Asian Journal of Chemistry Vol. 15, No. 1 (2003), 229–234

HPLC in Standardization of Herbal Drugs: Studies on Triphala Powder

MRS. ANAGHA S. BAHULIKAR†, MRS. R.V. KASHALKAR† and M.D. PUNDLIK*
Bhide Foundation for Research and Education in Chemistry
Department of Chemistry, S.P. College, Tilak Road, Pune-411 030, India

High performance liquid chromatography (HPLC) is a very efficient method for the analysis of herbal drugs and herbal preparations, because of its ability to simultaneously separate, identify and analyse the complex mixture of organic substances. In the present communication we report the HPLC analysis of the ayurvedic drug, Triphala powder and demonstrate the utility of the technique in standardizing the ayurvedic drug.

Key Words: HPLC, Standardization, Herbal drug, Triphala.

INTRODUCTION

High performance liquid chromatography (HPLC) is the most powerful analytical technique and is widely used in pharmacopoeial analysis. Allopathic pharmacopoeia relies more on HPLC and allied chromatography techniques for impurity profile, quality control and assay. By virtue of its ability to simultaneously separate, identify and analyze the complex mixture of organic substances, HPLC will be highly useful for the analysis of herbal drugs and formulations. It is an efficient method to ensure identity and purity of the herbal materials and can playa vital role in standardizing the herbal drugs. At present the ayurvedic pharmacopoeia[1] gives methods for characterization of crude herbal drugs, which are based on macroscopic and microscopic characteristics, water soluble extractives, alcohol soluble extractives, ash value, acid insoluble ash etc. In addition to these parameters, if HPLC is also used for the analysis of these materials, it will go a long way in establishing the methods for quality control and standardization of herbal drugs. With this point of view the present work was undertaken and to the best of our knowledge this is the first report on the analysis of ayurvedic drug Triphala using HPLC technique. The ayurvedic drug Triphala Churna (powder) is a mixture of crude herbal drugs-Amalaki *(Emblica officinalis)*, Beheda *(Terminalia bellerica)* and Haritaki *(Terminalia chebula)*, collectively known as myrobalans. The constituents of these myrobalans are tannins, polyphenolic compounds, gallotannins, gallic acid, tannic acid, etc. Gallic acid is the common constituent of the three herbal drugs, which constitute the drug Triphala. The present HPLC method is therefore based on the qualitative identification and quantitative estimation of gallic acid in Triphala Churna and demonstrates its

†Department of Chemistry, S.P. College, Tilak Road, Pune-411 030, India.

utility in analyzing market samples vis-à-vis standard mixtue of 1 : 1 : 1 of Amalaki, Beheda and Haritaki prepared as reference standard. Gallic acid has been analyzed by HPLC using mostly reverse phase columns and various mobile phases, in plant materials, tea, biological fluids, pharmaceutical formulations, etc.[2-11] In the present communication we report a simple isocratic method using aqueous phosphoric acid and methanol as mobile phase, which gives distinct separation of gallic acid (retention time 4.425 min) from other co-extractives.

EXPERIMENTAL

HPLC : Waters M-45 with Rheodyne 20 μL injection loop.

Column : Spherisorb, ODS2, particle size 5 μm, 25 cm × 4.6 mm i.d. with guard column (length = 5 cm).

Detector : Waters model 440 absorbance detector, fixed wavelength 254 nm.

Integrator : Chemito C-5000 [Toshniwal Instruments India Limited, Nasik, India].

Mobile phase : 0.1% H_3PO_4: Methanol (80 : 20).

Flow : 1 mL/min.

Gallic acid and orthophosphoric acid were of AnalaR grade and methanol of HPLC grade was used. Double distilled water (all glass assembly) was used throughout the work.

Amalaki, Beheda and Haritaki powders were procured from local ayurvedic agencies. Triphala, a mixture of these powders in 1 : 1 : 1 proportion, was prepared as reference standard and market samples of triphala were analyzed for comparative studies.

Standard gallic acid solution : 1 mg/mL in methanol.

Sample Preparation: 5 g sample was soaked in 50 mL distilled water by keeping overnight. It was then centrifuged and supernatant was collected separately and made up to 100 mL with distilled water. This was divided into two parts of 50 mL each. The 50 mL aqueous solution was extracted with ethyl acetate. The ethyl acetate extract was evaporated to dryness and residue was dissolved in 25 mL of methanol. 0.10 mL of this methanolic solution was diluted to 10 mL for HPLC analysis. All samples, *i.e.*, Amalaki, Beheda, Haritaki, Triphala standard and Triphala I and II market samples were processed as above.

RESULTS AND DISCUSSION

Standard gallic acid solution of 1 mg/mL in methanol was appropriately diluted with the mobile phase to obtain 0.01 mg/mL, 0.02 mg/mL, 0.03 mg/mL and 0.04 mg/mL and these solutions were analyzed (in triplicate) for studying linearity. Average area counts and the graph showing linearity are presented in Table-1 and Fig. 1 respectively. Gallic acid showed good linearity in the range 0.01 mg/mL to 0.04 mg/mL with a correlation coefficient of 0.9996. The precision of the method was also studied by injecting a single sample solution 5 times (Table-2) and finding out the coefficient of variation (C.V.). The C.V. was found to be 0.5567. Limit of detection and limit of quantitation were calculated by analyzing a 10 ppm solution of gallic acid by the proposed method and it was found to be 0.0093 ppm [LOD = (2 × N × L.D. conc.)/S] and limit of quantitation was found to be 0.0465 ppm [LOQ = 5 × L.D.].

TABLE-1
AVERAGE AREA COUNTS FOR GALLIC ACID

S.No.	Conc. of gallic acid (mg/mL)	Area count
1.	0.01	70,0000 (695947)
2.	0.02	13,00000 (12,71762)
3.	0.03	21,00000 (2114614)
4.	0.04	27,00000 (2665228)

TABLE-2
DETERMINATION OF COEFFICIENT OF VARIATION FOR GALLC ACID

S.No.	Conc. of gallic acid (mg/mL)	Area count
1.	0.01 mg/mL	7,55000
2.	0.01 mg/mL	7,54000
3.	0.01 mg/mL	7,57000
4.	0.01 mg/mL	7,64000
5.	0.01 mg/mL	7,54000

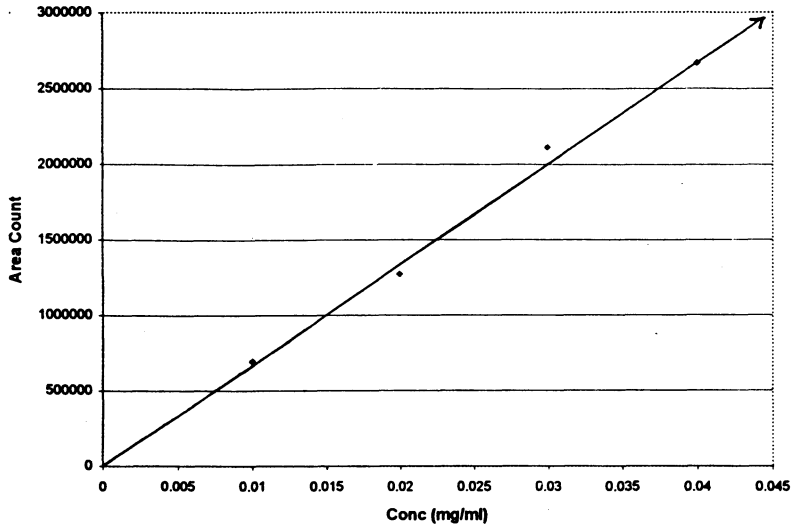
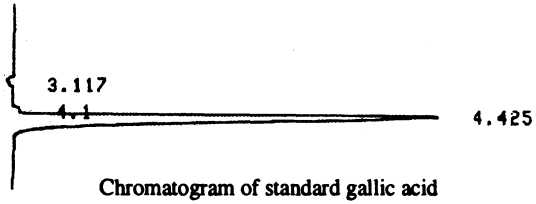


Fig. 1

Gallic acid is a common constituent of the three herbal drugs, which constitute Triphala. Apart from gallic acid, other constituents of these myrobalans are

tannins, polyphenolic compounds, gallotannins, etc., which are all acidic and thus imparting 'ionic' nature to the extract of Triphala. An 'ionic sample' is any mixture containing one or more ionized or ionizable compounds[12]. Such samples can be analyzed by ion suppression, ion pair or ion exclusion chromatography techniques. Ion suppression chromatography is a relatively simple extension of standard reverse phase chromatography that enables it to be used for ionizable solutes. The approach is to suppress the ionization of solutes by adjusting the pH of the mobile phase. In this way the solutes are either rendered neutral or only partially charged, thereby increasing their retention on non-polar stationary phases, which results in achieving better separation. At pH values (2.34) substantially less than the pKa value of the acid (gallic acid pKa = 4.41) the solute is present in its neutral form and shows longer retention[13]. Hence this technique was used to achieve distinct separation of gallic acid from all other coextractives from Triphala standard within a reasonable time. A typical chromatogram of standard gallic acid is shown in Fig. 2.



Chromatogram of standard gallic acid

Fig. 2

The typical chromatogram of the extract of Triphala standard is shown in Fig. 3. The extracts of Amalaki, Beheda, Haritaki, Triphala standard and Triphala I and II market samples were analyzed for gallic acid content by the proposed method and the results are given in Table-3. The results show that by following the proposed method of extraction the highest content of gallic acid was found in Beheda *(T. Bellerica)* amongst the three myrobalans constituting Triphala. The standard 1 : 1 : 1 mixture prepared as reference standard has 0.888%, market

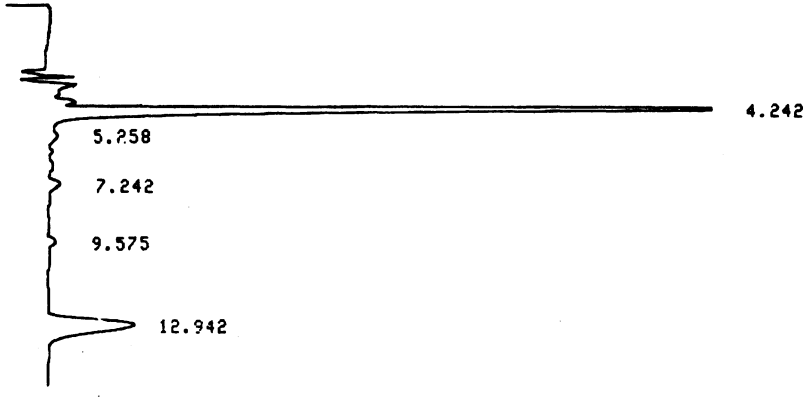


Fig. 3. Chromatogran of the extract of standard Triphala powder

sample I and II have 0.144% and 0.178% gallic acid respectively. A typical chromatogram of Triphala market sample is shown in Fig. 4.

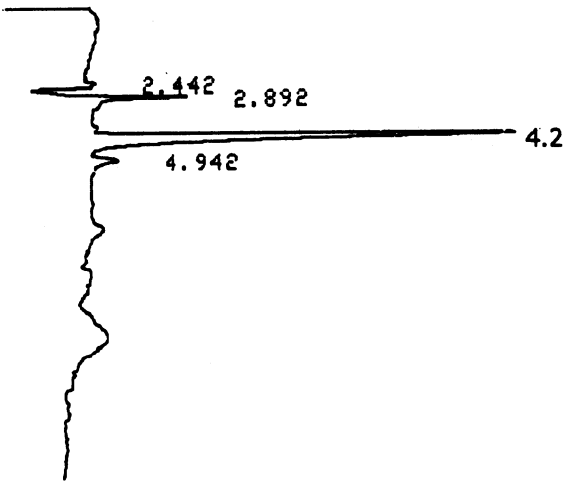


Fig. 4. Typical chromatogram of the market sample of Triphala powder

TABLE-3
GALLIC ACID CONTENT IN DIFFERENT SAMPLES

Sr.No.	Herbal drug (5 g)	Gallic acid content
1.	Amalaki	0.00860 (0.1725%)
2.	Beheda	0.02100 (0.42%)
3.	Haritaki	0.00965 (0.193%)
4.	Triphala standard	0.04440 (0.888%)
5.	Triphala market sample I	0.00720 (0.144%)
6.	Triphala market sample II	0.00890 (0.178%)

A review of all the pharmacopoeias including phytotherapy monographs, WHO monographs show that all recommend marker compound testing. Our pharmacopoeias, however, do not recommend the same as chromatographic techniques, as yet not used in our pharmacopoeia. Standardization using marker compound is one of the best methods of standardizing herbs and herbal preparations[14]. In the present work we have used gallic acid, a common constituent of all three myrobalans constituting Triphala, as marker compound for the standardization. The proposed method of extraction and analysis of the extracts of single herbs and herbal preparations shows that the gallic acid content of the samples can be successfully used to assess the quality of the market samples vis-à-vis reference standard.

Conclusion

The proposed method can be used to standardize Triphala powder on the basis of gallic acid as a marker compound. The method can be further improved in

terms of sensitivity and linearity by using a variable wavelength UV-vis detector or more sophisticated PDA detector as it will be possible to estimate the gallic acid content at its λ_{max} value (272 nm) instead of at 254 nm fixed wavelength.

ACKNOWLEDGEMENTS

Authors' thanks are due to Prof. A.G. Bhave for providing necessary facilities. One of us (ASB) is grateful to Department of Chemistry, S.P. College, Pune for the award of late Dr. T.R. Ingle fellowship.

REFERENCES

1. The Ayurvedic Pharmacopoeia of India, Govt. of India, Ministry of Health and Family Welfare, Dept of Indian Systems of Medicine and Homeopathy, Vol I, Part I, pp. 5, 26, 47 (2001).
2. H.F. Wang, K. Helliwel and X.D. You, *Food Chem.*, **68**, 115 (2000); *Anal. Abstr.*, **62**, 5H446 (2000).
3. J.K. Lin, C.L. Lin, Y.C. Liang, S.Y. Lin Shiau and I.M. Junan, *J. Agri. Food Chem.*, **46**, 3635 (1998); *Anal. Abstr.*, **61**, 2H260 (1999).
4. L. Zong, M. Inoue, M. Nose, K. Kojima, N. Sakaguchi, K. Isuzugawa, T. Takeda and Y. Ogihara, *Bios. Pharm. Bull.*, **22**, 326 (1999); *Anal. Abstr.*, **61**, 9G204 (1999).
5. S. Shahrzad and I. Bitsch, *J. Chromatogr. B., Biomed Appl.*, **705**, 87 (1998); *Anal. Abstr.*, **60**, 6F238 (1998).
6. D. Yu. Korulkin, V.B. Omarkamzinova and G.A. Bektennova, *Chem. Nat.*, **33**, 498 (1998), *Anal. Abstr.*, **60** 7H171 (1998).
7. H.D. Li, X.H. Yan and H. Ouyang, *Yaowu Fenxi Zazhi*, **16**, 105 (1996); *Anal. Abstr.* **58**, 8Gi52 (1996).
8. F.Q. Xiong, L.J. Yuan, G.D. Wu and C.Y. Lu, *Sepu*, **11**, 366 (1993); *Anal. Abstr.*, **56** 6H312 (1994).
9. T.K. Annapurnani, *Curr. Sci. (India)*, **58**, 190 (1989).
10. J.L. Wolfender and S. Rodriguez, *Planta Medica*, **63**, 2 (1997); Medicinal and Aromatic Plants Abstract No. **19**, 2555 (1997).
11. S.K. Cauhan, B. Singh, S. Agarwal, *JAOAC Int.*, **83**, 1480 (2000); *Anal. Abstr.*, **63**, 6H237 (2001).
12. L.R. Snyder, J.J. Kirkland, J.L. Glajeh, Practical HPLC Method Development, John Wiley & Sons Inc., New York, p. 293 (1997).
13. K. Robards, P.R. Haddad and P.E. Jackson, Principles and Practice of Modern Chromatographic Methods, Academic Press, London, pp. 342–43 (1994).
14. R.M. Dobriyal and D.B.A. Narayana, *Estern Pharmacist*, **41**, 31 **(1998)**.

(*Received*: 14 June 2002; *Accepted:* 19 August 2002) AJC-2823