International Journal of Phytopharmacy
Vol. 2 (5), pp.149-153, Sep-Oct 2012
©Scholar Science Journals

Research Article

QUANTITATIVE ESTIMATION OF ANDROGRAPHOLIDE BY REVERSE PHASE-HIGH LIQUID CHROMATOGRAPHY METHOD FROM *ANDROGRAPHIS PANICULATA* NEES.

Dilip B. Jadhao and Bhaskar N. Thorat

Advanced Drying Laboratory, Department of Chemical Engineering, Institute of Chemical Technology (Formerly UDCT), N. P. Road, Matunga (E), Mumbai 400 019, India.

Corresponding Author: dbjadhao@gmail.com

Abstract

Reverse Phase High performance liquid chromatographic method with UV array detection was established for the determination of Andrographolide. The Andrographolide was separated using isocratic solvent system consisting of isopropyl alcohol, formic acid and water (70:10:20 v/v) at flow rate of 1.0 ml/min and the detection wavelength of 223 nm. The method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). The linearity of the proposed method was obtained in the range of 4.5-70 μg/ml for andrographolide with regression coefficient of 0.9999. Intraday and interday precision studies showed the relative standard deviation less than 2.5%. The accuracy of the proposed method was determined by a recovery study conducted at 3 different levels. The average recovery was 97-99%. The LOD and LOQ were 0.03 and 0.05 μg/ml for andrographolide, respectively. The contents of andrographolide obtained in the dried leaves powder was within the ranges of 0.98 – 1.15% w/w, respectively. The proposed method is simple, sensitive yet reproducible. It is therefore suitable for routine analysis of andrographolide in *A. Paniculata* Nees.

Keywords: *Andrographis Paniculata*; andrographolide; Quantitative HPLC

1. Introduction:

Andrographolide (3 - (2 - (Decahydro - 6 -hydroxyl - 5 - (hydroxymethyl) - 5, 8a – dimethyl - 2 – methylenenaphthyl) ethylidene) dihydro - 4 - hydroxyfuran - 2 (3H) - one) is the active principle of *Andrographis paniculata extracts.* The molecular structure of Andrographolide is shown in the **Figure 1.** *Andrographis paniculata* Nees, locally known as Kalmegh, Kalupnath, Kiriat and Mahatila. Its common name is King of Bitters. It grows widely in the tropical area of Asia, and belongs to the acanthaceae family[1]. It has an annual growth of 30–70 cm height. In India, this plant has been extensively used for traditional medicine and helps against fever, dysentery, diarrhea, inflammation, and sore throat[2]. Furthermore, it is a promising new way for the treatment of many diseases, including HIV, AIDS, and numerous symptoms associated with immune disorders [3-4]. Moreover, *Andrographis Paniculata* is reported to possess a wide spectrum of biological activities such as antipyretic, potential cancer therapeutic agent, anti-viral, anti-hyperglycemic[5-8].

Furthermore, the increased interest in plant-derived drugs in recent years is because of their undisputed efficacy as Phytomedicine and because active principles from natural products serve either as templates or as intermediates for synthetic drugs [9]. Despite the sophistication of modern organic synthesis, it is not always economically feasible to synthesize drugs that are similar to these active ingredients. Accordingly, most plant drugs are cultivated and are used clinically as standardized extracts. A standardized extract is an herbal extract that has been processed so that it contains a specified amount of a certain compound, usually the one thought to be the active compound. The amount is then listed on the label with the intent to inform consumers that the product contains the listed amount of active compounds. To achieve this, various methods such as spectrophotometric, thin layer chromatographic, HPLC, HPTLC and electrophoresis method have been suggested for quantitative estimation of andrographolide in *Andrographis paniculata*. The spectrophotometric method proposed by Gained *et.al* [10] proposed a spectrophotometric method by extracting pure andrographolide from kalmegh measuring its absorbance at 226 nm but the extraction process was

very tedious. Maiti *et.al* [11] suffers from the disadvantage that the red color formed with the addition of alcoholic potassium hydroxide to the solution of andrographolide is unstable and fades away quickly. Subbarao [12] has suggested a chemical method involving a lactone titration but the method has been reported to be not suitable for detecting minute quantities. Thin layer chromatographic methods were also described for estimation of andrographolide in *Andrographis paniculata* extracts [13] and also reported by using capillary electrophoresis chromatography[14-15]. High performance liquid chromatographic methods were reported for estimation of andrographolide in *Andrographis paniculata* [16-19].

Even though, a reasonably good literature exists on the separation of andrographolide as seen in the foregoing section, however, no reports have been found in acidic medium describing the separation of andrographolide using small proportion of alcohol (Isopropyl alcohol) in permutation with formic acid and water in the mobile phase system, since andrographolide is having good solubility in alcohol[20] and also the methods described above have several limitations like preparation of samples for estimation of andrographolide. Moreover, the development of suitable mobile phase is an important step in devising an analytical procedure.

In the present study, accurate, simple, specific and reproducible HPLC have been developed and validated [21] for the determination of andrographolide in *A. paniculata* herb, extracts and dosage forms.

2. Experimental

***2.1 Plant Material and chemicals*:** Standard andrographolide was procured from Sigma Aldrich (purity 99.5%). Dried leaves of Andrographis Paniculata nees were purchased from two different herbal suppliers Amrut-Lal (A) and All India stores (B) from the local market in Mumbai. The sample was authentified and the sample specimen was preserved. The dried leaves were ground into powder, passed through a sieve (20 meshs). The samples were separately kept in air tight container and protected from light until used. HPLC grade Isopropyl alcohol from Merck Specialty Private Ltd (Mumbai, India). Deionized water was obtained from in-house Milli-Q Nanopure (Millipore, Bedford, MA, USA).

2.2. Method:

***2.2.1 Preparation of extract*:** Weighed about 10 g of dried leaves powder was extracted with 35 ml of methanol in Soxhlet apparatus at 55°C for 2 hr. The process was repeated for one more time for complete extraction of andrographolide. The two fractions were pooled together and concentrated by distillation to get dry residue.

2.2.2 Chromatography: Agilent (Germany) HPLC system, consisting of a model G1329A standard auto-sampler, model G1316A thermostat column, model G1322 A vacuum degasser, quaternary pump, model G1314B variable wavelength detector, was used. The separation was achieved on a stainless steel silica based Zorbax Eclipse XDB–C18 column (ф4.6 mm×150 mm, 5 μm). The absorption was measured at 223 nm for andrographolide. The chromatographic data was recorded and processed with EZChrom Elite software.

2.3 Sample preparation

***2.3.1 Standard solution preparation*:** Standard andrographolide 10 mg was accurately weighed and transferred to a 10 ml volumetric flask and the volume was made with water. Solutions of 10, 20, 30, 40 and 50 μg/ml was made by transferring the aliquot from stock solution and the volume was made with water in each case. Further standard solutions were prepared freshly each day by appropriate dilution of stock solution with water for intraday as well as interday analysis.

***2.3.2 Test sample preparation*:** 25 mg of water extract was accurately weighed and transferred to a 25 ml volumetric flask and the volume was made by distilled water. The final stock solution of 100 μg/ml concentration was made by transferring 1 ml of above solution to 10 ml volumetric flask and the volume was made with distilled water. Then 10 μl of the stock solution was subjected to HPLC analysis and the concentration of andrographolide was calculated based on the calibration curve equation.

2.4 Validation of the Method: Validation of the analytical method was done according to the International Conference on Har-monization guideline (ICH, 1996). The method was validated for linearity, precision, and accu-racy, limit of detection (LOD) and limit of quantitation (LOQ).

2.4.1 Linearity: Linearity was determined by using andrographolide standard solution. 4.5 to 70 μg/ml of the standard solution was prepared (n = 3). The calibration graphs were obtained by plotting the peak area versus the concentration of the standard solutions.

***2.4.2 Precision*:** The precision was determined by analyzing 25, 50, and 75 µg/ml of standard solution of andrographolide (n = 3) on the same day for intraday precision and on 3 different days for interday precision by the propose method. The precision was expressed as relative standard deviation (RSD).

3. Results and Discussion

3.1 Chromatography: Under the current conditions, andrographolide along with other phytoconstituents of *A. Paniculata Nees* leaves extract were eluted within 10 min. The peaks in the HPLC chromatogram of leaves extract were identified by comparing the retention time and UV spectra of andrographolide in the samples with andrographolide standard. The peak purity was above 97 %. Figure 2 shows the chromatograms of andrographolide standard and methanol extract of *A. Paniculata* nees sample at 223 nm. The quantification data is as shown in Table I.

3.2 Validation of chromatographic method: The method was validated for its linearity, precision, accuracy, LOD and LOQ. The calibration graph for andrographolide was within the concentration range of 4.5-70 µg /ml, with a correlation coefficient (r^2) of 0.9999 (Table 1). The interday and intraday precisions of andrographolide are presented in Table 2. The results showed acceptable precision of the method, with RSD values much lower than 2%. The recovery at 3 different levels of andrographolide was 96.60, 97.36, and 98.94% with an average of 97.64% (Table 3).These values indicate the accuracy of the method. The LOD and LOQ for andrographolide were found to be 0.06 and 0.17 µg/ml, respectively, which indicate a high sensitivity of the method which was calculated by using the following formulae.

LOD =3.3 σ/ S and LOQ = 10 σ/ S.

Where σ is the standard deviation of the response and S is the slope of the calibration plot.

3.3 Sample analysis: Andrographolide content in the samples of *A. Paniculata Nees* obtained from two different herbal suppliers in Mumbai during October 2011 determined by the newly proposed HPLC method are given in Table 4. The contents of andrographolide in the dried powder were 1.12 ± 0.08 and 0.9 ± 0.04% *w/w* (Table 4). HPLC chromatograms of both extracts showed similar pattern with a major peak of andrographolide at retention time of 3.67 min (Figure 2). The identity of the peak of andrographolide in the sample chromatograms was confirmed with the standard and the corresponding retention time.

Conclusion:

The developed RP-HPLC method for assay of andrographolide in *A. Paniculata Nees* is simple, precise, specific and highly accurate and less time consumption for analysis could be recorded. So, it can be employed for the routine analysis for simultaneous estimation. Hence this RP-HPLC method is suitable for quality control of raw materials and formulations, and also for dissolution studies. It can be used for bioequivalence studies in plasma.

Acknowledgements

The authors would like to be thankful to Rajiv Gandhi Commission for Science and Technology, Government of Maharashtra, India for providing funding for this research work.

References:

1. The wealth of India, raw materials, New Dehli; Publications and information Directorate, CSIR, 1985, vol.1.pp.264-266.
2. Handa, S. S., and Sharma, A. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride. *Indian J of Medic Research* B 1990; 92: 276-283.
3. M. Rajani, N. Shrivastava, M.N. Ravishankara, A rapid method for isolation of andrographolide from *Andrographis paniculata* Nees (Kalmegh), Pharmaceut. Biol. 2000; 38; 204.
4. Calabrese, C., Berman, S.H., Babish, J.G. "A phase **I** trial of andrographolide in HIV positive patients and normal volunteers", *Phytother. Res*., 2000; 14, 333-338.
5. Madav, S., Tripathi, H. C., Tandan, S. K., and Mishra, S. Analgesic, antipyretic and antiulcerogenic effect of andrographolide. Indian *J of Pharmaceu Sci,* 1995; 57: 121-125.
6. Rajagopal, S., Kumar, R. A., Deevi, D. S., Satyanarayana, C., and Rajagopalan, R. Andrographolide, a potential cancer therapeutic agent isolated from Andrographis paniculata. *Journal of Experi and Therape Oncology*, 2003; 3: 147-158.

7. Chang, R. S., Ding, L., Chen, G. Q., Pan, Q. C., Zhao, Z. L., and Snith, K. M. Dehydrographolide succinic acid monoester as an inhibitor against the human immunodeficiency virus. Proceedings of Society of *Experim Biol and Medicine*, 1991; 197: 59-66.
8. Bu-Chin, Y., Chen-Road, H., Wang-Chuan, C. and Juei-Tang, C. Antihyperglycemic effect of andrographolide in streptozotocin- induced diabetic rats. *Planta Medica*, 2003; 69: 1075-1079.
9. Shreiber, W. L.; Scharpf, L. G.; Katz, I. Flavors and Fragrances: The Chemistry Challenges. (1997) *Chemtech*, 58-61.
10. Gaind, K. N., Dar, R. N., and Kaul, R. N. Spectrophotometric estimation of andrographolide in Kalmegh. *Indi J of Pharmacy*, 1963; 25: 225-226.
11. Maiti, P. C., Kanji, S. K., and Chatterjee, R. Studies in Kalmegh extract. *Indian J of Pharmacy*, 1959; 21: 169-171.
12. Bhat, V. S., and Nanavati, D. D. Andrographis paniculta (Burm) Wall ex Nees (Kalmegh). *Indian Drugs*, 1978; 187-190.
13. Saxena, S., Jain, D. C., Gupta, M. M., Bhakuni, R. S., Mishra, H. O., and Sharma, R. P. High-performance thin layer chromatographic analysis of hepatoprotective diterpenoids from Andrographis paniculata. *Photochemic Analy*, 2000; 11:34-36.
14. Cheung, H. Y., Cheung, C. S., and Kong, C. K. Determination of bioactive diterpenoids from Andrographis paniculata by micellar electrokinetic chromatography. *J of Chromatogr A*, 2001; 930: 171-176.
15. Zhao, Y., Ming, Y., Zhang, H., Luo, X., Chen, L., and Li, Y. Rapid determination of diterpenoids in Andrographis paniculata by microemulsion electrokinetic capillary chromatography with short-end injection. *Chromatographia*, 2005; 62: 611-615.
16. Wongkittipong, R., Prat, L., Damronglerd, **S.,** Gourdon, C., "Solid-liquid extraction of andrographolide fromplants-experimental study kinetic reaction and model*", Sep. Purif. Technol* 2004**;** 40, 147-154.
17. Sharma, A., Lal, K., and Handa, S., Standardization of the Indian crude drug Kalmegh by high pressure liquid chromatographic determination of andrographolide. *Phytochemi Analy*, 1992; 3:129-131.
18. Srivastava, A., Misra, H., Verma, R. K., and Gupta, M. M. Chemical finger printing of Andrographis paniculata using HPLC, HPTLC and densitometry. *Phytochemic Analy*, 2004; 15: 280-285.
19. Vijaykumar K., Papolu B., Murthy S., Sukalak K., Syamasundar B., and Subbaraju G., Estimation of Adrographolide in Andrographis paniculata Herb, Extracts and Dosage forms International *Journal of Applied Science and Engineering* 2007; 5, 1: 27-39.
20. Meili, C., Chunying., X, and Longxiao, L., Solubility of Andrographolide in Various Solvents from (288.2 to 323.2) K. J. Chem. Eng. Data 2010; 55, 5297–5298.
21. "*ICH Topic Q2B Validation of Analytical Procedures: Methodology*", London, 1996.

Figure 1: Structure of Andrographolide

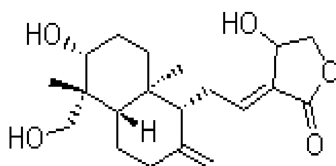

Table 1. Method of validation parameters for the quantification of Andrographolide by the proposed method

Parameters	Results
Linear range (µg/ml)	5.0-75 µg/ml
Regression equation	y = 3.735x
Correlation coefficient (r^2)	0.9999
LOQ (µg/ml)	0.03
LOD (µg/ml)	0.05

Where, x is the concentration of Andrographolide in µg/ml; y is the peak area at 219 nm

Table 2. Precision and Stability of andrographolide

Analyte		Precision (RSD, %)				Stability
		Intra-day (n=3)		Inter-day (n=3)		RSD of
		R_t	P_a	R_t	P_a	P_a (%)
Andrographolide	25 µg/ml	2.1	0.13	2.14	0.15	0.16
	50 µg/ml	2.17	0.17	2.18	0.18	0.20
	75 µg /ml	2.19	0.19	2.21	0.21	0.24

Table 3. Recovery of andrographolide from *A. Paniculata* Nees

Analyte	Contained (µg/ml)	Added (µg/m)	Found (µg/ml)	Recovery (%)	Mean (%)	RSD (%)
Andrographolide	11.3	100	105.67	96. 67	97.64%	1.95
	11.3	100	109.52	96. 18		
	11.3	100	106.89	96. 94		
	11.3	50	56.98	97. 92		
	11.3	50	59.56	96. 18		
	11.3	50	56.78	97. 98		
	11.3	10	18.19	98. 65		
	11.3	10	20.89	99. 31		
	11.3	10	19.78	98.87		

Table 4. The content of andrographolide in dried powder of *A. Paniculata nees* by the proposed method

Sample	Andrographolide content (% w/w) in dried leaves powder
Herbal supplier A	1.15 ± 0.08
Herbal supplier B	0.98 ± 0.04

Figure 2. Chromatograms of standard andrographolide (upper chromatogram) and water extract (lower chromatogram) of *A. Paniculata* nees.