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Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF HEPATOPROTECTIVE DITERPENOID ANDROGRAPHOLIDE IN POLYHERBAL FORMULATIONS

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ABSTRACT

Objective: The present study was designed with an objective of the development and validation of a simple, rapid, precise, accurate and specific HPTLC method for the determination of hepatoprotective diterpenoid andrographolide from polyherbal formulations.

Methods: High performance thin layer chromatography (HPTLC) method was developed and validated for rapid analysis of determination of andrographolide. Chromatographic separation was achieved on precoated silica gel HPTLC aluminium plate 60 F254 using Chloroform: Methanol (9:1, v/v) as mobile phase. Detection was performed at 232 nm wavelength densitometrically.

Results: The Rf value for andrographolide was found to be 0.64. Linearity was observed in the concentration range of 200-1000 ng/spot for andrographolide. The limit of detection and limit of quantitation were found to be 3.05 ng/spot and 18.28 ng/spot respectively for andrographolide. The method was validated according to the ICH guidelines with respect to precision, accuracy, linearity, robustness and specificity.

Conclusion: The developed method can be used for routine quality control analysis of hepatoprotective diterpenoid andrographolide in polyherbal formulations.

Keywords: Andrographolide, HPTLC, Validation, Polyherbal formulations.

INTRODUCTION

From the past few decades compounds from natural sources have been gaining importance because of the vast chemical diversity that they offer. This has led to phenomenal increase in the demand for herbal medicines and a need has been felt for ensuring the quality, safety and efficacy of herbal drugs. Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. In the last two decades HPTLC has emerged as an important tool for the qualitative, semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. This includes developing HPTLC fingerprint profiles and estimation of chemical markers and biomarkers. The major advantage of HPTLC is that several samples can be analysed simultaneously using a small quantity of mobile phase [1].

Herbal medicines are increasingly used by the general population, Andrographis paniculata is one of the most frequently used ingredients in Ayurvedic and Chinese medicine for treatment of various liver diseases. Andrographis paniculata contains andrographolide, has been found to possess a variety of interesting pharmacological effects including immuno-stimulant, aperients, anti-parasitic, tonic, anti-biotic and anti-viral activities. It is used for treating hepatitis B, HIV, flatulence, gastric acidity, bowel complaints and common cold [2-3].

The constituent responsible for the hepatoprotective activity of Andrographis paniculata is andrographolide, has been used as a marker substance. Literature survey revealed that the estimation of andrographolide in marketed polyherbal formulations using validated HPTLC methods has not been reported. Most of these methods are not precisely validated. Hence the objective of this work is to develop and validate simple, rapid, precise and accurate HPTLC method for estimation of andrographolide in polyherbal formulations. The HPTLC fingerprint serves a highly useful purpose in evaluating the changes in chemical composition during storage [4-5].

MATERIALS AND METHODS

Chemicals and reagents

Standard Andrographolide was obtained from Yucca laboratories, Mumbai. All chemicals and reagents used were of analytical grade. Marketed formulation Amylcure (Brand-A), Livomyn (Brand-B), Liv-compound (Brand-C) were purchased from local market.

Equipment

A Camag HPTLC system comprising of Camag linomat-V automatic sample applicator, Twin trough development chamber, Hamilton syringe (100µl), Camag TLC scanner-3, win cats software version-1.3.3, Camag reprostar-3, Camag TLC plate heater were used during the study.

Preparation of standard solution

An accurately weighed quantity of andrographolide 1 mg was transferred to 10 ml volumetric flask and dissolves in methanol and made the volume up to 10 ml (100 ng/µl).

Preparation of sample solution

The twenty tablets were weighed and powdered. The 1 gm powder was withdrawn and dissolved in methanol (8 ml). The solution was refluxed for 30 min and filtered through whatman filter paper no.-41. The volume was made upto 10 ml with methanol.

Validation of the Method

After the development of HPTLC method for the estimation of the polyherbal formulations, validation of the method was carried out according to the ICH guidelines with respect to Precision, Accuracy, Linearity, Limit of Detection and Limit of Quantification, Robustness, Specificity etc [6-8].

RESULTS AND DISCUSSION

A wavelength of 232 nm was chosen for quantification. The Rf value of andrographolide after development with the mobile phase Chloroform: Methanol (9:1, v/v) was 0.64. When the concentrations and their respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2=0.997) was observed between the concentrations of andrographolide and the respective peak areas in the range 200-1000 ng/ spot. The regression equation for andrographolide was found to be Y=10.67x+198.9 where 'Y' is the peak area and 'X' is the concentration of andrographolide**.** The regression equations were used to estimate the amounts of andrographolide in tablet polyherbal formulations or in validation study (precision and

accuracy). The content of andrographolide present in polyherbal formulations were shown in Table 7. The chromatograms containing peaks of andrographolide in polyherbal formulations were shown in Fig: 2, 3 and 4 respectively [9-10].

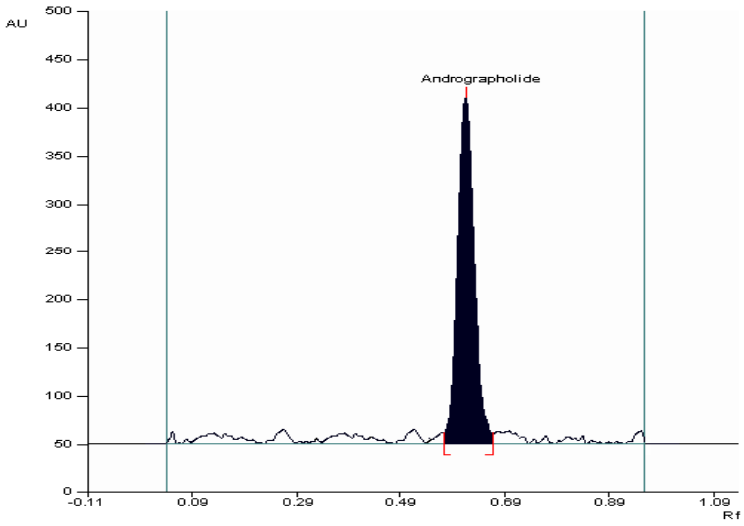

Fig. 1: Chromatogram of standard Andrographolide

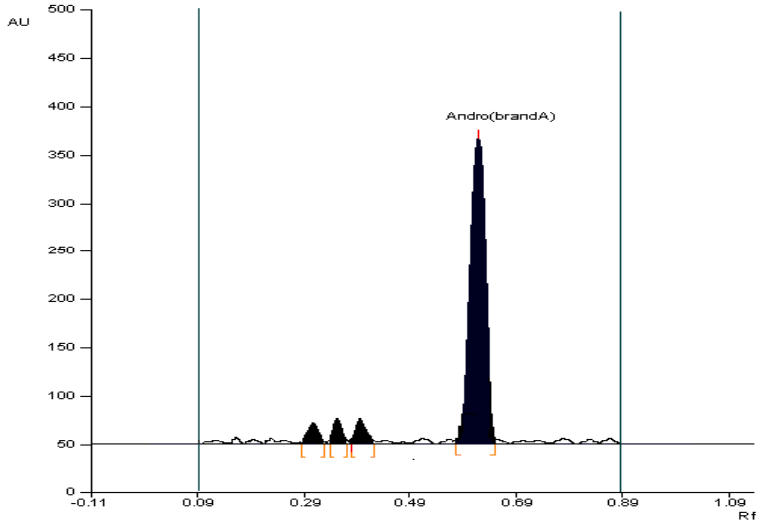

Fig. 2: Chromatogram of brand-A containing Andrographolide

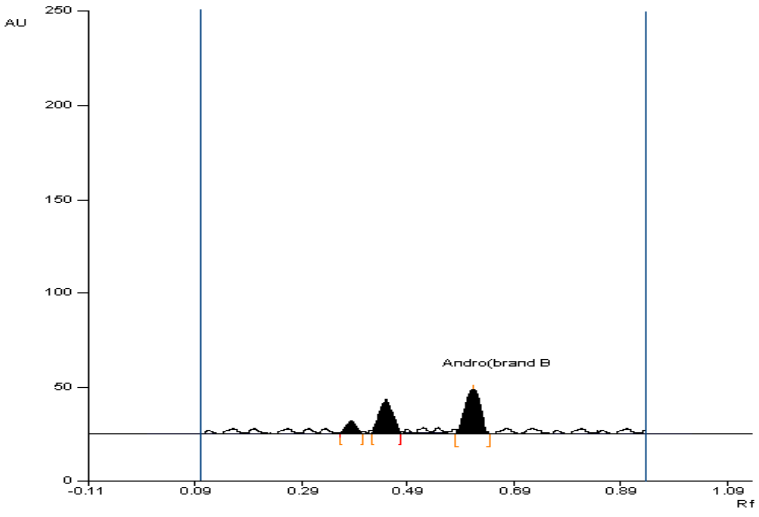

Fig. 3: Chromatogram of brand-B containing Andrographolide

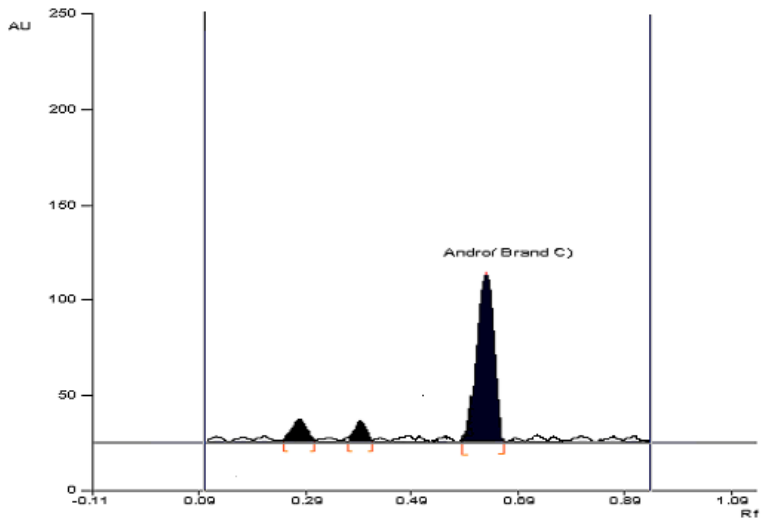

Fig. 4: Chromatogram of brand-C containing Andrographolide.

Table 1: Method Validation parameters for estimation of andrographolide by HPTLC

Parameters	Andrographolide biomarker
Calibration range (ng / spot)	200-1000
Detection wavelength	232 nm
Mobile phase	Chloroform: Methanol (9:1, v/v)
Rf value	0.64
Regression equation	Y=10.67x+198.9
Slope	10.67
Intercept	198.9
Correlation coefficient (R^2)	0.997
Limit of detection (ng/spot)	3.05
Limit of quantitation (ng/spot)	18.28
Specificity	Specific

Table 2: Analysis data of andrographolide in polyherbal formulations (n=5)

Sample	Amount of andrographolide estimated (ng)	% Mean estimated	SD	% RSD
Brand-A	585.24	99.25	0.55	0.551
	586.41			
	581.00			
	580.48			
	582.21			
Brand-B	175.23	99.51	0.50	0.501
	172.45			
	171.22			
	173.21			
	176.60			
Brand-C	407.14	99.31	0.58	0.588
	406.41			
	401.23			
	400.19			
	404.44			

n is number of determination, SD is standard deviation, RSD is relative standard deviation

Table 3: Intra-day and inter-day precision of the method (n = 6)

Sample	Amount (ng/spot)	Intra-day precision			Inter-day precision		
		Mean area	SD	% RSD	Mean area	SD	% RSD
Brand-A	200	2277.34	1.50	0.023	2279.23	1.51	0.062
	600	6890.33	1.55	0.058	6887.90	1.68	0.069
	1000	10890.69	1.48	0.061	10891.45	2.09	0.035
Brand-B	200	2275.68	1.67	0.045	2277.75	1.69	0.045
	600	6890.35	1.48	0.056	6891.45	1.43	0.056
	1000	10892.27	2.13	0.032	10894.23	1.45	0.063
Brand-C	200	2278.78	1.50	0.045	2280.18	1.51	0.067
	600	6893.55	1.56	0.056	6896.25	1.67	0.076
	1000	10894.23	1.45	0.063	10896.03	1.97	0.047

n is number of determination, SD is standard deviation, RSD is relative standard deviation

Table 4: Recovery study of andrographolide by HPTLC in Polyherbal formulations (n=6)

Sample	Initial amount (ng/spot)	Amount added (ng/spot)	Amount recovered (ng/spot)	Recovery ± SD (%)	% RSD
Brand-A	583.55	466.84	99.13	99.13±0.16	0.167
		583.55	101.29	101.29±0.24	0.245
		700.26	99.81	99.81±0.66	0.668
Brand-B	174.5	139.32	99.45	99.45±0.17	0.178
		174.15	101.38	101.38±0.30	0.301
		208.98	99.60	99.60±0.50	0.508
Brand-C	403.11	322.48	99.32	99.32±0.16	0.169
		403.11	101.07	101.07±0.25	0.251
		483.73	99.77	99.77±0.30	0.309

n is number of determination, SD is standard deviation, RSD is relative standard deviation

Table 5: Preparation of standard calibration curve (n=6)

Sr. No	Conc. (ng/spot)	Peak Area
1	200	2277.13
2	400	4469.26
3	600	6831.40
4	800	8524.01
5	1000	10928.21

n is number of determination

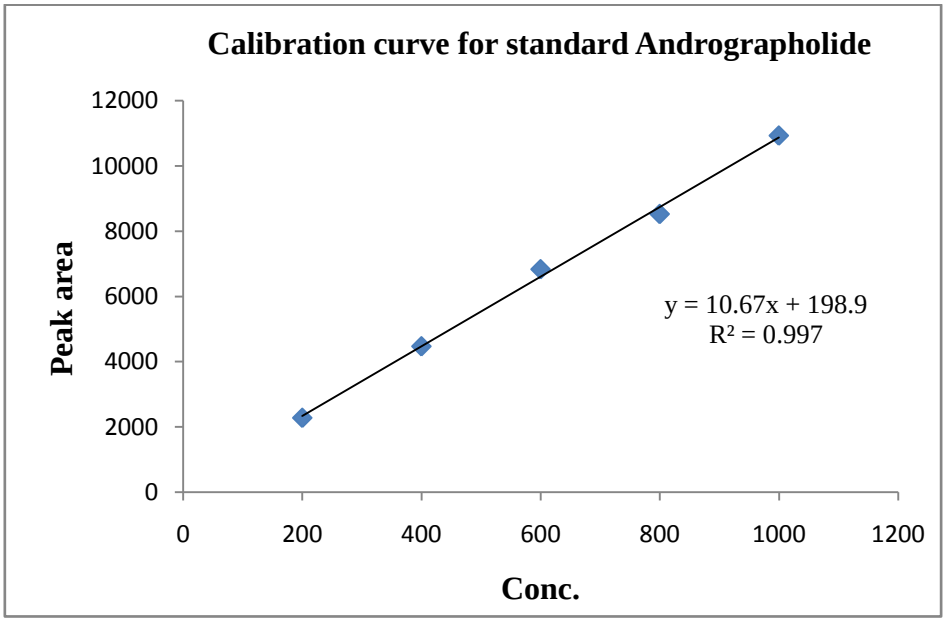

Fig. 5: Standard calibration curve for Andrographolide

Table 6: Detection/Quantification limits of andrographolide in formulations (n=3)

Parameters	Andrographolide (ng/spot)
LOD	3.05
LOQ	18.28

n is number of determination

Table 7: Content of andrographolide in formulations (n=3)

Sample	Content of andrographolide (%)
Brand-A	0.491
Brand-B	0.117
Brand-C	0.231

n is number of determination

Table 8: Robustness of the HPTLC method (n =3)

Parameters	Brand-A SD % RSD	Brand-B SD % RSD	Brand-C SD % RSD
Mobile phase composition	1.49 0.425	1.48 0.435	1.46 0.429
Mobile phase Volume (18,20,22 ml)	1.59 0.315	1.62 0.319	1.598 0.318
Duration of Saturation (20,30,40 min)	1.32 0.284	1.38 0.278	1.35 0.286
Activation of prewashed TLC plates (2.5 and 7 min)	1.31 0.167	1.30 0.171	1.32 0.176

n is number of determination, SD is standard deviation, RSD is relative standard deviation

Precision

The precision of the method in terms of intra-day precision (% RSD) was determined by analysing andrographolide standard solutions in the range (200-1000 ng/spot) three times on the same day. Inter-day precision (% RSD) was assessed by analysing these solutions (200 - 1000 ng/spot) on three different days over a period of one week. The results of the precision studies are shown in Table 3.

Accuracy

Determination of method accuracy by the standard addition method at three concentrations (80%, 100%, 120%) levels. This was done to check for the recovery of the andrographolide at different levels in the formulations. The results are shown in Table 4.

Linearity

The linearity was found in the concentration range of 200-1000 ng/spot. The correlation coefficient was found to be 0.997 for andrographolide. The results are presented in table 5. The standard solution (100 ng/µl) was applied as 200-1000 ng/spot. The calibration curve was found to be linear in this concentration range.

Limit of Detection and Limit of Quantification

The LOD and LOQ of andrographolide were found to be 3.05 ng/spot (minimum concentration at which andrographolide is detected) and 18.28 ng/spot (concentration at which andrographolide is quantified) respectively. The content of andrographolide in formulations were calculated and reported in Table 7. The drug peak-area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area.

Robustness of the method

By introducing small changes in the mobile phase composition, mobile phase volume, duration of mobile phase saturation and activation of prewashed TLC plates with methanol, the effects on the results were examined. Robustness of the method was done in triplicate at a concentration level of 600 ng/spot and the % RSD of peak areas were calculated.

Specificity

The specificity of the method was ascertained by analysing the standard drug and marketed polyherbal formulations. The spots for andrographolide in the samples were confirmed by comparing the Rf values of the spot with that of the standard. The peak purity of the andrographolide was assessed by comparing the spectra at three different levels, viz. peak start (S), peak apex (M) and peak end (E) positions of the spots [11-12].

CONCLUSION

In the present study, the estimation of andrographolide in marketed polyherbal formulations by HPTLC, wide variations in the content of andrographolide in the formulations to be administered or prescribed by the physicians were observed. This shows that polyherbal formulations are not standardized. This leads to marked differences in the therapeutic efficacy of the formulations when administered. Hence, the newly developed method for the estimation of andrographolide in polyherbal formulations can be adapted to standardize the formulations and the content andrographolide can be altered during the formulation stage, thus ensuring desired therapeutic efficacy of the herbal product. This would also minimize or avoid batch-to-batch variations in the therapeutic efficacy of such polyherbal formulations.

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REFERENCES

1. Singh VK, Govil JN. Standardization of Herbal/Ayurvedic formulations: Recent progress in medicinal plants, Vol 24, 3rd ed. Stadium press: LLC publishers; 2006. p. 25-44.
2. Indian Pharmacopoiea, Ministry of health and family welfare, Vol. I, 5th ed. Indian Pharmacopoeial commission, Ghaziabad, India; 2007. p. 178-183.
3. Mukherjee PK. Quality Controle of Herbal Drugs. Business Horizons; 2003. p. 701-704.
4. Shinde VM, Onalwal K, Biradas YS, Mahadik KR, Rajani YM. Phytochemical evaluation and validation of polyherbal formulation using HPTLC. Phcog Mag 2008; 4: 218-224.
5. Shah P, Trivedi P, Pundarikakshudu K. Spectrophotometric determination of Andrographolide in *Andrographis Paniculata Nees* and its formulation. Indian Journal of Pharmaceutical Sciences 2001; 60(4): 457-459.
6. Chauhan SK, Singh BP, Kimathi GP, Agrawal S. Determination of Andrographolide in *Andrographis Paniculata* by High Performance Thin Layer Chromatography. Indian Drugs 1998; 36(2): 130-132.
7. Gautam V, Ajudhia NK. High performance thin layer chromatography method for simultaneous estimation of vicine, trogonelline and withaferin-A in polyherbal antidiabetic formulation. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(2): 367-371.
8. Yadav SS, Rao JR. Simultaneous HPTLC analysis of bisoprolol fumarate and hydrochlorthiazide in pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(2): 286-290.
9. Raina AP, Kumar A, Pareek SK. HPTLC Analysis of Hepatoprotective Diterpenoid Andrographolide from *Andrographis Paniculata Nees (Kalmegh).* Indian Journal of Pharmaceutical Sciences 2007; 69(3): 473-475.
10. Rajini M, Bagul MS. Phytochemical evaluation of classical formulation, A case study of Prabhakar Vati, Indian Drugs 2005; 42(1): 173-175.
11. International Conference on Harmonization (ICH), Validation of Analytical Procedures; Methodology, Q2B, Geneva 1996, 3-9.
12. International Conference on Harmonization (ICH), Validation of Analytical Procedures; Methodology, Q2A, Geneva 1994, 1-5.