

Quantitative Estimation of Aloin in Aloe Vera and its Commercial Formulation by using UV Spectroscopy



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Abstract

In Indian Ayurvedic system of medicine the leaf of Ghritkumari has been extensively used as a cathartic agent to treat severe stomach constipation. The aloe vera plant is used as food supplement and also used in cosmetic products to treat different skin problems. In order to develop reliable quality control marker to check its potential toxicity and carcinogenic activity and to check the commercial samples of Aloe vera for authenticity and purity, UV Spectroscopy analysis has been done. It has been found that aloin is present in a few commercial samples. HPLC, Infra-Red and thin layer chromatography methods have been reported for aloin determination in commercial samples. Therefore, UV Spectrophotometric method was developed for determination of aloin in commercial formulations. This method gives reproducible results and found linear in the range 80-180µg/ml. The method was found to be simple, precise and accurate for estimation of aloin from aloe vera and commercial formulations containing aloin

Keywords: Aloin; UV-Spectroscopy; Commercial formulation

Introduction

It is an important medicinal leaf of plant of Aloe barbadensis miller, known as curacao aloes, belonging to family liliaceae. Aloe vera contains varieties of chemical constituents from which anthraquinone glycoside is major constituent. Aloin is the principle active constituent of aloe vera, which contain mixture of glycosides. Aloin is official drug in Indian Pharmacopoeia and British Pharmacopoeia. HPLC, UV Spectroscopy, Infra-Red and thin layer chromatography methods have been reported for aloin determination.

Aloin is also known as Barbaloin. Aloin is Bitter, yellow brown colour compound. Aloin an active compound has laxative, anti-inflammatory and anti-cancer effect. Aloe vera is used both internally and externally for curing various ailments. Aloin is used in various cosmetic products, used to treat acne, sunburns, itchy rash, psoriasis and oral submucous fibrosis. Therefore, in order to develop reliable quality control marker to check its potential toxicity and carcinogenic activity and to check the commercial samples of Aloe vera for authenticity and purity is essential.

International aloe science council (IASC) has developed a set of identify, purity and quality standards for aloin containing products certified by IASC are specifically prepared in manner to

assure aloin is present in above a minimum level and to ensure the absence of adulteration (Figure 1).

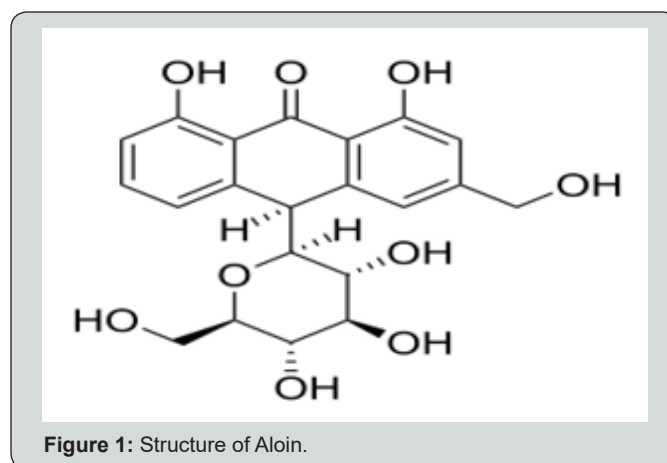


Figure 1: Structure of Aloin.

Mechanism of action of aloin

Effect on inflammation: Aloin, dose dependently inhibit inducible nitric oxide synthase mRNA expression and nitric oxide production. With inhibition of NO production, it did not suppress PEG2 production.

Effect on antioxidant system: Antioxidant effect of aloin on free radical induced DNA breaks. The greater reducing activity of aloin at lower concentrations, however, free radical scavenging activity of aloin gradually predominated over its reducing power, resulting in the protection of DNA.

Nutrients in aloe vera

- a) Vitamin: A, B, C & E, M,
- b) Choline: Helps metabolism,
- c) Minerals: Calcium, Phosphorus, Potassium, Iron, Sodium, Chlorine, Zinc,
- d) Mono and Polysaccharides: Aldopentose, Cellulose, Glucose, L-rhamnose, Mannose,
- e) Fatty acids: cures Burns and wounds,
- f) Salicylic Acid: Anti-Bacterial,
- g) Amino Acid: Body needs 22 amino acids, out of 20 amino acids are provided by Aloe Vera,
- h) Lignin: A strong ability to penetrate human skin,
- i) Saponins: Work saponifying agent and antiseptic,
- j) Enzymes: Phosphatase-Amylase,
- k) Bradykinase: Immune building, Catalase prevents accumulating of water in system,
- l) Cellulase: Cellulose digestion, Creatine,
- m) Phosphokinase: muscular enzyme
- n) Lipase: digestion, Nucleotidase, Alkaline phosphate,
- o) Protease: hydrolyses proteins into their constitute elements.

Materials and Methods

Apparatus and instrument

Spectrophotometric was carried out by using UV-Visible spectrophotometer having two matched quartz cells with 1cm light path.

Model: - shimadzu-1800, Software: -UV probe, version 2.42. Glass wares (Borosilicate) Electronic analytical balance (REPTECH), Ultrasonicator (LOBA Life), Magnetic stirrer.

Materials and reagents

Methanol, N-hexane, Dis. Water, Butanol all chemicals were used as an analytical grade. The aloe vera dry powder was collected from local market. The marketed sample viz., Neem Aloe vera Face Pack, Aloe Vera Cream, Hydrating Jelly, Aloe Vera Gel, Aloe Fresh Body Lotion, Aloe Vera Face Wash, Aloe Vera Shampoo, Aloe Vera Soap were produced from local market.

Extraction methods for commercial formulations

For commercial samples Neem Aloe vera Face Pack, Aloe Vera Cream, Hydrating Jelly, Aloe Vera Gel, Aloe Fresh Body Lotion, Aloe Vera Face Wash, Aloe Vera Shampoo, Aloe Vera Soap were dissolved in 100ml methanol by using magnetic stirrer for 1h and transferred into separating funnel respectively.

For the removal of oily and fatty material, to that added 100ml of n-hexane and shaken well and set aside for separation of two distinguish layers and collected methanol layer into a conical flask respectively. These concentrated methanol extracts were dissolved in a minimum amount of water and transferred into separating funnel respectively.

To that added 100ml of butanol and shaken well and set aside for separation of two distinct layers.

Collect the butanol layer into round bottom flask. Again, added 100ml of butanol to that water layer and collected the butanol layer into the same round bottom flask respectively and concentrated under sun light.

These butanol extracts Neem Aloe vera Face Pack, Aloe Vera Cream, Hydrating Jelly, Aloe Vera Gel, Aloe Fresh Body Lotion, Aloe Vera Face Wash, Aloe Vera Shampoo, Aloe Vera Soap were dissolved in the minimum amount of methanol and transferred into 10ml of volumetric flask and made up the volume with methanol.

Method

Preparation of sample solution: The sample solution of extracted aloin from different commercial formulation (Oil, Face pack, Cream, Jelly, Lotion, Gel, Face wash, shampoo, soap) were prepared by dissolving 1mg in 10ml of methanol in 10ml volumetric flask. The solution was scanned in UV range of 200-400nm

Preparation of standard solution: The standard solution of aloin were prepared by dissolving 1mg in 10ml methanol in 10ml volumetric flask. The solution was scanned in UV range of 200-400nm.

Method validation

Linearity: The solution was prepared by pipetting 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8ml from working standard stock solution in to 10ml volumetric flask and the volume was adjusted to mark with methanol to produce 80-180µg/ml respectively. The absorbance of solution was measured at 267nm. Calibration curve were generated by taking the response vs. concentration.

Precision: The precision of an analytical procedure expresses the closeness of agreement (results) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility.

Repeatability: Aliquots of 1.2ml of working standard solution of aloin (1000µg/ml) was transferred to 10ml volumetric flask and volume was adjusted to distilled water to get concentration of 120µg/ml. the absorbance of solution was measured spectrophotometry six times and %RSD was calculated.

Intraday and interday: Aliquots of 0.8, 1.0 and 1.2ml of working standard solution of aloin (1000µg/ml) was transferred to 10ml volumetric flask and volume was adjusted to distilled water to get concentration of 10, 20 and 30µg/ml.

The absorbance of solution was measured spectrophotometry three times and %RSD was calculated. For intraday, the analysis was carried out at different intervals on the same day and for interday, the analysis was carried on different days.

Result and Discussion

Calibration curve of standarnd aloin are shown in Figure 2. In all the sample maximum absorbance occur at 267nm (Table 1-5) [1-12].

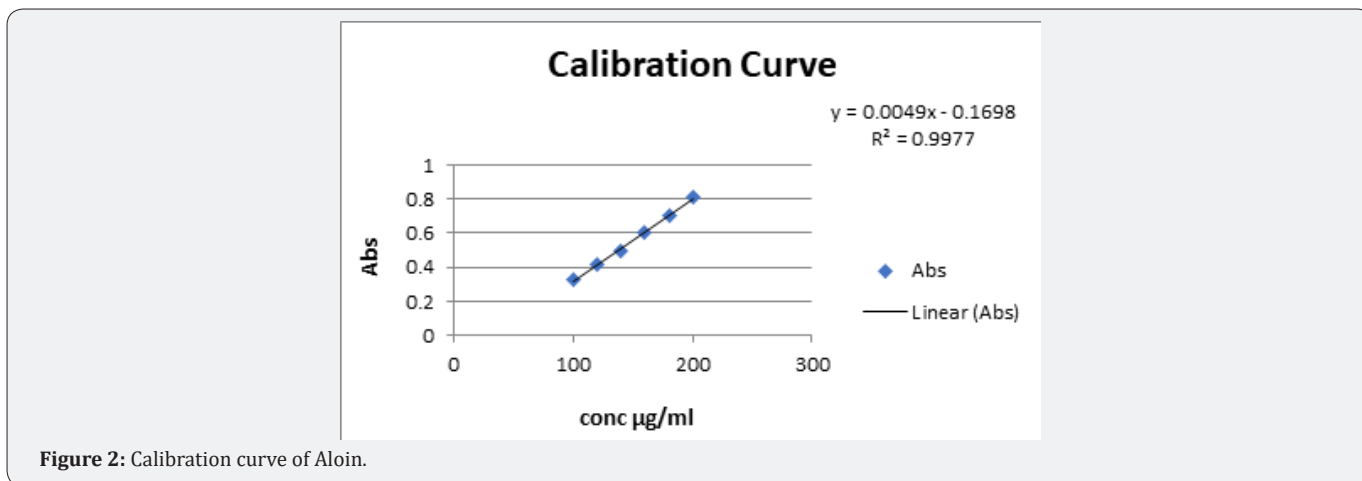


Figure 2: Calibration curve of Aloin.

Table 1: Linearity data of Aloin (n=6).

Sr. No.	Concentration (µg/ml)	Absorbance at 267nm
1	80	0.325
2	100	0.419
3	120	0.498
4	140	0.606
5	160	0.705
6	180	0.814

Table 2: Repeatability data of Aloin.

Concentration (µg/ml)	Absorbance
120	0.4961
120	0.497
120	0.4963
120	0.4981
120	0.497
120	0.4962
MEAN	0.4967
SD	0.000738
%RSD	0.1485

Table 3: Intraday precision of Aloin.

Concentration (µg/ml)	Absorbance At 267nm	Mean ± SD (n=3)	%RSD
100	0.4185	0.4187	0.0405
	0.4188		
	0.4189		

120	0.4983	0.4992	0.1646
	0.4991		
	0.5003		
140	0.6072	0.6074	0.0279
	0.6076		
	0.6075		

Table 4: Interday precision of Aloin.

Concentration ($\mu\text{g/ml}$)	Absorbance At 267nm	Mean \pm SD (n=3)	%RSD
100	0.4195	0.4195	0.0546
	0.4196		
	0.4194		
120	0.4998	0.5003	0.1703
	0.5015		
	0.4996		
140	0.6065	0.6066	0.0205
	0.6067		
	0.6068		

Accuracy: Accuracy of aloin was found to be 99.0-101%.

Table 5: Aloin content in different commercial sample.

Sr No.	Sample	Amount Found (mg)
1	Aloe Vera powder	110.36
2	Neem Aloe Vera Face Pack	37.53
3	Aloe Vera Cream	234.59
4	Hydrating Jelly	218.67
5	Aloe Body Lotion	815
6	Aloe Vera Face Wash	207.5
7	Aloe Vera Shampoo	657
8	Aloe Vera Soap	314.21

Conclusion

The spectrophotometric methods were developed and validated as per ICH guidelines. These validated methods were new, rapid, accurate, precise 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 in different commercial formulation.

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