLC

Linearity range	**100-1000 ppm**
Linear regression equation	**2506.x+41652**
Slope± SD	**2506.x**
Intercept±SD	**41652**
Correlation coefficient (r ^{2})	**0.9937**

Table 10: Specifity Parameters

Standard	Purity angle	Purity threshold	Purity flag
Andrographolide	**0.182**	**0.197**	**No flag found***

No Flag Found * means no interference in Andrographolide Peak

Table 11: System precision parameters

Concentration(µg/ml)	Area of standard
500	**1107215**
500	**1106764**
500	**1109924**
500	**1109030**
500	**1109508**
Average area	**1108488.2**
SD=1413.24	**RSD=0.13**

Table 12: Method precision for AP cream formulation

Sr. No.	Peak Area	Andrographolide Concentration found (µg/ml)	% Assay (w/w)
1.	**2694589**	**911.3**	**99.59**
2.	**2669526**	**901.3**	**98.50**
3.	**2665015**	**899.5**	**98.311**
4.	**2613385**	**878.9**	**96.05**
5.	**2689326**	**909.2**	**99.37**
6.	**2704865**	**915.4**	**100.04**
Peak area	**2672784**	**902.6**	**98.64**
S.D.	**32794.82**	**13.08**	**1.43**
%RSD	**1.23**	**1.45**	**1.45**

Table 13: Intermediate precision parameters

Sr. No	Name of Analyte	% RSD for System-1	% RSD for System-2	Average RSD
1.	**AP extract**	**0.18%**	**0.20%**	**0.19%**
2.	**Cream formulation**	**0.84%**	**0.82%**	**0.83%**

Table 14: Robustness parameters

Robustness parameter	Injection	Peak Area	USP Tailing	USP Plate count	% Assay (w/w)
Change in temperature					
25°C	2242952.4	1.134	44410.8	Pass	97.23
30°C	2140692.6	1.134	44411.8	Pass	96.54
35°C	2440534.2	1.15	43364	Pass	98.31
Change in wavelength					
218	2267180.2	1.11	41507	Pass	96.34
223	2277742.2	1.058	49428.4	Pass	97.13
228	2240606.2	1.122	39620.6	Pass	96.90

Table 15: Recovery level of AP Cream Formulation

%Level		% Recovered	Average recovery	% recovery	Average recover
80%	1	100.12	97.41	97.41	98.11
	2	96.44	97.28	97.28	
	3	97.82	99.65	99.65	
100%	1	101.14	102.61	102.61	100.57
	2	101.92	99.75	99.75	
	3	100.76	99.37	99.37	
120%	1	92.07	101.70	101.70	107.64
	2	91.90	108.82	108.82	
	3	90.30	104.41	104.41	

Table 16: Assay of extract and cream formulation by HPLC

Sr no.	Peak area (Cream)	% Content (w/w) (Cream)	Peak area (Extract)	% Content (w/w) (Extract)
1.	1696480	99.24	919527.3	95.33
2.	1694099	99.05	944239.4	99.96
3.	1661267	96.52	924665.2	96.29
4.	1660640	96.47	935818.2	98.38
5.	1646079	95.35	939652.9	99.10
6.	1657257	96.21	935442.3	98.31
Average	1669304	97.14	933224.2	97.89
± S.D.	20868.99	1.61	9329.238	1.75
RSD	1.250161	1.66	0.999678	1.78

Table 17: Linearity Table for Andrographolide by HPTLC

Sr. No	Conc. of Andrographolide (ng/band)	Average Peak area of Andrographolide
1.	100	586.63
2.	250	1379.66
3.	500	2663.43
4.	600	3076.1
5.	750	3896.2
6.	1000	5072.51

Table 18: Summary of linear regression data by HPTLC

Parameters	Andrographolide
Linear regression equation	4.9894x + 82.643
Slope± SD	4.9894± 0.001
Intercept±SD	82.643± 0.06
Correlation coefficient	0.99970

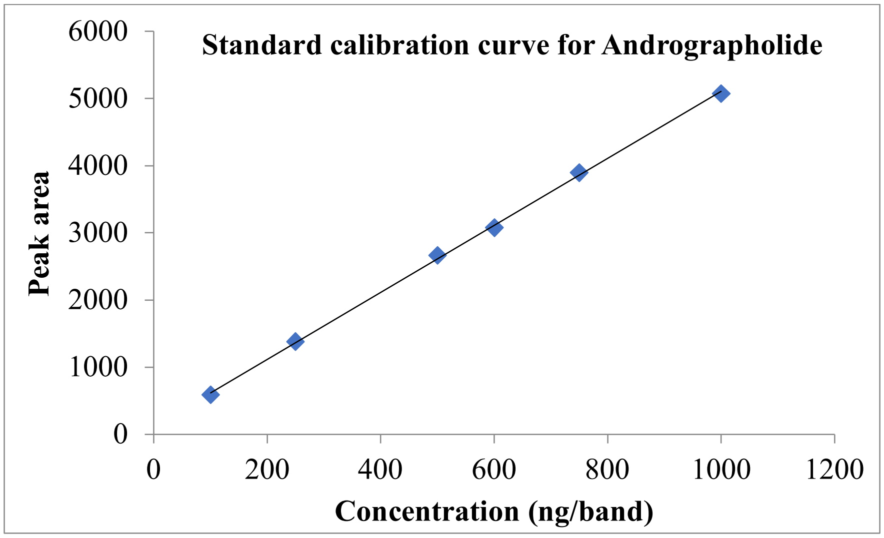


Figure 3: Linearity graph for standard Andrographolide

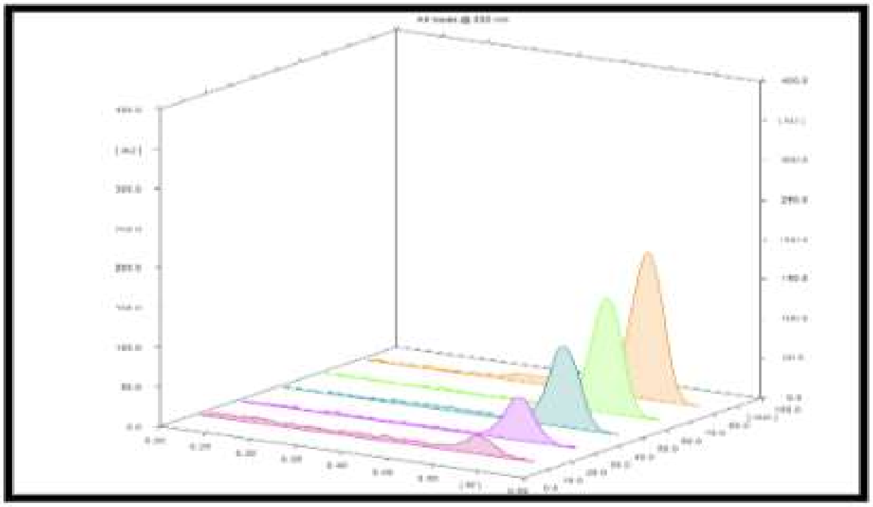
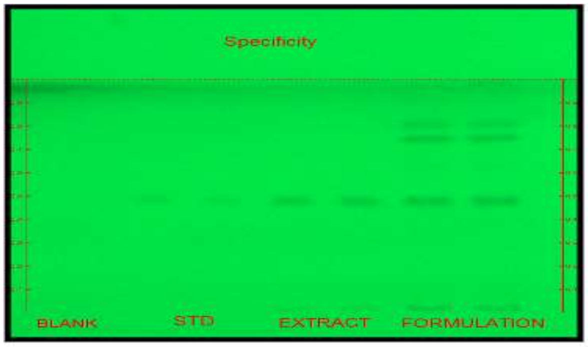
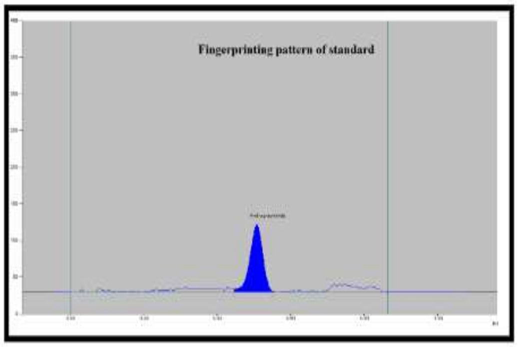


Figure 4: 3-D Chromatogram of Andrographolide





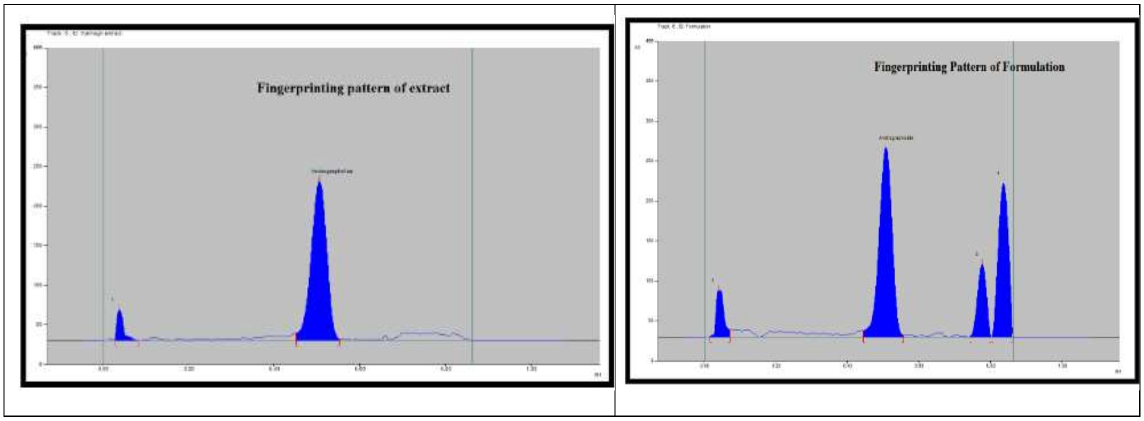


Figure 5: Comparative 2D chromatogram of standard Andrographolide, AP extract and cream formulation

Table 19: Robustness study for the developed HPTLC method

Sr. No.	Changes in the mobile phase composition 1	Changes in the mobile phase composition 2	Change in development distance 1	Change in development distance 2	Change in chamber saturation time 1	Change in chamber saturation time 2
	Peak area (Extract)					
1	2156.8	2190.41	2223.9	2195.21	2180.38	2143.51
2	2178.65	2176.15	2186.9	2119.43	2187.92	2109.17
3	2200.3	2207.3	2234.65	2106.65	2172.05	2143.11
Average	2178.58	2191.28	2215.15	2140.43	2180.12	2131.93
±S. D	21.75	15.59	25.04	47.86	7.93	19.71
% RSD	0.99	0.71	1.13	2.23	0.36	0.92
% Content	98.61	99.2	100.33	96.81	98.68	96.41
Peak area (Cream)						
1	2305.19	2327.16	2299.11	2292.23	2272.92	2290.14
2	2387.12	2346.05	2214.42	2282.03	2205.08	2265.68
3	2281.36	2275.36	2225.36	2282.41	2206.41	2286.82
Average	2324.56	2316.19	2246.30	2285.56	2228.14	2280.88
±S. D	45.30	29.88	37.61	4.72	31.67	10.83
% RSD	1.95	1.29	1.67	0.21	1.42	0.47
% Content	98.53	98.17	95.05	96.82	94.3	96.61

Table 20: Recovery study of HPTLC method for Andrographolide

Recovery level	Peak area (Extract)	% Recovery in Extract	Peak area (Cream)	% Recovery in (Cream)
80% - 1	1867.12	96.14	1990.11	98.53
80% - 2	1879.43	96.78	1993.90	98.68
80% - 3	1858.09	95.65	1945.36	96.20
Average	1868.21	96.19	1976.46	97.80
100% -1	2070.44	96.68	2167.19	97.61
100% - 2	2106.05	98.43	2186.73	98.50
100% - 3	2057.65	96.05	2148.92	96.72
Average	2078.05	97.05	2167.61	97.61
120% - 1	2279.91	97.39	2398.21	99.16
120% - 2	2245.86	95.88	2345.93	96.89
120% - 3	2259.21	96.50	2350.73	97.10
Average	2261.66	96.59	2364.96	97.72

Table 21: Assay of Extract and cream formulation by HPTLC

Sr no.	Peak area (Cream)	% Content (w/w) (Cream)	Peak area (Extract)	% Content (w/w) (Extract)
1.	2356.13	99.92	2205.89	99.89
2.	2387.12	101.28	2115.87	95.65
3.	2281.36	96.63	2172.3	98.31
Average	2341.53	99.28	2164.687	97.95
± S.D.	54.36	2.39	45.49	2.14
RSD	2.32	2.40	2.10	2.18

CONCLUSION

A cream formulation of Andrographolide leaves extract was developed. This cream may find anti-inflammatory or antiallergic use. HPLC and HPTLC method was successfully developed and validated for determination of Andrographolide from extract and cream formulation.

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