QUANTITATIVE ESTIMATION OF ALOIN FROM PHARMACEUTICAL DOSAGE BY HPTLC

Vinay Sharma, V. J. Shukla and P. K. Prajapati

Gujarat Ayurved University, Jamnagar – 361 008

ABSTRACT

Estimation of marker components in a given dosage form not only authentifies its genuineness but even indicate towards the inherent therapeutic potentials. In the present study, a simple, precise, accurate and rapid high performance thin layer chromatographic method was validated for the estimation of Aloin in liquid dosage forms. The stationary phase used was precoated silica gel 60F 254 whereas the mobile phase consisted of Ethyl acetate: Methanol: Water (10: 1.35: 1.0). The detection of spot was carried out at 254nm. The \( R_f \) value was found to be 0.65± 0.01. The linearity curve found to be linear in between 4-17 \( \mu \)g/spot. Proposed method can be used to determine the drug content from marketed formulations.

Keywords: HPTLC, Aloin, linearity curve.

INTRODUCTION

The plant Aloe vera is universally known and widely cultivated all over India. Therapeutically, Aloe vera is used both internally and externally for curing various ailments. The gel found in the leaves is used for soothing minor burns, wounds, and various skin conditions like eczema and ringworm. The extracted Aloe vera juice Aloe vera plant is used internally to treat a variety of digestive condition\(^1\). There are more than 200 different chemical constituent found in Aloe vera namely amino acids, Anthraquinone, Lignin, Mono-and polysaccharides, Saponins, sterols, Vitamins, Salicylic acid, Minerals, Enzymes, in which Aloin is reported as a main chemical constituent\(^2,3\). Aloe vera also contains several potentially bioactive compound including Salicylates, magnesium, lactate, acemannan, lupeol, campestrol, \( \beta \)-sitosterol, aloin A and anthraquinones \(^4\).

Aloe vera must be “biologically alive” and must be in bio-available from if customers around the world tend to experience full scope and range of benefits from this plant. The only way that Aloe vera can reach the customer in a fresh state and still be of
the highest quality is if the raw inner gel is stabilized immediately. This stabilization process must also preserve and retain all the active ingredients without the use of any damaging chemicals or exposing to excessive heat \[5\]. Earlier studies have proven that Aloin is not heat stable, it even degrades in sunlight \[6\]. Other research works show that there is very less or negligible amount of Aloin present in market samples \[7\] due to some wrong processing methods.

H.P.T.L.C is a sophisticated and automated form of TLC. H.P.T.L.C is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad no of compound both efficiently and cost effectively. Additionally numerous sample can be run in a simple analysis there by reducing analytical time with H.P.T.L.C. the analysis can be carried out using different wave lengths of light - 254nm short wave UV light, 366nm long-wave UV light, and 302nm mid wave light. In addition, white light of 400–750nm is also utilized when viewing derivatized plates., thereby providing by complete picture of plant then is typically observed with more specific analysis.

The objective of the present work was to develop an accurate, specific and reproducible method for the estimation of Aloin from pharmaceutical dosage forms.

**MATERIALS AND METHODS**

A Camag HPTLC equipped with a sample applicator Linomat IV, twin trough plate development chamber, TLC Scanner III, Reprostar and Wincats 4.02, integration software (Switzerland). and stationary phase precoated silica gel 60F 254 were used. Reference compound Aloin was purchased from TOTAL HERB SOLUTIONS, Mumbai Maharashtra. The aloe extracts were purchase from the local market. All chemicals were used are of AR grade.

**Chromatographic Conditions:**

Application mode : Camag Linomat V, Hamilton syringe
Development chamber : Camag Twin Through Chamber (20x10cm²)
Plates : Precoated silica plate
Chamber saturation : 30 min
5.0 µl was of sample was applied on E Merk Aluminium plate of 0.2 mm thickness pre-coated with silica-gel 60 F<sub>254</sub> using Linomat IV applicator. The plate was developed in the solvent system up to 8 cm and scanned at 254 nm and 359 nm using Deuterium tungsten lamp in a Camag µPhotographs were taken using Camag Photo Documentation System. The R<sub>f</sub> value and percentage area of each spot were calculated.

To calculate the concentration of Aloin in each sample following equation was developed:

Vol. made × concentration × total solubility

Wt. of dried extract × sample loaded × 1000

SAMPLE PREPARATION

Sample preparation

Different samples were soaked in 20 ml of methanol and refluxed on boiling water bath for 30 minute. The filtrates were concentrated and made up to 5 ml in a standard flask.

Standard Preparation

Standard Aloin solution (5mg/µL) It was prepared by dissolving 10mg of Aloin in 10ml methanol under ultrasonicator, which yields a solution of concentration 1mg/ml, which was further diluted with methanol to yield a concentration of 0.005mg/ml.

Mobile Phase

Ethyl acetate : Methanol : Water (10 : 1.35 : 1.0).

Sample Application

5µL, of each sample applied on E Merk Aluminium plate

Aliquot of Standard solution of Aloin was applied in duplicates 5µL, 10µL
RESULTS AND DISCUSSION

Quantitative Estimation

In the chromatogram of the drugs extracted from the aloe juice, many well resolved spots were observed, out of these spots one spot matched with the R_f value shown by standard Aloin and having the same \( \lambda_{\text{max}} \) 254 nm.

The results of percentage of Aloin calculated as per the formula is tabulated below

<table>
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<th>S. no</th>
<th>Samples for analysis</th>
<th>% of Aloin found</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sample A (standard)</td>
<td>85%</td>
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<tr>
<td>2.</td>
<td>Sample B (lab sample)</td>
<td>25%</td>
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PEAK TABLE

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<th>Start Rf</th>
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Calibration Curves

![Calibration Curves](image)

Calibration graph was found to be linear over the concentration range 4-17µg/spots. Linearity was evaluated by determining seven standard working solutions in duplicate. The peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation \( Y = 327.29 + 153.11X \) and regression coefficient (r²) was 0.999 (plate no. 1, 2).

CONCLUSION

The HPTLC method is applicable for the estimation of Aloin in *Aloe vera* sample. The comparison was done by comparing the Rf values of standard with that of sample the Rf value was found to be 0.65. The lab sample contains 25% of Aloin content when it compared with standard Aloin. When samples subjected for the calibration it showed linear graph.

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For Correspondence:
Vinay Sharma
Ph.D. Scholar,
Dept. of R.S. & B.K., I.P.G.T. & R.A.
Gujarat Ayurved University, Jamnagar – 361 008
Mob. No.- +919033867974
Email Add.- vinay.ayurveda@gmail.com