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Note

Determination of sennosides A and B in Senna extracts by high-performance liquid chromatography

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Senna leaves and pods have long been used as laxatives and purgatives[1], and numerous studies have been made to determine their principal constituents: anthracenic derivatives (anthronic, dianthronic and anthraquinonic forms) and flavonic derivatives. Sennosides A and B[2] (dianthronic glucoside derivatives) have proved to be the most active Senna constituents[3].

Several different methods, such as thin-layer chromatography (TLC)[4–11] and high-performance liquid chromatography (HPLC)[11–18], have been proposed for the specific evaluation of sennosides A and B. However, only a few of them result in simple, rapid and accurate quantitation, and are applicable to Senna extracts. A method was thus developed for the qualitative and quantitative assay of sennosides A and B in Senna extracts by ion-pairing HPLC.

MATERIALS AND METHODS

Reagents

Sennosides A and B, Standard quality (Sarsyntex, Mérignac, France) were used as reference samples. Methanol and ethanol, analytical-reagent grade were obtained from (Merck, Darmstadt, F.R.G.), phosphoric acid RP Normapur and ammonia solution RP Normapur from Prolabo (Paris, France). The ion-pairing reagent was obtained from a PIC A vial (Millipore-Waters) containing 5 m*M* tetrabutylammonium phosphate.

Sep-Pak C_{18} cartridges (Millipore-Waters) were employed. The mobile phase consisted of methanol–water plus PIC A (36.5:63.5); if necessary, the pH was adjusted to 7.5 with a few drops of phosphoric acid. It was filtered before use, through a 0.45-μm HAWP filter (Millipore), then degassed using helium. The flow-rate was 0.6 ml/min.

Apparatus

The liquid chromatograph consisted of a Spectra-Physics 8700 solvent-delivery system, a Rheodyne injection valve (sample loop of 10 μl) and a variable wavelength UV detector (UV/Visible SF 770 Schoeffel detector) operating at 215 or 270 nm, the absorbance maxima of sennosides A and B. A Hibar LiChrosorb RP-8 column (250 mm × 4 mm, 10 μm) (Merck) fitted with a pre-column packed with Corasil C_{18} (50 μm) (Millipore-Waters), was used.

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Sample preparation

Standard solutions of sennosides A and B. Accurately weighed quantities of sennosides A and B were diluted to known concentrations in the mobile phase. Where necessary, a trace of ammoniac solution can be added to effect complete dissolution of the sennosides.

Extracts of Cassia angustifolia leaves. Different extraction methods for sennosides A and B were tested on different batches of Senna leaves. Two of these processes were found to be efficient: aqueous extraction with heating under a reflux condenser for 45 min; 70% ethanol extraction at room temperature[3,4]. Where necessary, Senna extracts obtained using these procedures were diluted to 25 μg/ml in water or 70% ethanol respectively, then injected for HPLC.

Pre-treatment on Sep-Pak cartridge. A Sep-Pak C_{18} cartridge was conditioned with 5 ml methanol, then 5 ml water. The Senna extract (0.5 ml) was loaded on the cartridge. A 0.5-ml volume of methanol–water (10:90) and 1.0 ml methanol–water (30:70) were passed successively through the cartridge, and discarded. Then two 0.5-ml volumes of methanol–water (70:30) were passed, the fractions containing sennosides (fractions 1 and 2) were collected and examined by HPLC.

RESULTS AND DISCUSSION

Several methods using reversed-phase chromatography, ion pairing or ionic suppression techniques were tested.

The acidic character of sennosides A and B was used to form an ion pair between their carboxylic functional groups and the quaternary ammonium compounds. Then the separation method for standard sennosides was optimized by preparing different mobile phases (Table I). These tests demonstrated that sennosides A and B could easily be separated. Not all mobile phases, however, allowed the study of Senna extracts, because certain components were eluted with sennoside A and/or B. For all the Senna extracts tested, only methanol–water plus PIC A (36.5:63.5), flow-rate 0.6 ml/min, λ = 270 or 215 nm, at room temperature, allowed the separation of the peaks of sennosides A and B from the peaks of the other constituents, in a reasonable time (Fig. 1). We compared the identity of the sennoside peaks with those of standard substances: the absorbance ratio was found to be similar at two

TABLE I

SEPARATION OF SENNOSIDES A AND B WITH VARIOUS MOBILE PHASES

Mobile phase	*k'*	
	Sennoside A	*Sennoside B*
Methanol–water + PIC A (30:70)	10.6	5.4
Methanol–water + PIC A (35:65)	5.8	3.4
Methanol–water + PIC A (36.5:63.5)	4.1	2.3
Methanol–water + PIC A (38:62)	3.4	1.9
Methanol–water + PIC A (40:60)	2.0	1.3
Methanol–water + PIC A (45:55)	0.9	0.6

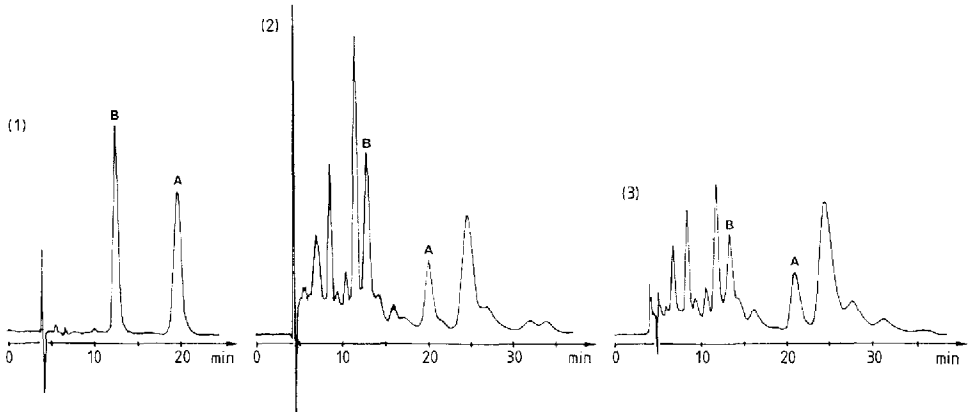

Fig. 1. HPLC chromatograms of Senna extracts. Column: LiChrosorb RP-8. Mobile phase: methanol–water plus PIC A (36.5 : 63.5); flow-rate 0.6 ml/min. Detection: UV, 270 nm. (1) Standard sennosides A and B; (2) aqueous extraction; (3) 70% ethanol extraction. Peaks: A = sennoside A; B = sennoside B.

wavelengths, 270 and 215 nm; the absorbance ratio for standard sennosides A and B is 3.11.

This analysis method can be used for a quantitation of sennosides A and B. Its linearity was tested for sennoside A or B concentrations up to 30 μg/ml, which corresponded with the areas for the concentrations under study.

The constitution of Senna extracts is very complex (Fig. 1). It can be modified according to the Senna part employed (leaves or pods) or by drying of the leaves (which has a great influence on the formation of sennosides)[19]. The pre-treatment on a Sep-Pak C_{18} cartridge that we propose allows chromatogram clarification and confirms the presence of sennosides in the sample, especially when they are present in low concentrations. However, such pre-treatment of Senna extracts has not often been described[18].

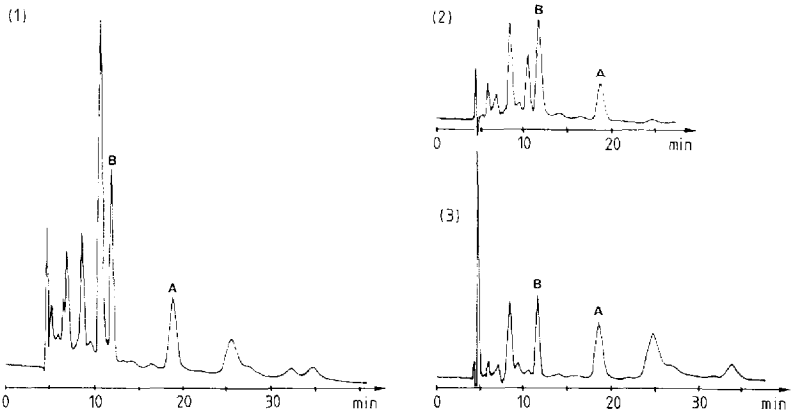

Fig. 2. HPLC chromatograms of Senna extracts. Conditions as in Fig. 1. (1) Without pre-treatment; (2) fraction 1 after pre-treatment with Waters Sep-Pak C_{18} cartridge; (3) fraction 2 after pre-treatment with Waters Sep-Pak C_{18} cartridge. Peaks: A = sennoside A; B = sennoside B.

Sennosides A and B were present in fractions 1 and 2 (Fig. 2). When these fractions were concentrated, traces of sennosides A and/or B could be detected after such simple and rapid pre-treatment.

Satisfactory results were obtained by the present procedure for the qualitative and quantitative determination of sennosides A and B in Senna extracts.

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