ORIGINAL RESEARCH ARTICLE

Current Research on Biosciences and Biotechnology **4** (2) 2023 269-274

Current Research on Biosciences and Biotechnology

www.crbb-journal.com

Development and validation of analysis method for sennoside B in *Cassia angustifolia* using UPLC-MRM/MS

Amrianto[ab*], Kurnia Nastira Ningsih[a], Sumail Sidik Ode Ishak[a], Ula Aulia Fitrian[ab], Syefira Salsabila[a], Diah Astari Salam[a]

[a]EBM Scitech Research Center, EBM Scientific and Technology Ltd., Bandung 40142, West Java, Indonesia
[b]University Center of Excellence for Nutraceuticals, Bioscience and Biotechnology Research Center, Bandung Institute of Technology, Bandung 40132, West Java, Indonesia

ABSTRACT

Cassia angustifolia is a commonly found wild plant from the family Caesalpinaceae that originates from Yemen and Hadramaut province in Southern Arabia, where it is called Arabian senna. The leaves of the plant have been used to treat a variety of ailments such as constipation, malaria, anemia, loss of appetite, and indigestion. Sennosides A and B are the major glycosides found in the leaves and pods of *C. angustifolia* and are important ingredients in purgative medicines. These compounds are considered as the major active components of Cassia plants and are responsible for their therapeutic activities. To assess the quality and quantity of sennosides A and B, an appropriate analytical method is required, which must be simple, accurate, precise, and widely used. The UPLC-ESI-MRM/MS method was used in this study to validate the analytical method in determining the contents of Sennoside B in senna leaves extract. The validation parameters included specificity, system suitability, linearity, sensitivity (LOD, LOQ), accuracy, and precision. The results indicated that the optimization of MRM using the direct infusion method provided good separation when eluted using liquid chromatography. The validation parameters for system suitability obtained RSD under 2%. The linearity of sennoside B showed excellent results (R2 = 0.999) in the concentration range of 0.98–62.5 µg/ml. The LOD and LOQ values of sennoside B were 0.011 µg/mL and 0.034 µg/ml, respectively. The accuracy values of sennoside B met the predetermined criteria, with RSD < 2% and % recovery of 97-102%. The quantitative analysis revealed that Cassia angustifolia extract contained 0.43 ±0.06 mg/g of sennoside B.

Article history:

Received 20 Feb 2023
Revised 24 Feb 2023
Accepted 27 Feb 2023
Available online 28 Feb 2023

Keywords:

Cassia angustifolia
sennoside B
method validation
UPLC
MRM

*Corresponding authors:
amrianto.amin@gmail.com

DOI: 10.5614/crbb.2023.4.2/22AMMLH7

1. Introduction

Cassia angustifolia Vahl is a wild plant commonly found in the Caesalpinaceae family, which is believed to have originated in Yemen and the Hadramaut province of Southern Arabia, where it is commonly referred to as Arabian senna (Tripathi, 1999). Senna cultivation has now expanded to Western Europe, North Africa, and various countries in South-East Asia, where it is being grown successfully (Husain, 1992). Senna leaves have been traditionally utilized for treating a variety of conditions including constipation, loss of appetite, hepatomegaly, splenomegaly, indigestion, malaria, jaundice, and anemia. The leaves and pods of *C. angustifolia* contain two major glycosides, sennosides A and B, which are widely recognized as key components in purgative medicines (Anon, 1966). These sennosides are of the aloe-emodin di-antraone di-glucosides type. In addition to these, the leaves and pods of senna also contain other glycosides of anthraquinones, rhein, and chrysophenic acid (Upadhyay et al., 2011).

Sennosides are a group of natural anthraquinone derivatives and dimeric glycosides, which serve as the primary bioactive compounds found in medicinal plants such as senna, and are mainly used in traditional herbal laxatives. Among the various sennosides, sennoside A and B are the primary purgative components, which were initially isolated and identified from the leaves of senna, and

later identified as members of the anthraquinone family (Le et al., 2021). According to reports, sennosides exhibit multiple pharmacological effects, such as having laxative properties that help in treating common gastrointestinal disorders (Jones et al., 2002); anti-obesity effect by decreasing energy intake from intestine by accelerating transit of luminal contents in the distal colon (Rumsey et al., 1993); hypoglycaemic effect in the treatment of type 2 diabetes (T2DM) (Wei et al., 2020); hepatoprotective effect by inhibiting the proliferation of hepatic stellate cells (HSCs) and suppressing the progression of liver fibrosis (Zhu et al., 2020); anti-inflammatory effect in the pathogenesis of pancreatitis (Chen et al., 1999); and anti-tumor effect in human osteosarcoma cells (Chen et al., 2009).

Senna is a compact plant that possesses leaves and pods, which are comprised of at least 2.5% anthraquinone glycosides, specifically sennosides A and B. These active compounds are essential to the medicinal properties of Senna. Sennoside A is characterized by R=COOH (threo form), while sennoside B has R=COOH (erythro form) (Metha and Ladda, 2009). Sennosides are a type of laxative that exerts purgative effects by converting rhein anthrone. Although both sennoside A and B are active components of Senna, they exhibit different biological activities. Sennoside A has been found to improve insulin resistance, whereas sennoside B has been shown to inhibit cell proliferation in human osteosarcoma and the growth of *Entamoeba histolytica* trophozoites (Chen et al., 2009; Espinosa et al., 2020).

Both of these compounds have diverse pharmacological activities, especially sennoside B. According to a study by Chen et al. (2009), sennoside B has been identified as a specific inhibitor of PDGFR-β activation caused by PDGF-BB, and it is capable of down-regulating downstream signalling and cell proliferation. This particular molecule may be considered as a promising option for therapeutic use in diseases where excessive PDGF-dependent cell proliferation is involved, such as cancer, atherosclerosis and restenosis.

Determining the quality and quantity of sennosides is crucial for assessing their potential therapeutic uses in other diseases, and therefore an appropriate analytical method is necessary (Bhope et al., 2010). Validation is a crucial process that is necessary to ensure that a method or process meets the established requirements and is suitable for its intended use. In the context of drug manufacturing and laboratory testing, validation is essential to comply with current good manufacturing practices (CGMP) and good laboratory practices (GLP) requirements (Chikanbanjar et al., 2020). A more practical method for determining plant constituents is necessary, which is less complicated, affordable, time-efficient, and can be used on a larger scale (Tiwari et al., 2010). Ultra-Performance Liquid Chromatography (UPLC) is a potential method that can be utilized for the estimation of plant constituents. UPLC works based on the same principle as HPLC, but with improved speed, sensitivity, and resolution, making it a more efficient and accurate method for analysis (Sheliya and Shah, 2013). UPLC, or Ultra-Performance Liquid Chromatography, is a type of liquid chromatography that uses columns with particles smaller than those used in traditional HPLC (2.5-5 μm). This allows for improved speed, sensitivity, and resolution in the separation of components. When combined with a mass spectrometer (MS or MS/MS), UPLC can be used for a wide range of clinical applications, such as bioanalytical method and metabolite studies, stability-indicating method study, impurity profiling of drugs, separation of isomers, and separating the impurities and degradation products of drugs and metabolites (Rathod et al., 2019). Additionally, a different research paper outlined that the combination of UPLC and mass spectrometry using multiple reaction monitoring (MRM) modes is a highly effective method for quickly analyzing a mixture in Traditional Chinese Medicine (TCM) (Liu et al., 2012; Li et al., 2021). The objective of this study is to validate analytical methods for quantifying the levels of sennoside B in extracts of *C. angustifolia* leaves using the UPLC-MS/MS technique. The development and validation of this method involved using MRM modes.

2. Materials and methods

2.1. Chemicals and reagents

Sennoside B was purchased from Markherb® (Bandung, Indonesia). *C. angustifolia* was obtained from the local market. Deionized water was generated using Milli-Q laboratory water purification system (Millipore®, Bedford, MA, USA). All reagent and solvents were analytical and HPLC grade (Merck®).

2.2. Preparation of reference standard solution

The stock standard solution (1 mg/ml) of sennoside B was freshly prepared in methanol (MeOH). The resulting solution was sonicated for 2 min and then filtered through 0.22-μm PTFE.

2.3. Liquid chromatography-mass spectrometry analysis

2.3.1. Instrumentation

Analysis was performed on an Acquity UPLC H-Class system equipped with binary solvent manager, sample manager, and column heater (Waters, Milford, Michigan, USA) coupled triple quadrupole. The operation of the instrument and data analyses were carried out using analyst software Masslynx.

2.3.2. Liquid chromatography conditions

The mobile phase consisted of solvent A: water + 0.1% formic acid and solvent B: acetonitrile + 0.1% formic acid. The elution was performed in gradient mode during a time course of 10 min as follows: 0-1.0 min, 5% B; 1.0-1.5 min, 5-10% B; 1.5-2.0 min, 10-20% B; 2.0-3.5 min, 20-28% B; 3.5-5.0 min, 28-30.3% B; 5.0-5.5 min, 30.3-50% B; 5.5-10.0 min, held to 50% B at flow rate of 0.3 ml/min. After reaching the maximum gradient of 95% B, the composition was then returned back to the initial state (5% B) in just 0.01 minutes. The column was then left to equilibrate for 5 minutes before the next injection. The column was maintained at a temperature of 40°C, and the injection volume for both samples and standards was 3 μl.

2.3.3. Mass spectrometry conditions

The MRM detection was carried out using ESI in negative mode (ESI-) with the following source parameters: spray voltage, source gas

flow (desolvation 900 l/h), and source temperature (desolvation temp 450°C). The collision energies, capillary voltages, and cone voltages were adjusted for each standard to obtain optimal resolution and sensitivity. The method was optimized by directly infusing a standard solution of 10 μg/ml.

2.4. Method validation

2.4.1. Specificity

The term specificity in analytical method refers to its ability to accurately measure individual components in a complex mixture. In this study, the specificity of the UPLC-MS/MS method was evaluated by comparing the chromatograms and multiple reaction monitoring (MRM) spectral patterns of standard solutions, blank solutions (methanol), and the *C. angustifolia* extract (Hwang et al., 2017; Lee et al., 2021).

2.4.2. System suitability

Six replications of sennoside B at concentration of 7.81 μg/ml in the MeOH solution was simultaneously analyzed. Retention time (Tr), peak area (AUC), tailing factor (T), and theorical plat number (N) were determined. Relative standard deviation (RSD) values of these parameters were calculated to evaluate the system suitability of the developed method. The suitability test is accepted when the RSD values of these parameters are less than 2% (USP, 2009).

2.4.3. Linearity

To verify the linearity of the calibration curves, standard solutions of sennoside B were prepared with concentrations of 0.98; 1.98; 3.9; 7.81; 15.625; 31.25 and 62.5 μg/ml and analyzed using UPLC. The analysis was repeated three times for each concentration, and linear least squares regression was used to calculate the slopes, intercepts, and correlation coefficients. The acceptance criterion for the correlation coefficients was set at 0.998 (LoBrutto and Patel, 2007).

2.4.4. Precision and accuracy

To evaluate precision, the relative standard deviation (%RSD) was determined by analyzing the calibration standards with three concentration levels of standard solution. Accuracy was determined by calculating the percentage of recovery of the measured concentration with three concentration levels for each standard. The acceptance criteria for the percentage of relative standard deviation was set at a maximum of 2%, while the percentage of recovery was expected to be between 97-103% (FDA, 2020).

2.4.5. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined as the lowest concentrations of standard solutions or the lowest spiked concentrations in fortified samples that produce a signal-to-noise ratio of 3 and 10, respectively. The calculation of the LOD and LOQ was performed using the following formula (Hwang et al., 2017; Syukri et al., 2015).

$$LOD = 3.3 \times \frac{SD}{a} \quad (1)$$

$$LOQ = 10 \times \frac{SD}{a} \quad (2)$$

where,

SD = Standard Deviation

a = The slope of the calibration curve

2.5. Quantitative analysis of sennoside B extract and simplicial from *C. angustifolia* leaves

The leaves of *C. angustifolia* were cut into small pieces and dried in oven at 50°C for 48h. The dried samples were ground into powder and passed through a sieve (20 meshes). The dried sample (1 g) was extracted with MeOH (25 ml) using Ultrasound Assisted-Extraction (UAE) for 20 min at 40°C. The results of the extraction process and then centrifuge using 4000 rpm for 5 min. The supernatant obtained was filtered using a 0.22 μm PTFE filter and the calculation of sennoside B content in the extract was conducted using analysis method that has been validated.

3. Results and discussion

3.1. Optimization of MRM parameters

During the optimization of the MRM mode, two important parameters, cone voltage and collision energy, were adjusted to achieve optimal detection and selectivity of the target compounds. This was based on the precursor and product ions of the compounds, with the aim of maximizing the signal of the selected fragment ion and improving the accuracy of its detection. The results of optimization of mass detection were carried out on ESI Negative Mode (M-H) ionization with a capillary voltage of 2.5 KV, source temperature of 150°C, desolvation temperature of 450°C, nitrogen gas flow rate of 900 l/h, cone 1 l/h. The m/z ratios of the standard compounds were obtained (Table 1). The mass and fragmentation spectrum can be seen in Fig 1.

Table.1 MRM parameters for sennoside B

Parameters	Sennoside B
Parent ion (m/z)	861.47
Transition MRM	
Quantifier (m/z)	386.22
Qualifier (m/z)	224.27
Cone voltage (v)	80
Collision energy	
Quantifier (v)	40
Qualifier (v)	70

3.2. Method validation

Method validation according to USP was carried out to ensure that the analytical method is accurate, specific, reproducible, and resistant to the range of analytes to be analyzed. In summary, validation is an act of confirmation that the analytical method to be used is in accordance with the desired purpose.

3.2.1. Specificity

The method specificity is represented in Fig 2.. Specificity was determined by comparing the retention times and MRM spectral patterns of the standard solutions and blank solution. There were no peaks observed at the retention time of sennoside B in the blank sample. The result indicated the method specificity.

3.2.2. System suitability

The optimized UPLC-MRM method was subjected to system suitability test. A sample was injected six times in the system and the Retention time (Tr), peak area (AUC), tailing factor (T), and theorical plat number (N) were determined. As shown in Table 2, the RSD values of all these parameters for sennoside B as well were below 2% which indicated all parameters of the proposed UPLC-MRM method met the USP and ICH standards. Therefore, the developed UPLC method is concluded to be suitable and effective for the analysis.

3.2.3. Linearity

Linearity is the ability of analytical methods to provide a direct response, or with the help of a good mathematical transformation, proportional to the concentration of the analyte in the sample. Linearity testing was performed in triplicate by concentration. The experiment of linearity ($R^2 \geq 0.998$) was observed for sennoside B standard solutions in the concentration range of 0.98 – 62.5 µg/ml. The linear regression analysis of sennoside B generated y = 34.122x + 66.838. The LOD values of sennoside B was 0.011 µg/ml, while the LOQ was 0.034 µg/ml. The results of linearity as well as LOD and LOQ validation for sennoside B standard can be seen in Table 3.

3.2.4. Precision and accuracy

Accuracy is an analytical method that describes the closeness of the value obtained by the method to the actual concentration value. While the precision describes the closeness of the repeatability of the test sample measurement. Accuracy and precision were carried out by testing 3 variations of concentration. Accuracy and precision observations were carried out in three replications for each concentration. Accuracy is indicated by the percentage of recovery while precision is indicated by the percentage value of relative standard deviation (%RSD). Standard for acceptance of percentage of relative standard deviation (%RSD) lowest 2% and for percentage of recovery 97-103% (FDA. 2020). The results of the accuracy and precision of the indications obtained show that the requirements of the predetermined criteria were fulfilled. The results are shown in Table 4.

3.3. Quantitative analysis of sennoside B content in *C. angustifolia* leaves

C. angustifolia Vahl (family *Caesalpiniacea*) popularly known as Senna, is a valuable plant drug in Ayurveda and modern system of medicine for the treatment of constipation. This plant contains sennosides that have been useds marker compounds for quantitative analysis. Sennosides, a class of natural anthraquinone derivatives and dimeric glycosides, are main bioactive components from medicinal plants used for traditional herbal laxatives. There are several sennoside compounds found in Senna, including sennoside A, B, C and D. Sennoside B is a type of anthranoid, which is commonly used for treating constipation worldwide. These compounds are found in various plant-based drugs, mostly as O- or C-glycosides, and can have different substituents and aglycone structures such as anthraquinone, anthrone, or dianthrone. Despite their widespread use, there is limited information available on their metabolism and pharmacokinetic characteristics. Sennoside, a dianthrone O-glycoside found in senna leaves and pods, and its aglycone, rhein anthrone, are the most well-studied compounds in this class. After oral intake, sennoside is broken down only in the lower gastrointestinal tract, releasing the active pharmacological component rhein anthrone.

Table 2. System suitability test of the developed method

Parameters	Sennoside B
Retention time (min)	
Mean	4.091
RSD (%)	0.10
Peak area	
Mean (area/µg)	338.35
RSD (%)	1.27
Tailing factor	
Mean	1.00
RSD (%)	1.17
Theorical plate number	
Mean	15.99
RSD (%)	1.71

Table 3. Validation parameters of the analytical method: linearity. And sensitivity for sennoside B

Parameters	Sennoside B
Retention time (min. n=3)	4.09 ± 0.000
Regression equation	y= 34.122 x -66.838
Correlation coefficient (R^2)	0.999
Linear ranger (µg/mL)	0.98 – 62.5
LOD (µg/mL)[a]	0.011
LOQ (µg/mL)[b]	0.034

[a]LOD: 3.3 × (standard deviation of the response/slope of calibration curve; [b]LOQ: 10 × (standard deviation of the response/slope of calibration curve)

Table 4. Validation parameters of the analytical method: accuracy and precision

Compound	Concentration (µg/mL)	Parameter	
		Accuracy Recovery (%)	Precision RSD (%)
Sennoside B	62.5	99.30±0.89	0.90
	31.25	101.59±0.89	0.88
	15.625	102.78±0.14	0.14

Table 5. Sennoside B content in the of *C. angustifolia* leaves

Sample	Sennoside B content	
	(mg/g±SD)	(%±SD)
C. angustifolia leaves	0.43±0.01	0.043±0.00

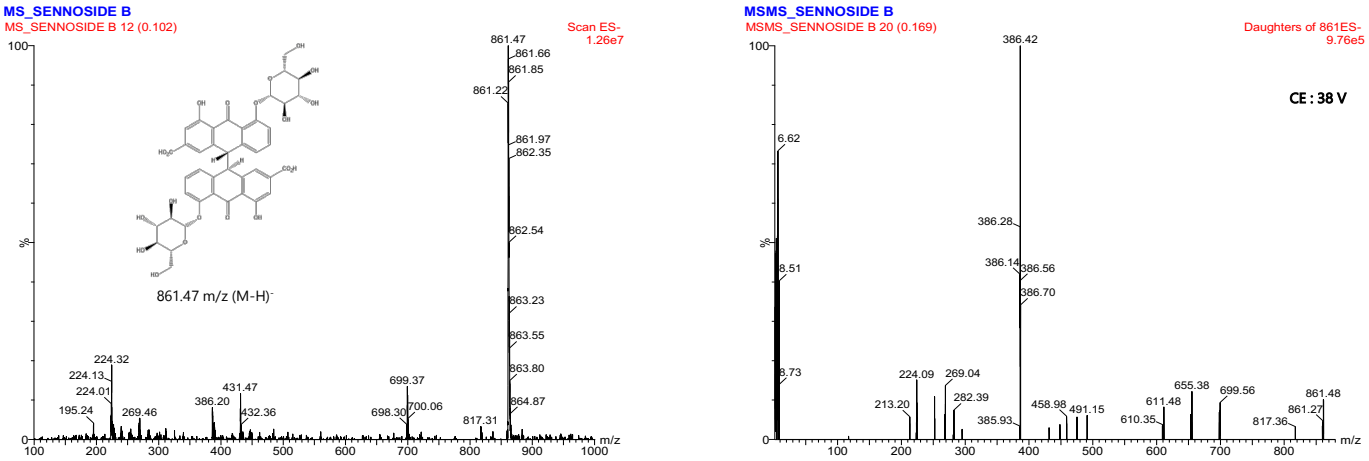

Fig. 1. Mass and fragmentation spectra of sennoside B

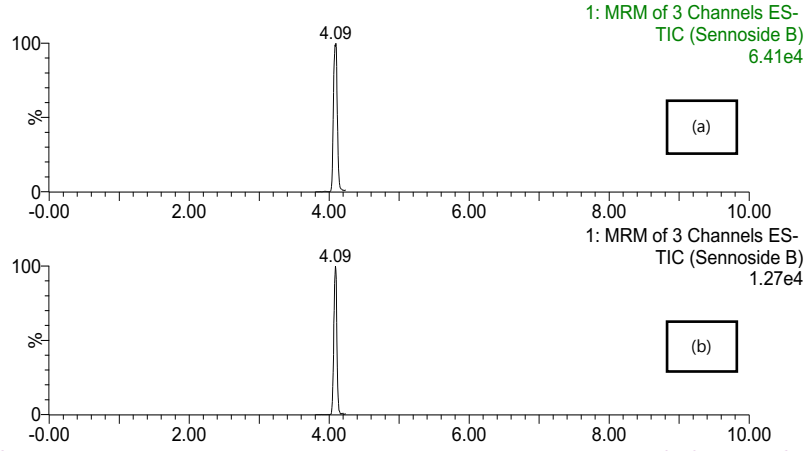

Fig. 2. Ultra-performance liquid chromatogram-MRM/MS (UPLC-MRM/MS) chromatograms, (a) sennoside B; (b) extract of *C. angustifolia* leaves

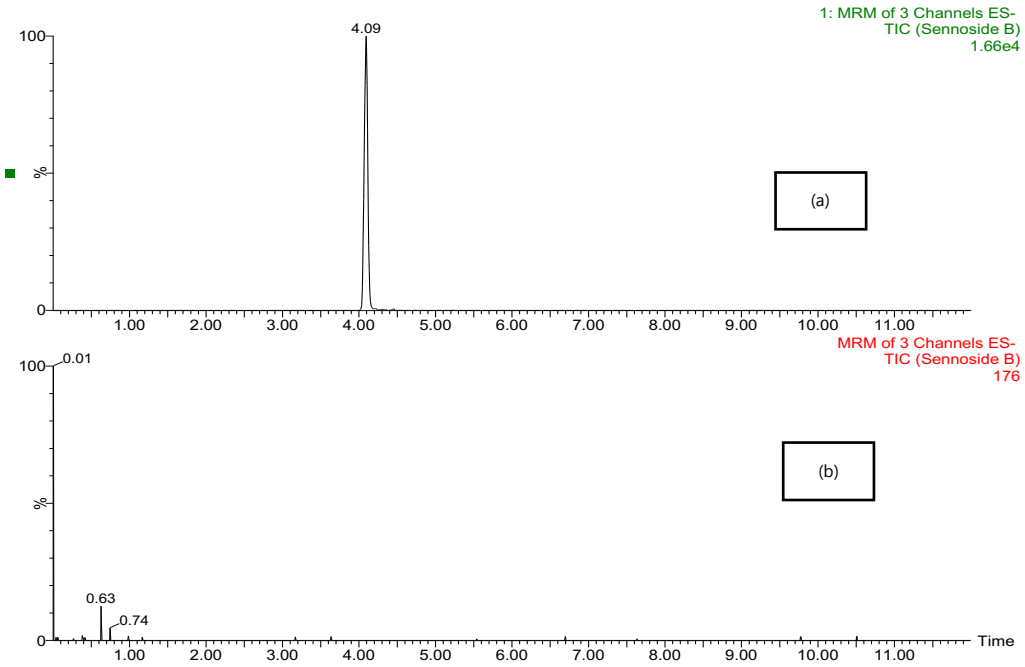

Fig. 3. Specificity test using UPLC-MRM/MS. (a) sennoside B, (b) blank solution (MeOH)

The analysis of sennoside B content was carried out using the analytical method that has been validated previously in this study. The instrument used is Ultra performance liquid chromatography with mode MRM which provide better separation due to the smaller column size and higher sensitivity compared to other instruments and other mode analysis. Based on the results of the analysis, the extract of *C. angustifolia* extracted using Ultrasound Assisted Extraction (UAE) contained 0.43 ± 0.01 mg/g of sennoside B. The calculation results are presented in the Table 5. In addition, UPLC-MRM/MS profiling was carried out to visually see the presence of sennoside B in the extract based on the chromatogram of each test instrument (Fig 3.).

4. Conclusion

In this study, the UPLC-MRM/MS method was optimized and validated according to ICH, USP, and FDA guidelines for drug development. The method used a gradient elution system with 0.1% formic acid in water and acetonitrile. The validation process included system suitability, specificity, linearity, LOD, LOQ, precision, and accuracy. The results of quantitative analysis showed that *C. angustifolia* extract contained 0.43±0.01 mg/g of sennoside B. Overall, the study suggests that the developed method is reliable and simple, and it could be useful for quality control and standardization of *C. angustifolia* leaves extract. Furthermore, the method can be applied for sennoside B analysis of other plant species.

Acknowledgements

This research was internally funded by EBM Scitech.

Conflict of interest

The authors declare there is no conflict of interest in this study.

References

Anon. 1966. Pharmacopoeia of India (2nd Ed.). Delhi, India: Manager of Government Publications.

Bhope S, Kuber V, Nagore D. 2010. Validated HPTLC method for simultaneous quantification of sennoside A, sennoside B, and kaempferol in *Cassia fistula* Linn. *Acta Chromatogr* 22(3): 481–9. doi: 10.1556/AChrom.22.2010.3.11

Chen X, Valente JF, Alexander JW. 1999. The effect of sennosides on bacterial translocation and survival in a model of acute hemorrhagic pancreatitis. *Pancreas* 18(1): 39–46. doi: 10.1097/00006676-199901000-00006

Chen YC, Chang CN, Hsu CH, Chiou SJ, Lee LT, Hseu TH. 2009. Sennoside B inhibits PDGF receptor signaling and cell proliferation induced by PDGF-BB in human osteosarcoma cells. *Life Sci* 84(25–26): 915–22. doi: 10.1016/j.lfs.2009.04.003

Chikanbanjar N, Semwal N, Jyakhwa U. 2020. A review article on analytical method validation. *J Pharm Innov* 1(1): 48–58.

Espinosa A, Paz-Y-Mino-C G, Santos Y, Ma H, Nadeau M, Seeram NP, Rowley DC. 2020. Anti-amebic effects of Chinese Rhubarb (*Rheum Palmatum*) leaves extract, the antraquinone rhein and related compounds. *Heliyon* 6(4). doi: 10.1016/j.heliyon.2020.e03693

FDA F, DA. 2020. Methods, method verification and validation, II, office of regulatory affairs laboratory manual.

Husain A. 1992. Status report on medicinal plants for NAM countries. United States: Centre for Science and Technology of NAM and other Developing Countries.

Hwang SH, Kim JH, Wang Z, Lee JY, Lim SS. 2017. Analytical method for the validation of three polyphenols as a marker compound for the standardization of *Solidago virgaurea subsp. gigantea* extracts and antiadipogenesis of harvesting time and location. *J Anal Methods Chem* 2017(2013). doi: 10.1155/2017/3047408

Jones MP, Talley NJ, Nuyts G, Dubois D. 2002. Lack of objective evidence of efficacy of laxatives in chronic constipation. *Dig Dis Sci*. 47(10): 2222–30. doi: 10.1023/a:1020131126397

Le J, Ji H, Zhou X, Wei Y, Chen Y, Fu Y, Ma Q, Han Y, Sun Y, Gao, Wu H. 2021. Pharmacology, toxicology, and metabolism of sennoside a; a medicinal plant-derived natural compound. *Front Pharmacol* 12(October): 1–14. doi: 10.3389/fphar.2021.714586

Lee SG, Ko H, Choi EJ, Oh DR, Bae D, Choi C. 2021. Isolation and analytical method validation for phytocomponents of aqueous leaf extracts from *Vaccinium bracteatum* thunb. In korea. *Processes* 9(11). doi: 10.3390/pr9111868

Li B, Fan S, Hu J, Ma Y, Feng Y, Wang F, Wang X, Niu L. 2021. Phytochemical analysis using UPLC-MS/MS combined with network pharmacology methods to explore the biomarkers for the quality control of lingguizhugan decoction. *eCAM* 7849032. doi: 10.1155/2021/7849032

Liu Y, Lu J, Zhang J, Wang Q, Wang F, Qiao Y, Zhang Y. (2012). Rapid determination of ten polyphenols in Kudiezi injection using ultra-performance liquid chromatography-tandem mass spectrometry in multiple reaction monitoring mode. Anal. *Methods* 4(12): 4230–6. doi: 10.1039/C2AY26093J

LoBrutto R, Patel T. 2007. Method validation. In HPLC for Pharmaceutical Scientists. John Wiley & Sons, Ltd. pp. 455–502. doi: 10.1002/9780470087954.ch9.

Mehta N, Laddha KS. 2009. A modified method for isolation of rhein from senna. *Indian J Pharm Sci* 71(2): 128-9. doi: 10.4103/0250-474X.54275

Rathod RH, Chaudhari SR, Patil AS, Shirkhedkar AA. 2019. Ultra-high performance liquid chromatography-MS/MS (UHPLC-MS/MS) in practice : analysis of drugs and pharmaceutical formulations. *Future J Pharm Sci* 5(6): 1–26. doi: 10.1186/s43094-019-0007-8

Rumsey RD, Squires PE, Read NW. 1993. *In vitro* effects of sennoside on contractile activity and fluid flow in the perfused large intestine of the rat. *Pharmacology* 47(1): 32–39. doi: 10.1159/000139840

Sheliya KG, Shah KV. 2013. Ultra performance liquid chromatography (UPLC): a modern chromatography technique. *PSM* 4(3): 78–99.

Syukri Y, Nugroho AE, Martien R, Lukitaningsih E. 2015. Validation of andrographolide analysis in Sambilot plant (*Andrographis paniculata* Nees) using HPLC (in Indonesian). *J Sains Farmasi & Klinis* 2(1): 8. doi: 10.29208/jsfk.2015.2.1.42

The United States Pharmacopeial Convention, 2021. USP chapter 621 Chromatography. USP31–NF26. pp 232.

Tiwari RK, Chanda S, Deepak M, Murli B, Agarwal A, Noida G, Pradesh U, Veerasandra. 2010. HPLC method validation for simultaneous estimation of madecassoside, asiaticoside and asiatic acid in *Centella asiatica*. *J Chem Pharm Res* 2(3): 223–229.

Tripathi YC. 1999. *Cassia angustifolia*, a versatile medicinal crop. *For Trees Livelihoods* 10(2): 121–9. doi: 10.1080/01435698.1999.9752999

Upadhyay A, Chandel Y, Nayak S, Khan NA. 2011. Sennoside contents in senna (*Cassia angustifolia* vahl.) as influenced by date of leaf picking, packaging material and storage period. *J Stored Prod Postharvest Res* 2(5): 97–103. doi: 10.5897/JSPPR.9000041

Wei Z, Shen P, Cheng P, Lu Y, Wang A, Sun Z. 2020. Gut bacteria selectively altered by sennoside A alleviate type 2 diabetes and obesity traits. *Oxid Med Cel Longev* 2020(2375676). doi: 10.1155/2020/2375676

Zhu H, He C, Zhao H, Jiang W, Xu S, Li J, Ma T, Huang C. 2020. Sennoside A prevents liver fibrosis by binding DNMT1 and suppressing DNMT1-mediated pten hypermethylation in HSC activation and proliferation. *Faseb J* 34(11):14558–71. doi: 10.1096/fj.202000494RR