

activity. The decreased steriodogenic activity due to the treatment with the plant extract is further established by the decrease in the activity of two enzymes, G6PD and HSD, which are related to the synthesis of steroid hormones.

ACKNOWLEDGEMENTS

We thank the University Grants Commission for extending financial support to the project.

REFERENCES

1. Doll, R. and Vessery, M.P., *Prit. Med. Bull.*, 1970, 26, 33.
2. Miale, J.B. and Kent, J.W., *Amer J. Obstet. Gynaecol.*, 1974, 120, 264.
3. Chatterjee, A. and Pakrashi, S. C., In; *The Treatise of Medicinal Plants, Vol II*, Publication and Information Directorate, New Delhi, 1992, 166.
4. Satyavati, G.V., Gupta, A.K. and Tandon, N., In; *Reviews of the Medicinal plants of India, Vol. II*, I.C.M.R. Publication, New Delhi, 1987, 409.
5. Kamboj, V.P., *Indian J. Med. Res.*, 1988, 87, 336.
6. Kamboj, V.P. and Dhawan, B.N., *Indian J. of Ethnopharmacol.*, 1982, 6, 181.
7. Litchfield, J.T. (Jr) and Wilcoxon, F., *J. Pharmacol Exp. Ther.*, 1949, 96, 99.
8. Oser, L.B., Eds., In; *Hawk's Physiological Chemistry, 14th Edn.*, Tata McGraw Hill Publishing Co. Ltd., New Delhi, 1971, 1062.
9. Oser, L.B., Eds., In; *Hawk's Physiological Chemistry, 14th Edn.*, Tata McGraw Hill Publishing Co. Ltd., New Delhi, 1971, 703.
10. Lohr, G.W. and Waller, H.D., In; Bergmeyer, H.U., Eds., *Method of Enzymatic Analysis, Vol. II*, Verlage Chemie, Florida, 1974, 477.
11. Lohr, G.W. and Waller, H.D. In; Bergmeyer, H.U. Eds., *Method of Enzymatic Analysis, Vol. II*, Verlage Chemie, Florida, 1974, 744.
12. Lowry, O.H., Resenbrough, N.J., Farm, A.L. and Randall, R.J., *J. Biol. Chem.*, 1951, 193, 265.
13. Woodson, R. F. In; *Statistical Methods for the Analysis of Biochemical Data. Probability and Mathematical Statistics*, Wiley, Chichester, 1987, pp 315-316.
14. Wills, J.W., Fuller, G.B. and Hansel, W., *Endocrinol.*, 1970, 87, 581.
15. Noach, E.L. and Van Reer, G.P., *Acta Endocrinol.*, 1958, 27, 502.

An Improved HPLC Method for Estimation of Sennosides in Senna

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Accepted 2 November 2001

Revised 16 October 2001

Received 28 May 2001

A simple high performance liquid chromatographic (HPLC) method for estimation of sennosides A and B in senna has been established. The aqueous extract of senna was directly injected and separated using reverse phase Shim pack CLC-CN column with UV detector monitoring at 220 nm. Compounds were well resolved when mixture of 20 mM sodium citrate buffer (pH 4.5) and acetonitrile in the ratio of 9:1 was used as mobile phase at the flow rate of 1.5 ml/min. The minimum detectable limit was 0.05 µg. The chemical analysis method supported the result. The present method is more suitable than the earlier reported HPLC method for routine analysis of sennosides.

Senna (Cassia augustifolia Vahl), locally known as *sonamukhi* has been found very much suitable for cultiva-

tion in arid region¹. It has been widely accepted by the farmers of the region because of its drought tolerance, perennial nature, non-palatability by animals, low cost of cultivation and yield stability. Because of its purgative action, it is widely

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used in Ayurvedic, Unani and Allopathic system of medicine and demand is also increasing in national and international market¹. Its price and acceptability in the market depends upon the concentration of sennosides mainly, sennosides A and B, the chemical constituents that are responsible for the purgative action of the crude drug². In this way, quantitative estimation of sennosides in senna has gained importance. It is therefore, a simple, quicker and sensitive technique capable of determining sennosides in short period with minimal sample preparation is required.

Several methods have been reported in the literature for estimation of sennosides in senna³⁻⁶. Different analytical techniques have also been used involving thin-layer chromatography⁷, column chromatography⁸. These techniques involve several steps and hence are time consuming and tedious. HPLC methods have also been reported⁹⁻¹². However, they have some discrepancies and they are not suitable for routine analysis. Present paper reports a simple, rapid and accurate HPLC method to determine sennosides A and B in senna.

A Shimadzu LC system fitted with LC-10AD pumps in isocratic mode, a Rheodyne 7161 injector with 20 μ l loop, Shim pack CLC-CN stainless steel column (15 cm x 4.6 mm ID with 5 μ m particle size), a variable wavelength ultraviolet spectromonitor (SPD-10A), and a CR-6A chromatopac integrator were used. The operating conditions were as follows: mobile phase, 20 mM sodium citrate buffer (pH 4.5) – acetonitrile (9:1) at flow rate 1.5 ml/min; detector wavelength, 220 nm; sensitivity, 0.05 AUFS. Suitable aliquots of test samples (5-10 μ l) were injected with a Hamilton syringe. The minimum detectable limit was 0.05 μ g. At the end of each working day the column was washed with 15 ml of each water, methanol and 60% methanol to improve the column life.

All solvents used in the study were of HPLC grade. Sennosides A and B were isolated from senna leaves and purified by the method of Tanaka *et al.*¹³ They were crystallized from 70% aqueous acetone. The color, crystal form and mp of sennosides were as follows: sennosides A, yellow plates, 218-228 $^{\circ}$; sennoside B, yellow needles, 195-201 $^{\circ}$. The 20 mM sodium citrate buffer (pH 4.5) solution was prepared by dissolving 10 mM each of sodium citrate and citric acid in water and the solution was diluted to one litre volume with water.

About 5 mg of each sennosides A and B were weighed accurately in to a 50-ml volumetric flask and diluted to volume with water, 5 ml of this solution was pipetted into a 50

ml of volumetric flask and diluted to volume with water. Graded concentrations (0.1, 0.3, 0.5, 0.7, 0.9, 1.0 μ g/ml) of standard solution of sennosides were injected into column. Peak area against concentration of sennosides was used for drawing standard curves.

Accurately weighed finely powdered senna material (500 mg) was extracted with 100 ml of water according to the procedure of Srivastava *et al.*¹² After centrifugation, the supernatant was filtered through G-4 glass filter and 10 μ l of the filtrate was subjected to HPLC. Ten microlitres of each standard or sample solution were accurately injected into the column. The quantity of sennosides in sample solution was calculated by reference to the standard solutions. Each analysis was carried out in three replications. Peaks of sennosides A and B were identified by retention time and confirmed by spiking in which standards were added to the sample. Increased peak area of sennosides A and B was found to be proportional to the added quantities of both sennosides.

In order to analyze large number of samples, it was desirable to have a simple and quick analytical method capable of determining sennosides A and B in a short period. The HPLC methods of Ernie and Frei⁹, Gorler *et al.*¹⁰, Hayashi *et al.*¹¹, Srivastava *et al.*¹² are time consuming, tedious as well as have some disadvantages. Ernie and Frei reported the combined use of gel permeation chromatography (GPC) and reversed phase chromatography while Gorler *et al.*¹⁰, used pair- ion chromatography for resolution of complex senna mixture. Therefore, they are lengthy and time consuming. Hayashi *et al.*¹¹ reported the use of 2% dioxane/pH 2.2 Britton-Robinson buffer as mobile phase. First, repeated use of low pH buffer solution as mobile phase decreases the self-life and efficiency of column. Second, preparation of Britton-Robinson buffer solution is cumbersome. It involves so many components like preparation of 0.2N NaOH solution, preparation of 0.04 M acid mixture composed of phosphoric acid, acetic acid and boric acid. The method developed by Srivastava *et al.*¹² has short analysis time, however, the peaks are not well resolved and the method is not environment friendly due to the use of tetrahydrofuran (THF) which is a hazardous chemical and it has unbearable smell. It seriously causes irritation to skin, eyes and respiratory system. It also prohibits the detection of sennosides at their maximum absorption region (220 nm) because the THF is not transparent at this region. Consequently, the quantity of minimum detectable is comparatively high.

Keeping all these problems in mind, several column

TABLE 1: COMPARISON OF SENNOSIDES CONTENT IN SENNA DETERMINED BY DIFFERENT METHODS*.

Sample no	Present HPLC Method		Total	Chemical Method Total Sennosides
	Sennoside A (%)	Sennoside B (%)		
Leaf				
1.	0.53 ± 0.05	1.65 ± 0.04	2.18 ± 0.05	2.25
2.	1.25 ± 0.02	1.70 ± 0.04	2.95 ± 0.06	3.04
3.	1.05 ± 0.02	1.79 ± 0.01	2.84 ± 0.04	2.96
Pod				
1.	1.25 ± 0.05	2.65 ± 0.07	4.04 ± 0.08	4.12
2	1.20 ± 0.03	2.54 ± 0.04	3.93 ± 0.05	4.04
3.	1.32 ± 0.02	2.48 ± 0.05	3.94 ± 0.03	4.05

*Percent sennosides content is w/w.

packing materials and mobile phases were examined. Shim pack CLC-CN column (15 cm x 4.6 mm I.D.) with a mixture of 20 mM sodium citrate buffer – acetonitrile (9:1) as mobile phase with UV detector set at 220 nm was found most suitable for the present purpose. Peaks of sennosides in the chromatogram (fig. 1) were well resolved. Since the solvent acetonitrile and buffer solution were transparent at the 220 nm region, sensitivity for detection of sennosides is maximum and the minimum detectable limit was found to be 0.05 µg.

Three samples of each senna leaf and pod were analyzed by both, HPLC method developed by the author and conventional chemical method¹⁴ and results were compared. Sennosides content determined by these two methods were comparable (Table 1). However, time taken by the present HPLC method is much less i.e. about 1 h. as compared to time taken by chemical method that is about 3 h. Thus the present method is simple and suitable for analysis of large number of senna samples.

REFERENCES

1. Sharma, A.K., Goyal, R.K. and Gupta, J.P., *Indian Farming.*, 1999, 49, 18.
2. Oshio, H., Naruse, Y. and Tsukui, M., *Chem. Pharm. Bull.*, 1978, 26, 2458.
3. Kussmaul, W. and Becker, B., *Helv. Chim. Acta*, 1947, 30, 59.
4. Lemli, J., *J. Pharm. Pharmacol.*, 1965, 17, 277.
5. Anonymous, *Analyst*, 1965, 90, 582.
6. Habib, A.M. and El-Sebakhy, A., *J. Nat. Prod.*, 1980, 43, 452.
7. Khafagy, S.M., Girgis, A.N., Khayyal, S.E. and Helmi, M.A., *Planta Med.*, 1972, 21, 304.
8. Denee, R. and Huizing, H.J., *J. Nat. Prod.*, 1981, 44, 257.

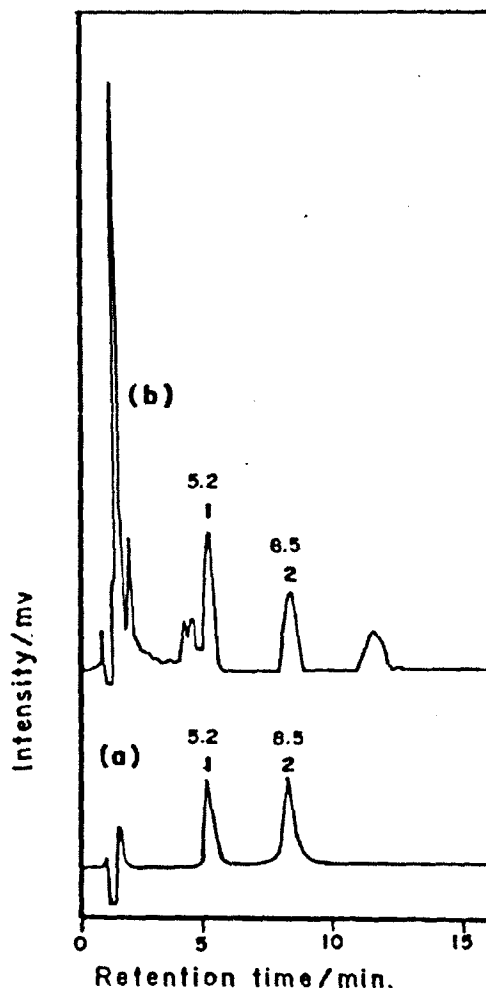


Fig. 1: HPLC Chromatograms of (a) Standards and (b) Sample. Peaks: 1, Sennoside B; 2, Sennoside A.

9. Ernie, F. and Frei, R., **J. Chromatogr.**, 1978, 149, 561.
 10. Gorler, K., Mutter, S. and Westphal, C., **Planta Med.**, 1979, 37, 308.
 11. Hayashi, S., Yoshida, A., Tanaka, H., Mitani, Y. and Yoshizawa, K., **Chem. Pharm. Bull.**, 1980, 28, 406.
 12. Srivastava, V.K., Maheshwari, M.L. and Mandal, S., **Indian J. Pharm. Sci.**, 1983, 45, 230.
 13. Tanaka, H., Murata, T., Yoshida, A., and Hayashi, S., **Chem. Pharm. Bull.**, 1982, 30, 1550.
 14. Anonymous, In; British Pharmaceutical Codex, The Pharmaceutical Press, London, 1973, 438.
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