International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-3, 2016 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Stability Indicating RP- HPLC Method for Determination of Andrographolide

Mrinalini. Damle*, Namrata Gujar

Department of Quality Assurance, AISSMS College of Pharmacy, Kennedy Road, Near R.T.O., Pune-411001, Maharashtra, India.

Manuscript No: IJPRS/V5/I3/00115, Received On: 14/07/2016, Accepted On: 24/07/2016

ABSTRACT

Andrographis Paniculata (Kalmegh) is used since ancient time for treatment of various diseases viz cancer, malaria, diabetes etc. Andrographolide is one of the major phytoconstituent responsible for the activity, which is a diterpene lactone. An approach for the stress degradation was successfully applied for the development of stability indicating HPLC method for the determination of Andrographolide. The system comprised of HiQ Sil C_{18} (150*4.6 mm particle size 5µ) column as stationary phase and 65:35 *v/v* mixture of methanol:water as mobile phase(flow rate 1ml/min) and a UV detector at wavelength of 226nm. The retention time of Andrographolide was found to be 3.85 ± 0.2min. The marker was subjected to acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. Stress testing of Andrographolide was carried out according to the International Conference of Harmonization (ICH) guideline Q1A (R2). The method was successfully validated according to ICH guidelines Q2 (R1). The calibration curve was linear over the concentration range of 10 -50 µg/ml (r^2=0.993). The accuracy of the method was established based on the recovery studies. The LOD and LOQ of Andrographolide was found to be 0.60 µg/ml and 1.82 µg/ml respectively. Among various stressed conditions, Andrographolide showed considerable degradation under alkali and acid catalyzed hydrolysis, oxidative and photolytic condition.

KEYWORDS

Andrographis Paniculata, Andrographolide High Performance Liquid Chromatography, Stability Indicating Method

INTRODUCTION

Kalmegh (Andrographis paniculata (Burm. f.) Nees) is wildly distributed medicinal plant in India and is being used since ancient times in traditional ayurvedic system of medicine[1]. It is contains diterpenes, lactones and flavonoids. The plant is traditionally used as antibacterial, antioxidant, antipyretic, antidiabetic, and in treatment of various diseases ranging from malaria to dysentery[2].

***Address for Correspondence:**
Mrinalini Damle
Department of Quality Assurance, AISSMS College of Pharmacy, Kennedy Road, Near R.T.O., Pune-411001, Maharashtra, India.
E-Mail Id: mcdamle@rediffmail.com

The primary bioactive component of the medicinal plant Andrographis Paniculata is Andrographolide, which is colourless crystalline bicylic diterpene lactone[3]. Andrographolide, which is grouped as an unsaturated trihydroxy lactone has the molecular formula of $C_{20}H_{30}O_5$. The molecular structure of andrographolide is shown in Figure 1[4].

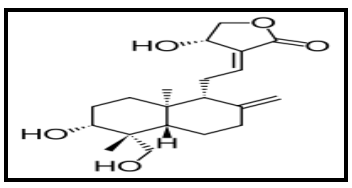

Figure 1: Structure of Andrographolide

Literature survey for Andrographis Paniculata reveals HPTLC[5-8], HPLC[9-10], UV[11] methods reported for estimation of Andrographolide, but there is no stability indicating HPLC method reported. Hence, an isocratic HPLC method has been developed in the present work for quantitation of Andrographolide.

MATERIAL AND METHODS

Chemicals and Reagents

Working Standard Andrographolide (Marker), along with the Raw Material (Stems of *Andrographis Paniculata*) was purchased from Yucca Enterprises, Mumbai. The marker was used as such, without any further purification. Stems of *Andrographis Paniculata* were authenticated from Agharker Research Institute, Pune. Methanol (HPLC grade), Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2 30% *v/v*), NaOH Pellets, were purchased from S. D. fine chemical Laboratories, Mumbai.

Instruments

Jasco (Model V-550) UV-Visible Double beam spectrophotometer, Elga Lab water (PURELAB UHQ-II) HPLC water purification system, Shimadzu (Model AY-120) Balance and Jasco HPLC system comprising: Model PU 2080 Plus pump, Rheodyne sample injection port, HiQ Sil C18 (150*4.6 mm particle size 5µ) column, UV detector, Borwin-UV software (version 1.5) , Newtronic Photostability chamber (Model IC DAC version 1.2) was used.

Chromatographic Conditions

The mobile phase consisting Methanol:water in the ratio of 65:35*v/v*, was filtered through 0.45µm membrane filter, sonicated and was pumped from the solvent reservoir. The flow rate of mobile phase was maintained at 1ml/min and the response was monitored at 226nm with a run time of 7 min. The volume of injection loop was 50µl. The column and the HPLC system were kept at ambient temperature.

Preparation of Standard Stock Solution

Standard stock solution of Andrographolide was prepared by dissolving 10 mg of marker in 10 ml of methanol to get concentration of 1000 µg/ml, which was further diluted appropriately to get a concentration of 300µg/ml. From the standard stock solution, working standard solution was prepared containing 10-50µg/ml of Andrographolide in methanol.

UV spectrum of the working standard solution was obtained as shown in Figure 2. From the spectrum of Andrographolide, 226nm was selected as detection wavelength. Standard chromatogram at λ_{max} 226 nm is as shown Figure 3.

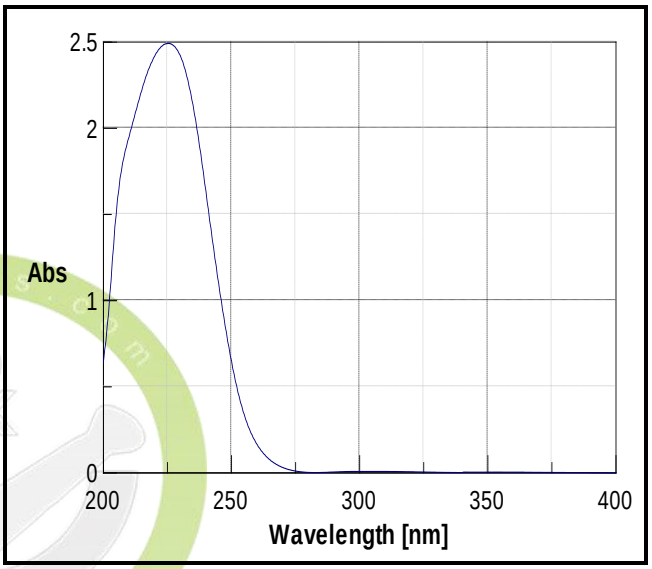

Figure 2: UV Spectrum of Andrographolide

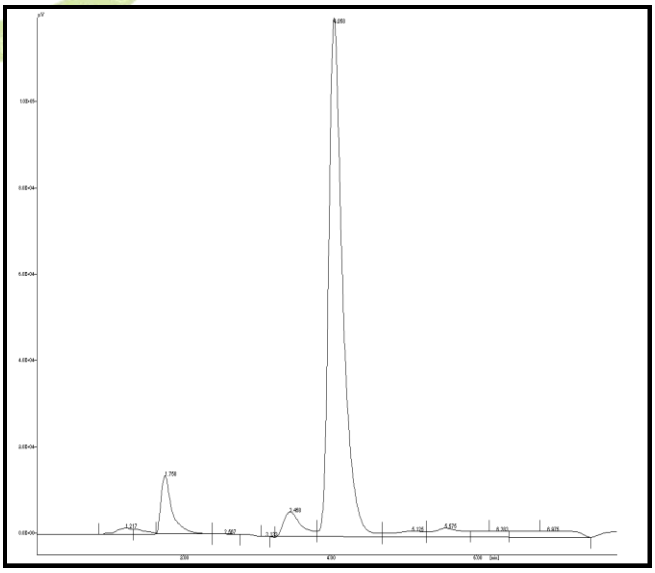

Figure 3: Chromatogram of Andrographolide (10 µg/ml)

The system suitability parameters are given in Table 1.

Table 1: System Suitability Parameters

Name	RT (Min)	Concentration (µg/ml)	Area	Theoretical Plates	Asymmetry
Andrographolide	3.95	10	1353790.79	2502.89	1.34

Stress Degradation Study of Andrographolide

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis as per ICH Q1A (R2) guideline. For each study, samples were prepared as follows[12,13]:

1. The blank subjected to stress in the same manner as the marker solution
2. Andrographolide working standard solution subjected to stress condition.

Optimized conditions[14] reported by us earlier were used to obtain about 10-30 % degradation

Alkaline Hydrolysis

To 1 ml of 300 µg/ml solution of Andrographolide, 1 ml of 0.1 N NaOH was added. The volume was made up to 10 ml with methanol. The above solution was kept 30 mins at room temperature in dark place.

Acidic Hydrolysis

To 5 ml standard stock solution of Andrographolide (300 µg/ml) with 5 ml of 1N HCl was added. The volume made up to 50 ml with methanol. Solution was refluxed for 1 hr.

Neutral Hydrolysis

To 5 ml standard stock solution of Andrographolide (300 µg/ml) with 5 ml water and volume made up to 50 ml with methanol. The solution was refluxed for 1.

Oxidation

To 5 ml standard stock solution of Andrographolide (300 µg/ml), 5 ml of 30% v/v solution of H_2O_2 was added and volume made up to 10 ml with methanol. Solution was refluxed for 1 hr.

Degradation under Dry Heat

Dry heat studies were performed by keeping drug sample in oven (60^0 C) for 8 hrs.

Photo-Degradation Studies

Photolytic studies was carried out by exposure of marker to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hrs. Sample was weighed, dissolved in methanol and appropriate dilutions were made to get final concentration of 30 µg/ml.

RESULTS AND DISCUSSION

Under optimized chromatographic conditions, retention time of Andrographolide was found to be 3.85 ±0.2 min. Degradation was observed for Andrographolide during stress conditions like Acid, alkali, neutral hydrolysis, oxidation, dry heat and photolysis.

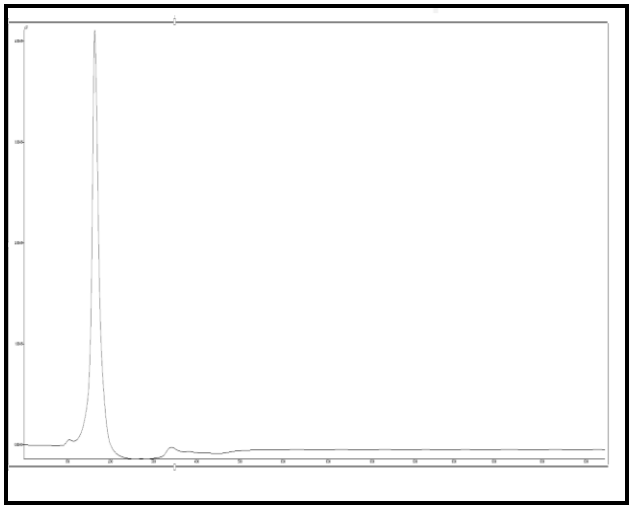

Figure 4: Blank after Alkali treatment

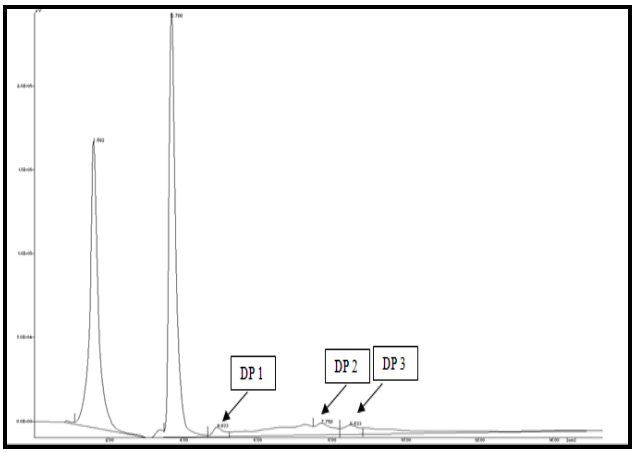

Figure 5: Alkali treated Andrographolide

Andrographolide showed 3 degradation peaks under Alkali hydrolysis. Summary of stress degradation results is given in Table.1. Peak purity results greater than 980 indicate that Andrographolide peaks are homogeneous in all stress conditions tested indicating noninterference of product of degradation. The unaffected assay of Andrographolide confirms the stability indicating power of the method.

Table 2: Summary of stress degradation study

Stress Degradation Condition	% Recovery	Rt of the degradation product
Acid (1 N HCl, refluxed for 1 hrs)	83.38	DP1-4.93, DP2-7.75, DP3- 8.53.
Alkali (0.1 N NaOH, kept for 30 min)	78.96	-
H_2O_2 30% (refluxed for 1 hr)	88.79	-
Neutral hydrolysis (refluxed for 1 hr)	97.80	-
Heat dry (60^0C, 8 hrs)	73.44	-
Photo stability	85.03	-

Method Validation

Method was validated as per ICH Q2A (R1) guideline[15].

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 980, indicating the non interference of any other peak of degradation product, impurity or matrix.

Linearity

From the standard stock solution (1000µg/ml) of Andrographolide working standard solutions were prepared containing 10-50µg/ml of Andrographolide. These solutions were used for injection. Five replicates per concentration were injected.

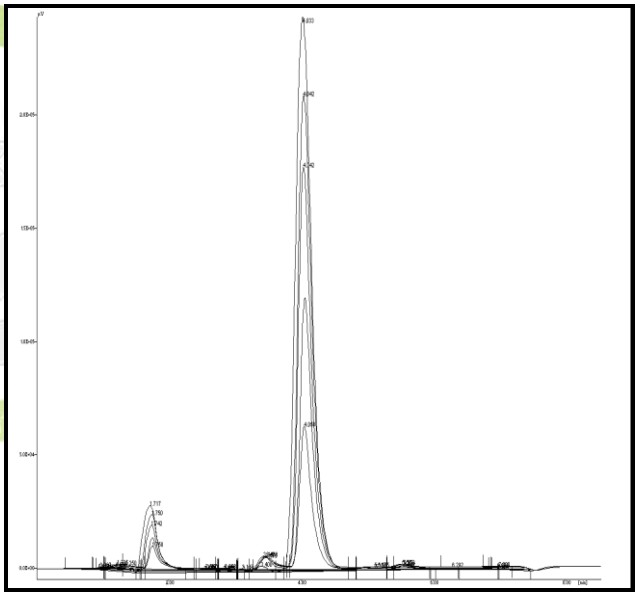

Figure 6: Overlay spectra of linearity of Andrographolide

Table 3: Linearity of Andrographolide

Sr. No	Concentration (µg/ml)	Avg. Peak Area
1	10	1334766.77
2	20	2422758.43
3	30	3762623.24

4	40	5588530.76
5	50	6767292.88

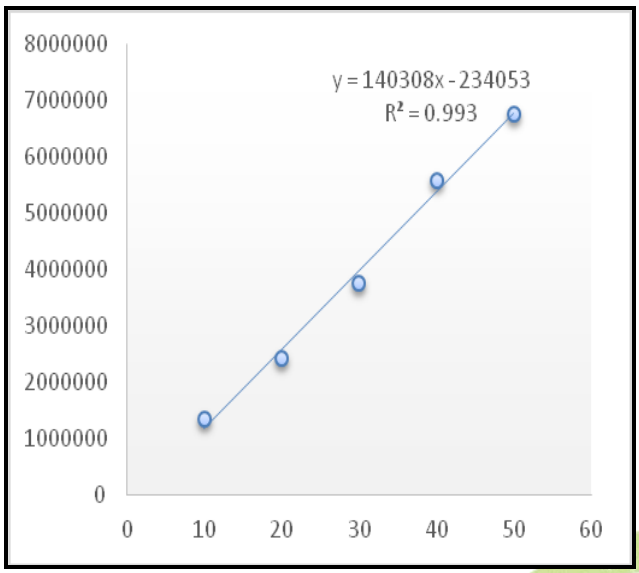

Figure 7: Calibration curve of Andrographolide

Precision Interday Precision

Precision of the system was evaluated by analyzing three independent standard preparations on three different days and % RSD value was calculated to determine system precision. Result are tabulated in.

Intraday Precision

Precision of the system was evaluated by analyzing six independent standard preparations in a day and % RSD value obtained was calculated to determine system precision. Results are tabulated in Table 4.

Table 4: Inter-day precision

Concen-tration (µg/ml)	Mean Area	SD	%RSD
10	1347111.70	19863.65	1.47
20	2433946.10	43765.75	1.79
30	3763388	58677.81	1.55

Table 5: Intra-day precision

Replicate	Intraday
1	1376526.49
2	1339721.54
3	1327593.34
4	1352789.93
5	1345921.28
6	133478.21
Mean Area	1346222.632
SD	17214.63981
% RSD	1.27

Accuracy

Accuracy studies were performed by standard addition method by adding 80%, 100%, and 120% with respect to target assay concentration (16µg/ml).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD was calculated by = LOD=3.3 σ/s
LOQ was calculated by= LOQ=10 σ/s

Where,

σ = standard deviation of response for the lowest conc. in the range

S = slope of the calibration curve.

LOD of Andrographolide = 0.60µg/ml and

LOQ of Andrographolide = 1.82µg/ml

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which wavelength, flow rate, mobile phase composition were altered and the effects on the area was noted. The results obtained are shown in Table 6.

Table 6: Recovery studies of Andrographolide

Level %	Sample (µg/ml)	Standard (µg/ml)	Area	Mean Area	% Recovery
80	16	12	3694571.28	3751646.35	101.45
			3834879.13		
			3725486.64		
100	16	16	4396110.19	4384592.65	102.8
			4236419.15		
			4521248.63		
120	16	20	5097651.23	4909486.56	100.8
			4757343.21		
			4773465.26		

Table 7: Robustness study

Sr. No	Parameter	%RSD
1	Mobile phase composition- Methanol : water :: 65:35 ± 1ml	1.25
2	Flow rate (1ml/min) ± 0.05	0.92
3	Wavelength (226) ± 1nm	0.74

Table 8: Extract assay for Andrographolide

Injected extract (µg/ml)	Area	Mean Area	% Assay
200	1985614.23	2024048.72	8.04
	2061942.51		
	2024589.43		

Assay

Methanolic extract was prepared by dispersing the 4gm of Andrographis paniculata stem powder in 100 ml methanol. The solution for kept for 24 hrs, after which it was filtered and the methanol was evaporated to dryness at room temperature to obtain methanolic extract.

Assay of extract was carried out by preparing 1000 µg/ml stock solution. Analysis was repeated for three times. Sample solution was injected and area was recorded. Concentration was determined from linearity equation.

DISCUSSION

As compared to other methods in literature; the developed method has advantage that it is stability indicating, whereas other referred methods are simple assay methods.

CONCLUSION

The developed method was found to be simple, economic and stability indicating. This method can be used for monitoring stability of Andrographolide.

ACKNOWLEDGEMENT

Authors are thankful to the Principal, AISSMS College of Pharmacy, Pune for providing the necessary facilities for research work.

REFERENCES

1. Sharma, M., & Sharma, R. (2013). Identification, purification and quantification of andrographolide from Andrographis paniculata (burm. F.) Nees by HPTLC at different stages of life cycle of crop. *Journal of Current Chemical and Pharmaceutical Sciences*, *3*(1), 23-32.

2. Das, P., & Srivastav, A. K. (2014). Phytochemical extraction and characterization of the leaves of Andrographis paniculata for its anti-bacterial, anti-oxidant, anti-pyretic and anti-diabetic activity. *International Journal of Innovative Research in Science, Engineering and Technology*, *3*, 15176-15184.

3. Jarukamjorn, K., & Nemoto, N. (2008). Pharmacological aspects of Andrographis paniculata on health and its major diterpenoid constituent andrographolide. *Journal of Health Science*, *54*(4), 370-381.

4. Jadhao, D., & Thorat, B. (2014). Purification (crystallization) of bioactive ingredient andgrographolide from andrographis paniculata. *World Journal of Pharmacy and Pharmaceutical Sciences*, *3*, 747-763.

5. Manvitha. (2013) Determination of Andrographolide in Andrographis Paniculata Extract with and Without Human Serum by High Performance Thin Layer Chromatography. *International Research Journal of Pharmacy*, 4(12), 41-45.

6. Jadhao, M. (2010). Estimation of Andrographolide in Herbal Powder and Polyherbal Asava by HPTLC. *International Journal of Pharma and Bio Sciences*, *1*, 242-245.

7. Mamatha, A. (2011). Quantitative HPTLC analysis of andrographolide in Andrographis paniculata obtained from different geographical sources (India). *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(2), 42-44.

8. Zade, S. R., Priya, B., Bagmar, U. R., & Ganjiwale, R. O. (2013). Development and validation of HPTLC method for estimation of hepatoprotective diterpenoid andrographolide in polyherbal formulations. *International Journal of Pharmacy and Pharmaceutical Science*, *5*(3), 976-980.

9. Patarapanich, C., Laungcholatan, S., Mahaverawat, N., Chaichantipayuth, C., & Pummangura, S. (2007). HPLC determination of active diterpene lactones from Andrographis paniculata Nees planted in various seasons and regions in Thailand. *Thai Journal of Pharmaceutical Sciences*, *31*(3-4), 91-9.

10. Sharma, M., Sharma, A., & Tyagi, S. (2012). Quantitative HPLC analysis of Andrographolide in Andrographis paniculata at two different stages of life cycle of plant. *Acta Chimica & Pharmaceutica Indica*, *2*, 1-7.

11. Suneetha, A., & Manasa, K. (2014). Development and Validation of Visible Spectrophotometric Method for Determination of Andrographolide in Kalmegh Plant Extract. *Asian Journal of Pharmaceutical Analysis*, *4*(2), 85-88.

12. Guideline, I. H. T. (2003). Stability testing of new drug substances and products. *Q1A (R2), Current Step*, *4*, 1-24.

13. ICH, Q. B. (1996). Stability Testing: Photostability Testing of New Drug Substances and Products. *Geneva, November*. 1-12.

14. Damle, M., & Gujar, N. (2016). Development and validation of stability indicating hptlc method for determination of andographolide. *Indo American Journal of Pharmaceutical Research*, *6*(6), 5816-5823.

15. ICH Expert Working Group. (2005, April). Validation of analytical procedures: text and methodology Q2 (R1). In *Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use* (pp. 25-27).

