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Estimation of Individual Sennosides in Plant Materials and Marketed Formulations by an HPTLC Method

SHAILESH A. SHAH, M. N. RAVISHANKARA, ANJOO NIRMAL, CHAMANLAL J. SHISHOO, ISHWARSINH S. RATHOD AND BHANUBHAI N. SUHAGIA

L. M. College of Pharmacy, Navrangpura, Ahmedabad-380 009, India

Abstract

Senna is a well-known drug, used in the Ayurvedic and Allopathic systems of medicine, and is a treatment for constipation. The purgative action of senna and its formulations is due to the presence of sennosides A and B. An HPTLC method has been developed for the determination of individual sennosides (A, B, C, D) without any derivatization in marketed formulations (three tablet formulations, two granule formulations and one liquid formulation) and plant materials (senna leaf and pod).

The methanolic solution of a sample was applied on a pre-coated silica gel G60 F_{254} TLC plate (E. Merck.) and was developed using n-propanol : ethyl acetate : water : glacial acetic acid (3 : 3 : 2 : 0·1 v/v) as the mobile phase. The relative band speeds (R_f values) obtained were 0·35, 0·25, 0·61, 0·46 for sennosides A, B, C and D, respectively. The densitometric response was monitored at 366 nm. Calibration curves were found to be linear in the concentration ranges 193–1356, 402–2817, 71–497 and 132–927 ng per spot for sennosides A, B, C, and D, respectively. The correlation coefficients were found to be 0·9978, 0·9987, 0·9939 and 0·9983 respectively for sennosides A, B, C and D. The result obtained with the HPTLC method for total sennoside content was compared with the results using the pharmacopoeial methods (spectrophotometric (British Pharmacopoeia) and spectrofluorimetric (United States Pharmacopeia) using the 'F' test). The results revealed no significant difference in the three different methods for estimation of total sennoside.

The proposed HPTLC method was found to be simple, specific, precise, accurate and rapid. It can be used for routine quality control of sennosides or senna-containing formulations for individual sennosides.

The most popular herbal remedies in the health food industry are those which relieve constipation and correct bowel irregularities. Sennosides are active chemical constituents of *Cassia acutifolia* Delile and *Cassia angustifolia* Vahl, which are widely used for the relief of constipation. Sennosides are mainly present in the leaves and pods of senna. Formulations are available which deliver concentrated senna extracts in which sennosides are present as Calcium-salts. The purgative action of senna and its formulations is due to the presence of sennosides A and B (Fairbrain & Moss 1970; Dreesen et al 1981). Sennosides C and D have a synergistic effect, if they are present in 10-15% of the total glycoside fraction (Fairbrain 1953).

A literature survey revealed that there are very few methods available to estimate the amount of sennosides as intact molecules. The official (British Pharmacopoeia, European Pharmacopeia, United States Pharmacopeia) methods of analysis are non-specific for estimation of individual sennosides and give total sennoside content only in terms of sennoside B. Consequently, analytical methods that determine "total sennoside" may not be indicative of bioactivity. Therefore it was thought of interest to develop a simple, specific, precise and accurate method for the quantitative estimation of the individual sennosides A, B, C and D without derivatization using HPTLC.

Correspondence: S. A. Shah, L. M. College of Pharmacy, Navrangpura, Ahmedabad-380 009, India.
E-Mail: lmcp2000@hotmail.com

Materials and Methods

Materials

Reference sennoside (20%) was obtained from Cipla Ltd (India). Senna leaves and senna pods were obtained from commercial sources. They

were authenticated as *Cassia angustifolia* Vahl by our pharmacognosy department. Market formulations (three tablet formulations, two granule formulations, and one liquid formulation) were procured from the local market. Other chemicals were of analytical reagent grade and were obtained commercially.

HPTLC method

Instrumentation. The HPTLC system consisted of a CAMAG Linomat IV-automatic spotting device, a CAMAG twin-trough chamber (20 × 10 cm), a CAMAG TLC scanner-3, CAMAG Cats-4 software, and a 100-μL HPTLC syringe (SGE, Australia).

Standard stock solution of sennosides. Accurately weighed 10 mg (equivalent to 2 mg sennosides) reference sennoside powder was mixed with 5 mL chloroform and centrifuged for 10 min. The supernatant chloroform layer was decanted and 5 mL chloroform was added to the residue and the above procedure was repeated until the chloroform layer was colourless (3 × 5 mL). The chloroform layer was discarded. To the residue, 4 mL oxalic acid solution in methanol (0·05% w/v) was added and mixed using a cyclomixer at high speed for 20 min. After mixing, the mixture was centrifuged for 10 min, the supernatant was transferred to a volumetric flask, and the volume was adjusted to 5 mL with methanol. The concentration of the final solution was 400 μg mL^{-1}.

Sample solution. Accurately weighed powdered (or volume) market formulation, or senna leaf or senna pod powder equivalent to 2 mg sennosides was taken and treated as described in the procedure of the standard stock solution of sennosides.

HPTLC. The standard sennoside solution (0·8–5·6 μg; 2–14 μL) or sample solution (10 μL) was applied on a pre-coated TLC silica gel G60 F_{254} plate (E. Merck), with a CAMAG Linomat IV-automatic sample spotter. The plate was developed with n-propanol : ethyl acetate : water : glacial acetic acid (3 : 3 : 2 : 0·1 v/v) in a glass twin-trough chamber previously saturated with the solvent system for 45 min. The development distance was 45 mm. The plate was removed from the chamber, air dried, and scanned and quantified at 366 nm using a CAMAG TLC scanner-3 and Cats-4 software. Data consisting of the peak area of each sennoside spot was recorded. The amount of each sennoside A, B, C and D was calculated from the peak area using their respective calibration curves.

Estimation of total sennoside by British Pharmacopoeial and United States Pharmacopeial methods

The total amount of sennosides was estimated also by spectrophotometry (British Pharmacopoeia 1993) and spectrofluorimetry (United States Pharmacopeia 1995).

Results and Discussion

Various methods (colorimetry, spectrophotometry, fluorimetry, TLC and HPLC) can be used for the determination of sennosides in senna-containing products. Most of the reported HPLC procedures lack good peak separation or require high column temperatures (Komolafe 1981; Duez et al 1984) or gradient elution with relatively long running time (Ohshima & Takahashi 1983; Metzger & Reif 1996). Various pharmacopoeias (British (1993) and European (1998)) describe a method for analysis, in which all the sennosides get converted to 1,8-dihydroxyanthraquinone derivative by oxidation followed by hydrolysis of the sennosides. Minor differences in other reported methods are centred in the initial extraction steps, in the reagents utilized in oxidative cleavage and hydrolysis, and in solvents recommended for extraction of genins (Kussmaul & Becker 1947; Auterhoff & Sachdev 1962; Muller et al 1962; Frederich & Baier 1973). The United States Pharmacopeia (1995) describes the fluorimetric estimation of sennosides after their reduction to anthranol. Those methods estimate total sennoside content only in terms of sennoside B and not the individual sennosides. TLC is able to separate the sennoside mixture into sennosides A, B, C and D. This observation led to the development of an HPTLC method for the estimation of individual sennosides and total sennoside content in the sample.

In this study the quantitative HPTLC method was developed for the estimation of sennosides A, B, C and D. The solvent system, comprising n-propanol : ethyl acetate : water : glacial acetic acid (3 : 3 : 2 : 0·1 v/v), was found to give good separation and resolution of sennosides A, B, C and D without interference from other materials. The relative band speeds (R_f values) obtained were 0·35, 0·25, 0·61 and 0·46 for sennosides A, B, C and D, respectively (Figure 1). Densitometric measurement was performed in absorbance/reflectance mode using a CAMAG TLC Scanner-3 and Cats-4 software. The

absorbance/reflectance spectra of sennosides were scanned in-situ between 200–600 nm. A wavelength of 366 nm was selected for scanning the plate of sennosides. The peak areas on the chromatogram were used for quantitative determination. It was also possible to identify the sennoside spots by spraying with an alcoholic potassium hydroxide solution, and also by comparing the UV spectrum of each spot of sample material with a reference standard spot. They were identical with a correlation coefficient in the range 0·991–0·996 (Figure 2).

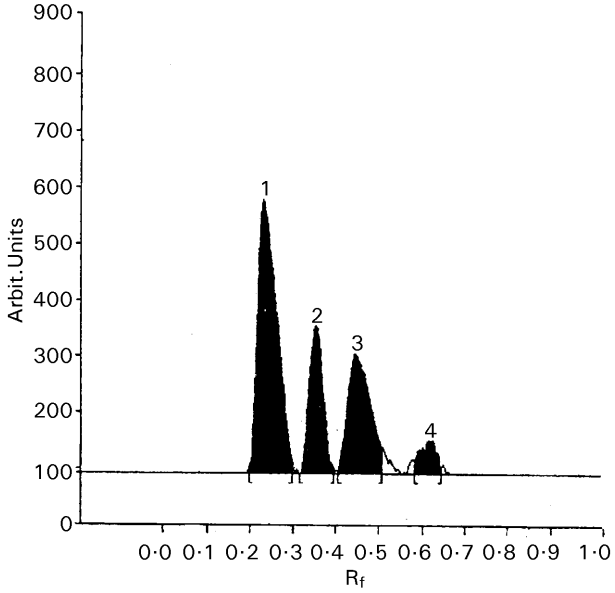

Figure 1. Chromatogram showing the resolution of sennosides A, B, C and D from senna formulations (concentration equivalent to 4 μg per spot of total sennoside) indicated by peak 2, 1, 4 and 3, respectively.

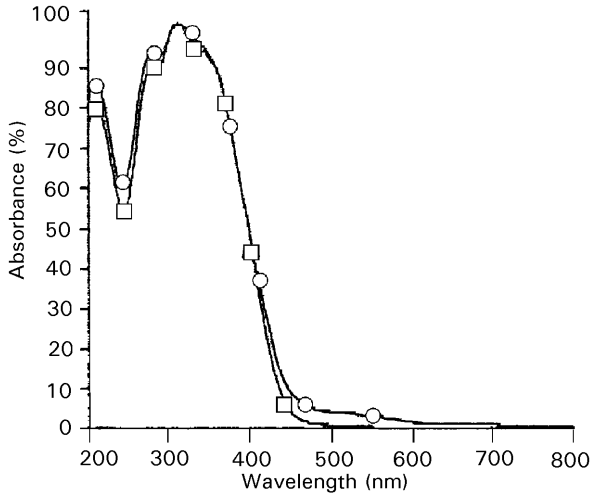

Figure 2. Comparison of the spectrum of reference sennoside B (○) and sample sennoside B (□).

During the development of the HPTLC method it was observed that a pre-saturation of the TLC chamber with mobile phase for at least 45 min was required to obtain a good separation with reproducible R_f values. The chromatographic plate was developed to obtain a 45-mm migration distance from the starting position of the spots. After development the TLC plate was air-dried.

The content of sennosides A, B, C and D in reference standard was first calculated using an area normalization method. The areas of the individual sennosides (A, B, C and D) were measured and their total was considered equal to the total sennoside present in the applied reference standard. The amount of each sennoside present in reference standard was calculated using the following formula:

Concentration of sennoside y =

(area of sennoside y × amount of reference sennoside applied)/total area of all sennosides

where sennoside y is sennoside A, B, C or D.

This is based on the assumption that the four sennosides had the same molar extinction coefficient and gave the fraction of the sennosides in 4 μg reference standard as sennoside A (0·967 ± 0·008 μg), sennoside B (2·012 ± 0·006 μg), sennoside C (0·358 ± 0·005 μg) and sennoside D (0·660 ± 0·005 μg) (n = 5).

The content of sennosides A, B, C and D in samples were determined by calibration curves of individual sennosides, prepared using reference standard (Table 1).

Validation

Specificity. The method is specific as it resolved the peak of sennosides A, B, C and D well with R_f values 0·35, 0·25, 0·61 and 0·46, respectively, even in the presence of other components of the formulations (Figure 1).

Linearity range of sennosides

Linearity was determined in the ranges 193–1356, 402–2817, 71–497 and 132–927 ng per spot for sennosides A, B, C and D, respectively. The correlation coefficients for the calibration curves are listed in Table 2.

Precision

The replicate analysis of 5 × 10-μL samples of the reference solutions was performed to test the effect of chromatography on the precision of the method. The results show % CV was in the range of ± 0·308 to 1·286 for individual sennosides (Table 2). The

Table 1. Analysis of individual sennosides by the HPTLC method.

Sample analysed	% w/w of sennosides ± s.d. (n = 4)[a]				
	A	B	C	D	Total sennosides
Senna pod	0·771 ± 0·0582	0·827 ± 0·0573	0·124 ± 0·0285	0·162 ± 0·0621	1·884 ± 0·072
Senna leaf	0·625 ± 0·0523	0·810 ± 0·0951	0·255 ± 0·0272	0·462 ± 0·0672	2·152 ± 0·060
Ca-sennoside tablets[b]					
T1	2·795 ± 0·0929	5·931 ± 0·020	1·057 ± 0·0065	1·883 ± 0·0191	11·666 ± 0·096
T2	0·094 ± 0·0021	0·209 ± 0·0071	0·039 ± 0·0007	0·072 ± 0·0008	0·414 ± 0·022
T3	1·880 ± 0·0089	3·975 ± 0·0424	0·709 ± 0·0068	1·278 ± 0·0051	7·842 ± 0·020
Granules containing senna extracts					
G1	0·183 ± 0·0034	0·334 ± 0·0020	0·060 ± 0·0014	0·110 ± 0·0016	0·687 ± 0·005
G2	0·292 ± 0·0040	0·612 ± 0·0059	0·109 ± 0·0112	0·197 ± 0·0031	1·210 ± 0·099
Liquid preparation (senna extract)	0	0	0	0	0

[a]Replicates of single extract. [b]Tablets: T1, uncoated tablet of Calcium-sennoside; T2, coated tablet of senna extract; T3, uncoated tablet containing senna extract along with acephenolisatin.

Table 2. Method validation parameters of the proposed HPTLC method for the determination of sennosides.

Parameter	Sennoside			
	A	B	C	D
Precision (% CV) (n = 5)	± 0·780	± 0·308	± 1·286	± 0·705
Repeatability (% CV) (n = 7)	–	± 0·043	–	–
Specificity	Specific	Specific	Specific	Specific
Linearity range (ng/spot)	193–1356	402–2817	71–497	132–927
Correlation coefficient (r)	0·9978	0·9987	0·9939	0·9983

precision of the densitometric determination of peak area was determined by carrying out seven determinations of the spot for sennoside B. The % CV was found to be ± 0·043.

Application of method

The proposed method was used to determine the content of sennosides A, B, C and D in senna leaf, senna pod and different marketed formulations (three tablet formulations, either alone or in combination with other drugs, two granule formulations and one liquid formulation; Table 1). All formulations were analysed by the spectrofluorimetric (United States Pharmacopeia 1995) and spectrophotometric (British Pharmacopoeia 1993) procedures for their total sennoside content (Table 3).

Table 3. Comparison of total sennoside content estimated using HPTLC, British Pharamacopoeial and United States Pharmacopeial methods.

Sample analysed	HPTLC method	British Pharmacopoeial method	United States Pharmacopeial method	Labelled/expected amount of total sennosides (% w/w)
	Total sennoside (% w/w ± s.d.) (n = 4)*			
Senna pod	1·884 ± 0·072	1·897 ± 0·078	1·889 ± 0·086	2·2
Senna leaf	2·152 ± 0·060	2·160 ± 0·081	2·142 ± 0·098	2·5
Ca-sennosides tablets				
T1	11·666 ± 0·096	11·815 ± 0·072	11·962 ± 0·171	13·480
T2	0·414 ± 0·002	0·341 ± 0·008	0·311 ± 0·163	2·188
T3	7·842 ± 0·020	7·840 ± 0·109	7·734 ± 0·068	8·695
Granules containing senna extracts				
G1	0·687 ± 0·005	0·638 ± 0·015	0·577 ± 0·021	3·00
G2	1·210 ± 0·099	1·210 ± 0·015	1·200 ± 0·013	1·25
Liquid preparation (senna extract)	0	0	0·047 ± 0·001	0·76

*Replicates of single extract.

Comparison of methods

The results obtained using the two different pharmacopoeial methods, i.e. spectrophotometry (British Pharmacopoeia) and spectrofluorimetry (United States Pharmacopeia), and the HPTLC method, were compared using the 'F' test. Results showed that the total sennoside content obtained with the HPTLC method compared favourably with the pharmacopoeial methods. The observed F value is in the range 1·720–3·756, which is less than the table value (4·26, n = 4) at 95% limit of confidence. This revealed that there was no significant difference between the proposed HPTLC method and official pharmacopoeial methods, indicating that the proposed method is as precise and accurate as the official methods.

When the liquid senna preparation was subjected to the HPTLC method and the spectrophotometric method the presence of sennosides was not shown, but the United States Pharmacopeial method did show a small amount of sennoside. This may be due to the reduction of some of the constituents present in the preparation, which may give fluorescence. Thus, the United States Pharmacopeial method is non-specific compared with other methods.

References

Auterhoff, H., Sachdev, R. (1962) Suggested improvements in the determination of anthraquinones. Deut. Apotheker Ztg. 102, 30: 921–923

British Pharmacopoeia (1993) HMSO, London, Vol.II, p. 1095

Dreesen, M., Eyssen, H., Lemli, J. (1981) The metabolism of sennosides A and B by the intestinal microflora: in-vitro and in-vivo studies on the rat and the mouse. J. Pharm. Pharmacol. 33: 679–681

Duez, P., Vanhelen, M., Vanhaelen Fastre, R., Hanoeg, M., Molte, L. (1984) Comparison between high performance thin layer chromatography and high performance liquid chromatography for the determination of sennosides A and B in Senna (Cassia sp.) pods and leaves. J. Chromatogr. 303: 391–395

European Pharmacopoeia (1998 Supplement) Third edition, Council of Europe, Strasbourg, pp 456–460

Fairbrain, J. W. (1953) Some recent advances in the knowledge of the constituents of vegetable drugs. J. Pharm. Pharmacol. 5: 281–292

Fairbrain, J. W., Moss, M. J. (1970) The relative purgative activities of 1,8-dihydroxyanthracene derivatives. J. Pharm. Pharmacol. 22: 584–593

Frederich, H., Baier, S. (1973) Untersuchungen uber die inhalisstofee der sennesblatter. Planta Med. 23: 74–87

Komolafe, O. O. (1981) High pressure liquid chromatographic analysis of sennosides A and B purgative drugs. J. Pharm. Sci. 70: 727–730

Kussmaul, W., Becker, B. (1947) Colorimetric determination of the glucosides of senna. Helv. Chem. Acta. 30: 59

Metzger, W., Reif, K. (1996) Determination of 1,8-dihydroxyanthranoids in senna. J. Chromatogr. A. 740: 133–138

Muller, K. H., Christ, B., Kuhn, G. (1962) Comparative study of the photometric determination of sennosides in drugs. Arch. Pharm. 295: 41–55

Ohshima, Y., Takahashi, K. (1983) Separation method for sennosides. J. Chromatogr. 258: 292

United States Pharmacopeia (1995) The United States Pharmacopeial Convention Inc., Rockville, MD. 23: 1407–1408