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Determination of sennosides A and B in tablets: comparison between a proposed HPLC procedure and the USP fluorimetric method

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A number of methods are used for determination of sennosides in senna-containing products. The methods include colorimetry, derivative spectrophotometry, fluorimetry, TLC and HPLC. Most published HPLC procedures lack good peak separation or require high column temperatures (Komolafe, 1981, Duez et al., 1984). Other procedures use gradient elution and require relatively long run times (Metzger & Rief, 1996). Inspite of the presence of an HPLC assay in the BP monograph of standardised senna granules, the BP deseribes a lengthy and tedious colorimetric method for the assay of total sennosides in senna tablets. Some reports confirmed that the most active constituents of anthracene derivatives of senna are sennosides A and B (Dreesen et al., 1981, Hattori et al., 1982). Consequently, analytical methods that determine" total sennosides" may not be indicative of bioactivity.The present study describes an isocratic reversed-phase HPLC procedure, capable of separate assay of sennosides A and B. The method is essentially based on the BP conditions described for assay of standardised senna granules.

The chromatographgic conditions used were as follows : C_{18} column (100 x 4.6 mm) packed with 3 um particles and a mobile phase consisting of 19 volumes of acetonitrile and 81 volumes of a 1% v/v solution of glacial acetic acid. Detection wavelength was 350 nm. Retention times were 4.3 min (sennoside B) and 8.2 min (sennoside A). The identification of the eluted sennosides was confirmed by spectral analysis using photodiode array detector and sennosides A and B standards. The method was validated for linearity, precision, accuracy and limit of quantitation. Linearity was found between peak areas and concentrations over the range 0.01 - 0.2 mg ml^{-1} (r = 0.995- 0.998) for sennosides A and B. Replicate runs (n = 10) for lowest and highest concentrations gave RSD values 0.96 and 1.20%. Analyses of synthetic mixtures containing the two sennosides showed percentages recovery between 97.5 and 101.7 Limit of quantitation for both sennosides was 20 ng (RSD : 3.60%). The proposed method was applied to the determination of sennosides A nd B in six brands of senna tablets. After grinding to a fine powder, an amount was extracted with 70% methanol. After membrane filtration, an aliquot was injected. The results obtained were compared with those of the USP spectrofluorimetric method (Table 1).The data indicate that the results of the USP method were relatively higher than the proposed HPLC procedure. This may be attributed to possible interferences of other anthracene species in the spectrofluorimetric assay. The HPLC procedure is suitable for the selective determination of sennosides A and B in senna tablets and for content uniformity and dissolution testing specified in the USP monograph of sennosides tablets.

Table 1. Results of analysis of six brands of senna tablets by the proposed HPLC procedure (I) and the USP method (II)

Brand	Total sennosides (mg/tablet)	Total Sennosides (%) I *	II
A	7.5	102.0	116.9
B	7.5	98.0	119.7
C	12.0	101.7	126.3
D	12.0	94.5	108.6
E	12.0	107.6	120.5
F	20.0	101.5	129.0

* As the sum of sennosides A and B

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