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Research Article

Quantification of Sennosides By Reverse Phase High Performance Liquid Chromatography Coupled With Electro Spray Ionization Tandem Mass Spectrometry in Unani Formulations

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ABSTRACT

Unani system is a science which deals with the preventive and promotive aspects of health of human beings and health problems occurred by the Ecological and Environmental factors, which may vitiate humours i.e. Blood, Phlegm, Yellow bile and Black bile, the fluids circulating in the body vessels. There is considerable demand in drug testing for a specific and precise analytical method for the identification of sennosides in various Unani formulations. A combination of high performance liquid chromatography and mass spectrometry (LC-MS/MS) will provide unambiguous fingerprint information for chemical structural confirmation and estimation of sennosides. The objective of the present investigation is to develop a simple, economical and reliable high performance liquid chromatography method for the quantification of sennosides in Unani formulations. The validated method allows quantification of sennosides in 1 – 100.00 µg/mL. The correlation coefficient was ≥0.9990 for the sennosides. The simplicity of the assay and rapid liquid-liquid extraction make it an attractive procedure in estimation of sennosides in Unani formulations. The separation was achieved in C18 column and negative ion mode was used for detection in ESI-MS detection.

Keywords: Sennosides; Hydrochlorothiazide and RPHPLC

INTRODUCTION

Sennosides A and B [CAS No: Sennoside A(81-27-6) & sennoside B(128-57-4)], chemically 2 (5 , 5 ' - b i s (B - D - g l u c o - p y r a n o s y l o x y) - 9 , 9',10,10'-tetrahydro-4,4'-dihydroxy-lO,lO'-dioxo(R,R')-[9,9'-bianthracenel-2,2 '-dicarboxylic acid and (5,5'-bis(B-D-glucopyranosyloxy)-9,9',10,10'-tetrahydro-4,4" - d i h y d r o x y - l O , l O ' - d i o x o (R , S ') [9 , 9 ' - b i - anthracene]-2,2 '-dicarboxylic acid, respectively),is a anthraquinone derivative used for treatment of skin diseases, constipation, dysentery, etc[1-3]. Liquid chromatography-electrospray-mass spectrometry (LC-ES-MS) has emerged as a sensitive and accurate analytical technique. Electrospray generates ions under atmospheric pressure and at relatively low temperature which minimizes thermal decomposition of labile compounds. In addition, mass spectrometry offers highly selective measurement by detecting specific mass-to-charge (m/z) ion related to analytical component; hence, more precise assignment of each eluted component. Various methods for estimation of Sennosides by HPTLC and HPLC have been reported[4-9]. The present paper reports a simple, precise and accurate method for the quantification of sennosides by LC-ESI-MS/MS with negative ion mode in Unani formulations.

MATERIALS AND METHODS

Sennosides A (CAS No. 81-27-6) and sennoside B (CAS No.128-57-4) (QTP-99.88%w/w) and Hydrochlorothiazide (CAS No: 58-93-5) (HYD-99.50%w/w) reference standard were a purchased from Sigma Aldrich, Bangalore and Varda Biotech., Mumbai, India respectively. Genesis C18 RP (100 mm x 4.6 mm i.d., 4µ), Grace Vydac was used as stationary phase. All chemicals and reagents used were of super gradient and purchased from Labscan Asia, Samutsakorn Province, Thailand. HPLC-grade water was prepared with a Milli-Q water purification system. A Thermo Finnigan (USA), HPLC system containing TSQ Quantum Discovery Max mass spectrometer (USA) was used for present study. Unani formulations were procured from Hamdard India Limited.

Preparation of reagents and solutions: Stock solutions of sennosides and hydrochlorothiazide were prepared by dissolving weight equivalent to 2.50 mg in acetonitrile and diluting upto 5 mL separately. Intermediate and working solutions were prepared by further diluting these stock solutions with acetonitrile: water (40:60, %v/v).

Table-1 Linearity Range with LOD

Compound	Retention lime	Linearity range	r^2	Detection limit
Sennoside	4.25	1-100 µg/mL	≥0.99	0.2 µg/mL

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Table-2 Percentage Recovery

Compound	Amount spkied (µg/mL)	Recovery (Average of three results)
Sennoside	10.0	98.10%

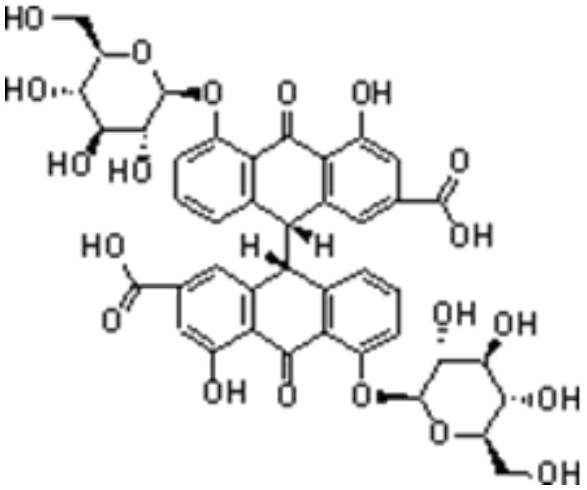

Fig.1 Structure of Sennoside

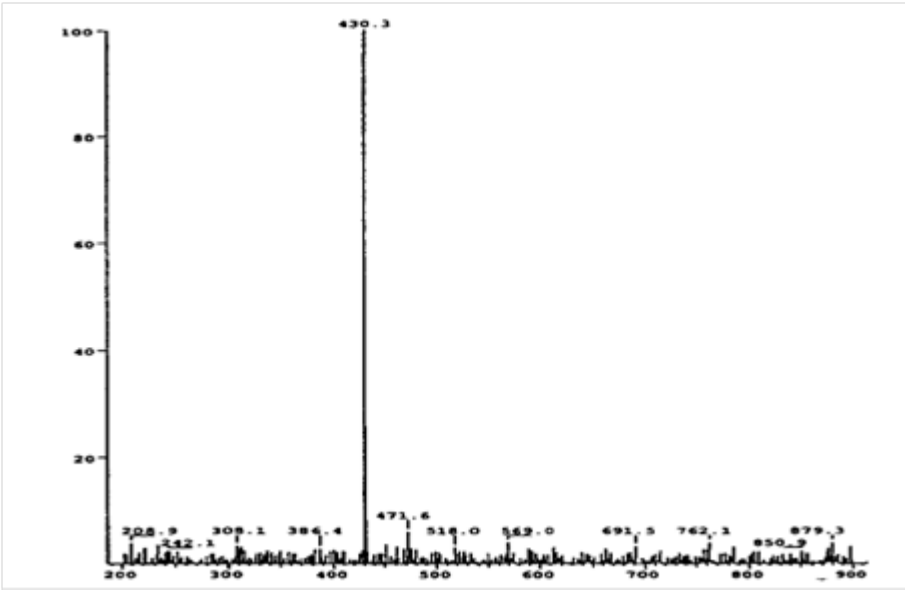

Fig.2 Representative negative ion spectra of Sennoside

The mobile phase consisted of a mixture of acetonitrile (ACN):0.05%v/v acetic acid in water (60:40%v/v/v).

Sample preparation: The Unani formulations were extracted with 70% methanol (each 5 mL) by stirring at room temperature for 30 min. Extraction was repeated three times. The extracts were combined and filtered through a No. 1 filter. The solution was stored in the refrigerator (4 °C). A micro-porous filter (0.45 µm) was utilized to filter the solution prior to LC-MS analysis.

High performance liquid chromatography and mass spectrometric conditions: Chromatographic separation was carried out on a Thermo Finnigan HPLC with a Genesis C18 100 RP, (100 mm x 4.6 mm i.d., 4µ) column. A mobile phase consisting of mixture of acetonitrile (ACN):0.05%v/v acetic acid in water (60:40%v/v/v) was delivered with a flow rate of 0.500 ml/min isocratically. The total run time for each sample analysis was 5.50 min and column oven temperature was maintained at 40º C with injection volume of 20µL. Detection of sennoside and hydrochlorothiazide was via LC--MS/MS.

The LC-MS/MS experiments were performed with a Thermo Finnigan LC module equipped with TSQ Quantum Disovery Max Triple Quads mass spectrometer in negative ionization mode. Nitrogen was used as a sheath gas at 40psi and argon was used as auxiliary gas at 20 psi. an electrospray voltage of 4500v was applied and the capillary temperature was set at 350°C. The mass analyzer was set to monitor negative ions. Sennoside and Hydrochlorothiazide were monitored at m/z 386.50 and 223.00 respectively at collision energy of 10 and 11v respectively.

RESULTS AND DISCUSSIONS

A simple, specific, rapid and sensitive analytical method for the determination of sennoside has been developed. Electrospray is a soft-ionization technique, collision-induced dissociation (CID) has been used to enhance

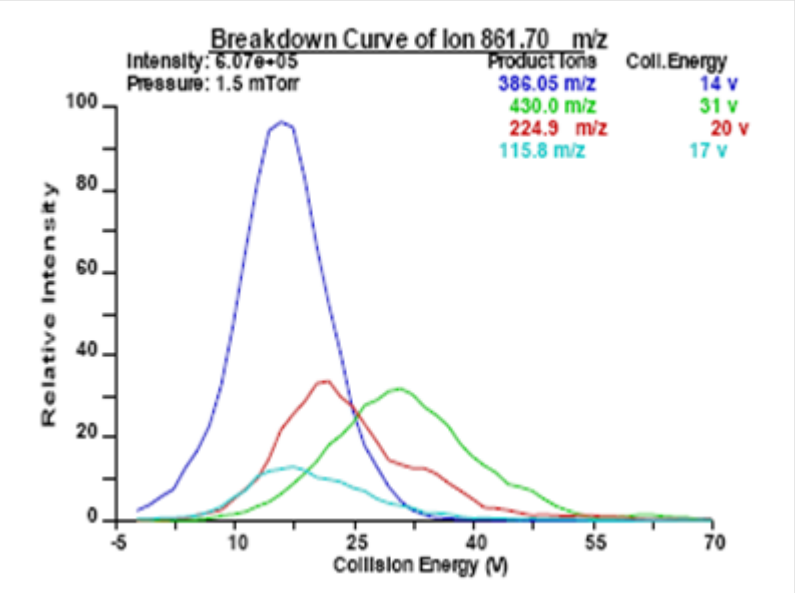

Fig. 3 Breakdown curve of Sennoside

molecular fragmentation. Negative ion mode was employed for the detection of Sennoside. The structure of the sennoside, negative ion spectra and breakdown curve of mass are captured in Figure-1, Figure-2 and Figure-3 respectively. The linearity range with limit of detection is summarized in Table-1. Adequate linearity ($r^2 \geq 0.9990$) was obtained through the range examined. The detection limit based on signal to noise of 5 was 0.2µg/mL in order to examine the matrix effect of the Unani formulation extract on determination of sennoside a known amount of pure sennoside was spiked to see the percentage recovery which was found to be 98.10% which is summarized in Table-2. It demonstrated that this sample extraction procedure is effective and indicated that there is no matrix effect on the measurement.

In summary, this work has successfully demonstrated the potential of LC-ES-MS for quantitative determination of sennoside. Adequate linearity and detection limit were also obtained. In addition, the application of this newly developed method was demonstrated by analyzing Unani formulation samples. The other major advantage of this method over all those referenced is the short run time of 5.50 min with single step extraction technique as compared to already reported articles; LC-ES-MS method is a promising alternative to the analysis of sennoside in Unanai formulations.

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REFERENCES

1. Okabe H, Matsuo K, Nishioka I. Studies on Rhubarb (Rhei Rhizoma). II. Anthraquinone Glycosides *Chemical and Pharmaceutical Bulletin*. 1973; 21(6): 1254.
2. Oshio H, Imai S, Fujioka S, Sugawara T, Miyamaoto M, Tsukui M. Investigation of Rhubarbs. III. New Purgative Constituents, Sennosides E and F *Chemical and Pharmaceutical Bulletin*. 1974; 22(4): 823-831.
3. Hsu HY, Chen YP, Hsu SJ, Chen CC, Chang HC. Chin. Mater. Medica – A Concise Guide; Modern Drug Press, Taipei, Taiwan, 1985.
4. Azam MM, Limy T. An improved HPLC method for estimation of sennosides in Senna. *Indian Journal of Pharmaceutical Sciences*. 2002; 64(2): 178-181.
5. Bala S, Uniyal GC, Dubey T, Singh SP. An improved method for the analysis of sennosides in *Cassia angustifolia* by HPLC presented on national seminars on frontiers of research and development in medicinal plants. Lucknow, CIMAP, 2000.
6. Habib AA, EI-Sebakhy NA. Spectrophotometric estimation of sennosides and rhein glycosides in senna and its preparations. *Journal of Natural Products*. 1980; 43(4): 452-458.
7. Lin YT, Huang CY. Determination of sennoside A and B in Diet tea by HPLC. *Journal of Food Drug Analysis*. 1998; 6: 433-438.
8. Shah SA, Ravishankara MN, Nirmal A, Shishoo CJ, Rathod IS and Suhagia BN Estimation of individual sennosides in plant materials and marketed formulations by an HPTLC method. *Journal of Pharmacy and Pharmacology*. 2000; 52(4): 445-449.
9. Upadhyay A, Nayak PS, Dwivedi SK, Rao S. HPTLC Densitometric quantification of sennosides from *Cassia angustifolia*. *Genetics and Plant Physiology*. 2011, 1(1-2): 38-44.