Research Article

Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Aloin and Sennoside in Suppository Dosage Form

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ABSTRACT

The simple, sensitive and specific U.V. spectrophotometric methods were developed for simultaneous determination of aloin and sennoside in synthetic mixture. Spectrophotometric studies were carried out using double beam JASCO U.V. spectrophotometer with methanol as solvent. The present work includes two methods: method A- simultaneous equation method and method B- absorbance ratio method. Wavelengths selected are 262.5 nm for Aloin and 276 nm for Sennoside. In both the methods linearity were observed in the concentration range of 5-30 µg/ml and 5-30 µg/ml for Aloin and sennoside, with good correlation coefficient 0.997 and 0.998 respectively. Method B involved formation of absorbance equation at 265nm (isobestic point) and 276nm (λmax of sennoside). Aloin and sennoside are used for the treatment of constipation. Both methods showed high sensitivity with reproducibility in result.

Keywords: Aloin, Sennoside, Simultaneous equation method, Absorbance ratio method

INTRODUCTION

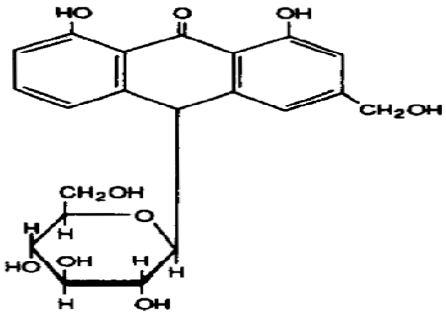
Aloe Vera (Aloe Barbandensis Miller) is a tropical succulent plant, of liliaceous family. It contains 100 separate constituents found in the leaf and mucilaginous gel inside the leaf. Some of the constituents found in the leaf such as Aloin, or the Emodins are recognised as having laxative and antimicrobial properties.[1] Aloin is a Folk Traditional medicine used for constipation, cuts, burns, bites and scares. It is an official drug in the Indian and British Pharmacopeia. Few analytical methods by HPLC and spectrophotometry have been reported for the estimation of Aloin.[3]

Senna (Senna alata L. or Cassia alata L.) is a plant belonging to the family Leguminosae. S. alata leaves have been traditionally used for the treatment of constipation and dermatophyte infections. Sennoside is demonstrating as the active constituents for the laxative properties. Sennoside is isolated from senna leaves. It has been used as folk medicines in many countries for treatment of constipation, stomach pain, ringworm and skin diseases.[9,4] It is an official drug in the Indian and British Pharmacopeia. Some analytical methods by spectrophotometry and HPLC have been reported for the estimation of Sennoside.

The combination of Aloin and Sennoside is very useful in the treatment of Constipation. On literature survey, it was found that not a single method reported for the simultaneous estimation of Aloin and Sennoside. And no method is available in the pharmacopoeias. In view of the need for suitable methods for routine analysis in combination, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in synthetic mixture.

Drug Profile

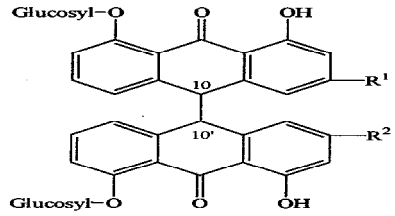
Aloin



Aloin is an anthraquinone glycoside and its IUPAC name: 8-Dihydroxy-10-(β-D-glucopyranosyl)-3- hydroxymethyl)-9(10H)-anthracenone. Its molecular weight: 418g/mol and has molecular formula: $C_{21}H_{22}O_9$. Descriptions: It is a yellowish brown powder extract, Solubility: Freely soluble in methanol, sparingly soluble in water, ethanol, acetone. Category: Antibacterial, Laxative, Wound Healing, food supplement, Cosmetics, Anti-inflammatory.[5]

Aloin used as a stimulant-laxative**,** treating constipation by inducing bowel movements & it increases peristaltic contractions in the colon, which leads to softer stools.[6]

Sennoside



	R^1	R^2	10-10'
Sennoside A	COOH	COOH	*trans*
Sennoside B	COOH	COOH	*meso*

IUPAC Name: 9-[2-carboxy-4-hydroxy-10-oxo-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-9H-anthracen-9-yl]-4-hydroxy-10-oxo-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-9H-anthracene-2-carboxylic acid. Molecular weight: 862.74g/mol. Molecular formula: $C_{42}H_{38}O_{20}$.Description: dark brown semi-solid powder extract. Solubility: Freely soluble in Methanol (90%), Ethanol, sparingly soluble in water and Acetone. Categories: Anti-inflammatory, Laxative, Anti-fungal.[7]

The breakdown products of sennoside act directly as irritants on the colonic wall to induce fluid secretion and colonic contraction. By irritating and stimulating intestinal cells, producing contractions in intestines, water influx to the intestines and bowel movement.[6]

MATERIALS AND METHODS

Isolation of Aloin

Aloe Vera leaves were collected from college botanical garden. The leaves washed with water and rinds were removed. The inner gel scrapped and cut into pieces, solar dried (30-40°C for 2 weeks) and dry gel particles were collected.

The dry gel particles were screened using sieves. Solvents used for the extraction and high performance liquid chromatography (HPLC) analysis were of AR and HPLC grade.[2]

Soxhlet extraction-The maximum recoverable aloin was estimated by Soxhlet extraction using methanol. 5 % (w/w) of aloe Vera powder were taken in Soxhlet with 200 ml methanol.

Extraction was carried out for 24 hours. Samples free from dry gel were collected at the end, stored in a freezer, and analyzed.[5,8]

Isolation of Sennoside

Senna leaves were collected from college botanical garden. Senna leaf powder (100 mg) was extracted with methanol (20mL) under reflux conditions for 1 h. The extract was then filtered and concentrated under reduced pressure. The sample was reconstituted and adjusted to 5 ml with methanol. Samples were analyzed immediately after extraction in order to avoidpossible chemical degradation.[4]

Instrumentation

For the present study JASCO double beam UV/Visible spectrophotometer (model-V630) was used with, spectral bandwidth of 2nm, wavelength accuracy ±0.5nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution. Weighing were done on electronic balance (Model ShimadzuAUW-220D), Ultrasonicator model 5.5L150H were used.

Materials

Standard samples of aloin and sennoside were taken. Combined dose aloe vera and senna suppository (200mg aloin and 100 mg sennoside) were taken.

Solvent

Methanol selected as solvent for developing spectral characteristics of the drug. The selection was made after assessing the solubility of both the drugs in different solvents.

Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solutions of both Aloin and Sennoside were prepared by dissolving 10 mg of Aloin and 10 mg of Sennoside separately in 20 ml of methanol in 100 ml volumetric flasks. Final volume was made up to 100 ml with methanol to get working standard solution of each 100 µg/ml. These stock solutions were used to prepare series of solution with concentration 5-30 µg/ml and 5-30 µg/ml of Aloin and Sennoside respectively for both methods.

Determination of Absorption Maxima

By appropriate dilution of standard stock solution of Aloin and Sennoside with methanol, solutions containing 20 µg/ml of Aloin and 20 µg/ml of Sennoside were scanned separately in the range of 200- 400 nm. Wavelength of maximum absorption was determined for both the drugs. Aloin showed maximum absorbance at 262.5 nm (λ_1) and Sennoside at 276 nm (λ_2).

Application of the proposed method for the determination of Aloin and Sennoside in suppository dosage form

Method A: Simultaneous equation method

Two suppositories were weighed and average weight was calculated. The suppositories were dissolve in methanol, from that 0.1ml of Aloin was transferred to 100ml volumetric flask and ultra sonicated for 10min. The volume was made up to the mark with methanol. The resulting solution was then filtered through a whatmann filter paper (No. 41). Aliquot portion was appropriately diluted with methanol to get final concentration of 20µg/ml. The concentration of both aloin and sennoside were determined by measuring absorbance of sample at 262.5nm, 276.0nm in spectrum mode and values were substituted in respective formulae to obtain the concentration.[10,11]

$$C_x = \frac{(A_2 a y_1 - A_1 a y_2)}{(a x_2 a y_1 - a x_1 a y_2)}$$

$$C_y = \frac{(A_1 a x_2 - A_2 a x_1)}{(a x_2 a y_1 - a x_1 a y_2)}$$

Where,

Cx = Concentration of Aloin,

Cy = Concentration of Sennoside;

A_1 = Absorbance of mixture at 262.5nm;

A_2 = Absorbance of mixture at 276nm;

ax_1= Absorptivity of Aloin at 262.5nm;

ax_2 = Absorptivity of Aloin at 276nm;

ay_1 = Absorptivity of Sennoside at 262.5nm;

ay_2 = Absorptivity of Sennoside at 276nm.

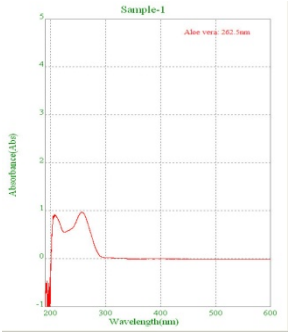


Figure 1: Spectra of Aloin in methanol.

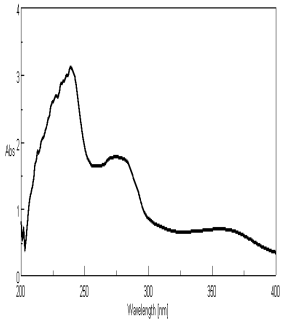


Figure 2: Spectra of Sennoside in methanol.

Method B: Absorbance ratio (Q – Absorbance) method

In the absorbance ratio method, from the over lay spectra of drugs (Figure 3), wavelength 265.0nm (Iso-absorptive point) and 276.0nm (λmax of Sennoside) were selected for analysis. The calibration curves for aloin and sennoside were plotted in the concentration range of 5-30µg/ml and 5-30µg/ml at both the wavelengths respectively.

The absorptivity values were determined for both the drugs at both the wavelengths. From the following set of equations the concentration of each component in sample was calculated,

$$C_x = \frac{(Q_m - Q_y)}{(Q_x - Q_y)} \times \frac{A_1}{ax_1} \qquad (1)$$

$$C_y = \frac{(Q_m - Q_x)}{(Q_y - Q_x)} \times \frac{A_1}{ay_1} \qquad (2)$$

Where,

Cx=concentration of aloin,

Cy=concentration of sennoside,

A_1=absorbance of sample at wavelength 276nm,

ax_1=absorbtivity of aloin at 262.5nm,

ay_1=absorbtivity of sennoside at 276nm,

Qm=ratio of absorbance of sample solution at 265.0nmand 276.0nm,

Qx=ratio of absorbtivities of aloin at 265.0nm and 262.5nm and

Qy=ratio of absorbtivities of sennoside at 265.0nm and 276.0nm.

Preparation of Synthetic Mixture

From the standard stock solution pipette out 2ml of aloin and 1 ml of sennoside solution in 10 ml of volumetric flask and diluted with distil water to get 10 µg/ml of aloin and sennoside solution. Absorbance of resulting solution was taken at both (262.5 nm and 276 nm) selected wavelength and values were substituted in respective formulae to obtain the concentration of drug.

Validation parameter

Validation

The method was validated according to ICH guidelines to study linearity, accuracy, sensitivity, LOQ and LOD.[9]

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Aloin and Sennoside. For both the methods, the Beer-Lambert's law was obeyed in the concentration range of 5-30 µg/ml and 5-30 µg/ml for Aloin and Sennoside respectively. The correlation coefficient was found to be 0.999 at 262.5 nm for Aloin and 0.998 at 276 nm for Sennoside. The results of the same are shown in Figure 3 and Figure 4.

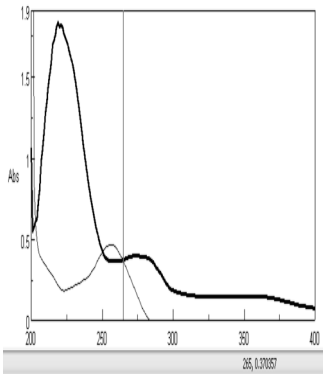


Figure 3: Overlay spectra of Aloin and Sennoside.

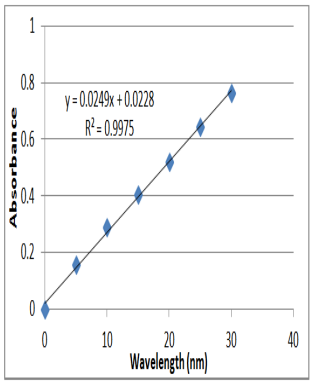


Figure 4: Linearity of Aloin at 262.5 nm in methanol.

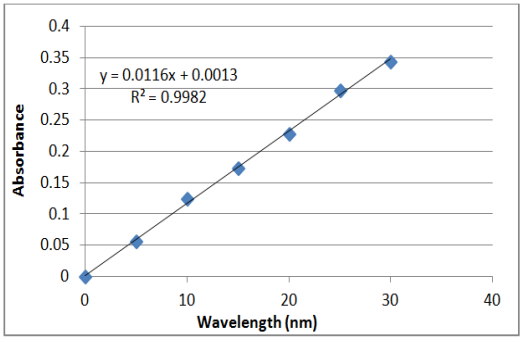


Figure 5: Linearity of Sennoside at 276 nm in methanol.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at Table 2 and Table 3.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3s/S and 10s/S, respectively, where S is the slope of the calibration curve and s is the standard deviation of response. The results of the same are shown in Table 2 and Table 3.

Results of Analysis of Suppository Formulation

In simultaneous equation method, wavelengths selected for analysis were 262.5 nm for Aloin and 276 nm for Sennoside.

In Q-method, wavelength selected for analysis was 265 nm. In both the methods linearity were observed in the concentration range of 5-30 µg/ml and 5-30 µg/ml for Aloin and Sennoside, with good correlation coefficient 0.997 and 0.998 respectively. Both methods are validated according to ICH guidelines and all validation parameters were studied for the proposed method, like linearity, accuracy, LOD, LOQ, sensitivity.

Table 1: Optical characteristics of Aloin and Sennoside

Optical characteristics	Aloin	Sennoside
Wavelength (nm)	262.5 nm	276 nm
Beer lambert's law limit (µg/ml)	5-30 µg/ml	5-30 µg/ml
Regression equation (y=mx+c)	Y = 0.024x + 0.022	Y = 0.011x + 0.001
Slope (m)	0.024	0.011
Intercept (c)	0.022	0.001
Correlation coefficient (R^2)	0.997	0.998

Table 2: Simultaneous equation method

Drug	Label Claim (mg)	Amount of drug taken (mg)	Amount found	% recovery	S.D.	%C.V.	LOD	LOQ
Aloin	200	20	19.98	99.9	0.00138	0.2755	0.1897	0.5751
			19.88	99.4				
			19.98	99.9				
Sennoside	100	10	8.89	88.9	0.00156	0.6872	0.1058	0.3208
			8.90	89				
			8.95	89.5				

S.D: Standard Deviation, **S.E:** Standard Error, **C.V:** Coefficient Variation

Table 3: Absorbance ratio method

Drug	Label Claim (mg)	Amount of drug taken (mg)	Amount found	% recovery	S.D.	%C.V	LOD	LOQ
Aloin	200	20	18.65	94.4	0.0018	0.4108	0.259	0.786
			18.50	94.2				
			18.54	94				
Sennoside	100	10	8.58	84.2	0.00078	0.7916	0.234	0.7090
			8.49	83.8				
			8.44	84.8				

S.D: Standard Deviation, **S.E:** Standard Error, **C.V:** Coefficient Variation

RESULTS AND DISCUSSION

The combination of Aloin and Sennoside plays vital role in the treatment of patient suffering from constipation. Hence the present work provides very simple and accurate method for simultaneous estimation of Aloin and Sennoside. From the proposed research, it was found that aloin and sennoside obeys linearity within the concentration range 5-30µg/ml and 5-30µg/ml respectively. Percentage label claim for aloin and sennoside in suppositories, by simultaneous equation and absorption ratio methods was found in the range of 99% to 99.9% and 88% to 90% respectively. For Coefficient of variation (CV) were calculated, which was found to be less than 2% indicating the both method has good reproducibility. Accuracy of proposed methods was ascertained by recovery studies and results are expressed as %recovery. Percent recovery for Aloin and sennoside by simultaneous equation and absorption ratio method was found in range of 99% to 99.9% and 88% to 90% respectively, values of standard deviation, coefficient of variation for both method were in range of 0.00138 to 0.00156 and 0.0018 to 0.00078; 0.2755 to 6872 and 0.4108 to 7916 respectively indicating the accuracy of proposed method.

CONCLUSION

The two spectrophotometric methods were developed and validated as per ICH guidelines. These validated methods are new, rapid, accurate, precise, sensitive, and

reproducible. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods.

Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of Aloin and Sennoside in bulk and formulation.

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