RESEARCH ARTICLE Am. J. PharmTech Res. 2012; 2(6) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

**Journal home page:** http://www.ajptr.com/

# Development of HPTLC and Colorimetric Analysis Techniques for *Aloe* Gel Evaluation

**Pranav Kumar Yadav[1], Vinod Gauttam[1], A. N. Kalia[*1]**

*1. Department of Pharmacognosy, ISF College of Pharmacy, Moga, Punjab (India)-142001*

## ABSTRACT

*Aloe* gel is commonly used in herbal medicines. Many of the *Aloe* products available commercially, do not demonstrate beneficial effects, indicating the poor quality of the *Aloe* gel. Therefore, an efficient, reliable and accurate method is needed to evaluate the quality of *Aloe* products. The present research work discussed the development of a colorimetric and HPTLC method for the determination of Glucomannan (a marker compound for *Aloe* gel) in *Aloe* gels and its preparations. Shimadzu 1700 UV-Visible spectrophotometer was used for the colorimetric estimation. In the colorimetric method developed obeyed the Beer's law in the concentration range of 50-90 $\mu$g-ml$^{-1}$ with $r^2$ of 0.999. CAMAG HPTLC instrument was used. The mobile phase comprised of n-butanol : ethanol : water : acetic acid (2 : 4 : 4 : 0.05 v/v) was used for the development of chromatogram. The densitometric scanning was done by TLC scanner III (CAMAG) in absorbance mode at the wavelength of 488 nm. After spraying with anisaldehyde sulfuric acid the system was found to give compact spots for glucomannan (Rf value of 0.69±0.02). The data obtained showed good linearity ($r^2 = 0.995$) with respect to peak area in the concentration range of 400-1400 ng spot$^{-1}$.

**Keywords:** *Aloe vera*, *Aloe perryi*, Glucomannan, Colorimetry, HPTLC

*Corresponding Author Email: ankalia_47@rediffmail.com

Received 17 October 2012, Accepted 25 October 2012

Please cite this article in press as Kalia AN *et al.*, Development of HPTLC and Colorimetric Analysis Techniques for *Aloe* Gel Evaluation American Journal of PharmTech Research 2012.

# INTRODUCTION

*Aloes* have been used therapeutically, certainly since Roman times.[12] Commercial species of *Aloe* are *barbadensis* (*Aloe vera*), *ferox*, *perryi* (*Aloe socotrina*), and *saponaria*.[10] The genus comprised of 500 species of *Aloe* (Lilliaceae).[16] Materia Medica mentions a wide range of therapeutic uses of *Aloe perryi* (*Aloe socotrina*), a species closely related to *Aloe vera*.[5] The genus *Aloe* species are used in the treatment of arthritis, skin cancer, burns, eczema, psoriasis, digestive problems, blood pressure problems, and diabetes.[9]

*Aloe* gel is the colourless mucilaginous gel obtained from the parenchymatous cells of the fresh leaves.[17] The raw pulp of *A. vera* contains approximately 98.5% water, while the mucilage or gel consists of about 99.5% water with a pH of 4.5. The remaining 0.5 – 1% solid material consists of a range of compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids.[7] More than 60% of the solid being made up of polysaccharides namely pectins, cellulose, hemicellulose, glucomannan and acemannan etc.[1,6,17] *A. vera* gel is commonly used in herbal medicines for various properties viz. antidiabetic, anticancer, anti-inflammatory, wound healing, gastrointestinal function, ulcer, antimicrobial, coughs, headaches, arthritis, immune-system deficiencies and also in burns and incisions.[2]

These uses of *Aloe vera* gel and its demand in the domestic as well as in International market attracted the adulteration. Therefore a validated and reliable method is required which can differentiate the gel of *Aloe vera* and others related species. Hence two method based on different principles were developed and validated. The chemical structure of GM (Glucomannan) consists, mainly, in mannose and glucose in the ratio 8:5 linked by beta (1-->4) glycosidic bonds.[3] GM is a hydrophilic molecule, and proven to accelerate wound healing, activate macrophages, stimulate the immune system, and have antibacterial and antiviral effects.[2,13] The commercial use of GM is in the various industries linked with the food items are in sugar, textile, pudding, pastry, ice-cream industry etc.[14]

The inconsistency in the efficacy of the *Aloe* product indicating the poor quality of products are reaching in to the market. To control fraudulent marketing of *Aloe* products and ensure that the consumer receives genuine beneficial effects of *Aloe*, it is necessary to establish a reliable composition standard for *A. vera* gel and aloe products based on the quality and quantity of GM in the sample. It is necessary for the *Aloe* industry to have a simple, reliable, sensitive, and validated assay method that is specific for the active component(s) of *Aloe.*

## MATERIALS AND METHOD

### Plant material

The plant of *Aloe vera* and *Aloe perryi* were procured from Punjab Agriculture University, Ludhiana and authenticated by Dr. Adarsh Pal Vig, head, deptt. of botanical and environmental sciences, Guru Nanak Dev University, Amritsar, Punjab (Reference No. : 882). Both the plants were cultivated in same environment at the medicinal plant garden of ISF College of Pharmacy, Moga, Punjab, in month of August 2011 (As per WHO guidelines 1999).

### Markers and chemicals

Glucomannan was provided as a gift sample from Neospark Pvt. Ltd. Hyderabad, India. Standard Glucose and Gallic acid were purchased from CDH, New Delhi. All other chemicals and solvents used were of analytical grade.

### Marketed Herbal Formulations

(A) Aloe vera juice with fibers, (Patanjali Ayurved Ltd., Uttrarakhand, India) (B) Aloe vera gel, (Patanjali Ayurved Ltd., Uttrarakhand, India)

## EXPERIMENTAL METHODS

### Collection of plants

The leaves of Aloe were harvested in month of December 2011 and May 2012 for Quantification.

### Preparation of *Aloe* gel

*Aloe* gel was separated from leaves by hand fillet method. Fresh leaves were collected and washed with distilled water, the inner gel components were removed with a sharp blade and gel was homogenized in an electric blender.[11]

### Quantitative Estimation of Glucomannan content using UV/Visible spectroscopy

### Calibration curve of standard Glucomannan

The accurately weighed (10 mg) GM dissolved in 10 ml distilled water to obtain a concentration of 1 mg/ml (stock solution). The working standard concentration of 50, 60, 70, 80 and 90 µg/ml were prepared from the stock solution with distilled water. 5 ml of each concentrations were transferred into volumetric flask. To each flask, 0.05 ml of 80% phenol was added followed by rapid addition of 5 ml of concentrated sulfuric acid, the steam of acid being directed against the liquid surface to obtain good mixing. The flasks were allowed to stand for 10 minutes, then vortexed and placed for 2 hours in a water bath at 40 to 45° C. The absorbance of the characteristic yellow-orange color was measured at 488 nm wavelength using UV/Visible

Spectrophotometer. Blanks were prepared by substituting distilled water for the solution. A plot of measured absorbance against the concentration of the standards was plotted and a calibration curve was obtained.[4]

### Preparation of sample solution for UV/Visible Spectroscopic method

In the separated *Aloe* fresh gel, 5 ml (10 fold dilution with distilled water) of gel was transferred into volumetric flask and vortexed for 30 min. Then same procedure was followed as described for the standard. The amount of GM may then be determined by reference to a standard curve.

### Marketed Herbal formulation A and B

To determine the content of Glucomannan in Marketed Herbal formulations (PATANJALI Ayurved Ltd., Uttrarakhand, India), 5 ml (10 fold dilution with distilled water) of sample was transferred into volumetric flask and vortexed for 30 min. Then same procedure was followed as described for the standard. The amount of GM may then be determined by reference to a standard curve previously constructed for the GM under estimation.

### Analytical method development

### Validation of the Method for UV/Visible spectroscopy

The reactions were carried out at different temperature ranging from (25–50$^0$c) in order to optimize the most appropriate reaction temperature to get the most appropriate results. The method constructed in this study was validated for linearity, precision, specificity, limit of detection (LOD), limit of quantification (LOQ) and accuracy.[8] The instruments was précised for its repeatability and intra and inter day variance in the results. For this, GM concentrations (70, 80 and 90 µg/ml) were analysed at different time intervals of 0, 4, 8 and 12 h. in a day (intra-day precision) and for three continuous days (inter-day precision). The results are expressed as % RSD. To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%) and the results are expressed as % R.S.D. The LOD and LOQ of the proposed method were determined, using standard deviation of the response and slope approach.

### Estimation of Sugar content using UV/Visible spectroscopy

### Calibration curve of standard Glucose

1mg/ml of stock solution for the standard Glucose were prepared in distilled water. The working standard solutions of 20, 30, 40, 50 and 60 µg-ml$^{-1}$ were prepared by diluting appropriate aliquots of the stock solution with distilled water. Each dilution was prepared in triplicates. To prepare a calibration curve, aliquot samples (5 ml) of each concentration were transferred into volumetric flask. To each concentration of 5 ml sample, 0.05 ml of 80% phenol followed by 5 ml of concentrated sulfuric acid was added rapidly, the steam of acid being directed against the liquid surface rather than against the side of the flask in order to obtain good mixing. The flasks were allowed to stand for 10 minutes, then vortexed and placed for 30 min. in a water bath at 25 to 30º C. The absorbance of the characteristic yellow-orange color was measured at 485 nm wavelength using UV-Visible Spectrophotometer. Blanks were prepared by substituting distilled water for the solution. The amount of sugars determined by reference to a standard curve previously constructed for the Glucose under examination.[4]

### Estimation of Total Phenolic content using UV/Visible spectroscopy

### Calibration curve of standard Gallic acid

The 10 mg of gallic acid standard solution was dissolved in 10 ml of distilled water to make 1 mg/ml stock solution**.** The working standard solutions of 10, 20, 30, 40 and 50 $\mu$g-ml$^{-1}$ were prepared by diluting appropriate aliquots of the stock solution with distilled water. To prepare a calibration curve, aliquot samples (1 ml) of each concentrations level were transferred into volumetric flask. Then an aliquot of the solution (2 ml) was mixed with Folin-Ciocalteu reagent (10-fold diluted with distilled water, 10 ml). After 5 min, a 7.5% (w/v) sodium carbonate solution (8 ml) was added and vortexed. After 2 h, the absorbance was measured at 765 nm against a blank prepared as described above with distilled water (2 ml), Folin-Ciocalteu reagent, and sodium carbonate solution. The content of total phenolics in *Aloe* gel was calculated using a calibration curve of gallic acid.[15]

### Quantitative Estimation of glucomannan content by HPTLC method

### Instrumentation and Chromatographic Conditions for HPTLC (Table 1)

**Table 1: Instrumentation and Chromatographic Conditions for HPTLC**

| | |
|---|---|
| HPTLC System | CAMAG, Switzerland. |
| Sample applicator | Camag Linomat V |
| Stationary phase | Pre-coated silica gel 60 $F_{254}$ TLC plate, 0.2 mm thick |
| Stock solution | 1mg/ml of glucomannan |
| Pre-chromatographic derivatization | 0.02 M sod. acetate solution (w/v) |
| Mobile phase | n-butanol : ethanol : water : acetic acid (2:4:4:0.05 v/v/v) |
| Saturation time | 20 min |
| Spraying reagent | Anisaldehyde sulfuric acid (5% v/v) |
| Scanning wavelength | 488 nm |
| Scanner | TLC Scanner III |
| Lamp | Tungesten |
| Slit dimension | 6 x 0.30 mm |
| Application position | 15 mm |
| Position of Solvent front | 90 mm |

### HPTLC method development and validation

### Sample application

The samples were applied in the form of bands of width 8 mm with a CAMAG Linomat V sample applicator (Switzerland) on the TLC plate. Application rate of 50 nl $s^{-1}$ was employed and space between the bands was 13 mm.

### Preparation of stock solution of standard glucomannan

A stock solution of glucomannan was prepared in water by dissolving 10 mg glucomannan and made up the volume to 10 ml with water (1 mg $ml^{-1}$).

### Preparation of samples

### Preparation of *Aloe* gel and formulation

Fresh succulent leaves of *Aloe* were collected and washed with distilled water, the inner gel component was removed with a sharp blade and gel was homogenized in an electric blender. The liquid was filtered through a nylon cloth and the fibrous residue was discarded.[11] 10 fold dilution of gel with distilled water was transferred into volumetric flask and vortexed for 30 min. 10 µl of the diluted solution was applied on the TLC plate followed by development and scanning as described in instrumentation and chromatographic section.

### Method Validation for HPTLC

The method was validated by establishing linear range, precision, robustness, specificity, accuracy, and limits of detection and determination. The linearity range of glucomannan was obtained by plotting the peak area of glucomannan against the concentration over a range (400 - 1400 ng $band^{-1}$). A stock solution of glucomannan (1 mg $ml^{-1}$) was prepared in water. Different volumes of stock solution 4, 6, 8, 10, 12 and 14 µL, were spotted on the TLC plate to obtain concentrations of 400, 600, 800, 1000, 1200 and 1400 ng $band^{-1}$ of glucomannan, respectively. Repeatability of the sample application and measurement of peak area were carried out using nine replicates of the same band (1000 ng $band^{-1}$of glucomannan) and was expressed in terms of percent standard deviation (%S.D.). The intra-day and inter-day variation for the determination of glucomannan was carried at three different concentration levels of 600, 800 and 1000 ng $band^{-1}$.

The specificity of the method was ascertained by analyzing the marker peak and corresponding peak in samples. The band for glucomannan in the sample was confirmed by comparing the Rf values and spectra of the band with that of the standard. By introducing small changes in the mobile phase composition, mobile phase volume (8, 10 and 12 ml), duration of mobile phase saturation (10, 20 and 30 min) and different analyst (1 - 3); the effects on the results were examined. Robustness study of the method was done in triplicate at a concentration level of 400 ng band$^{-1}$. LOD was experimentally verified by diluting the known concentrations of glucomannan until the peak was detectable. The pre-analysed samples were spiked with extra 80, 100 and 120 % of the standard glucomannan and the mixtures were reanalyzed by the proposed method. The experiment was conducted three times. This was done to check the accuracy of the method to evaluate the quantification range for the glucomannan.

### Analysis of glucomannan in *Aloe* gel and its Market formulations

The herbal formulations Aloe vera juice with fibers was selected for the study. 10 fold dilution of gel with distilled water was transferred into volumetric flask and vortexed for 30 min. 10 µL and 20 µL of the herbal formulations were taken respectively in micro-syringe and applied on precoated plates in the form of bands with sample applicator, followed by development and scanning as described earlier.

## RESULTS AND DISCUSSIONS

### Results of Method Validation of Glucomannan using UV/Visible spectroscopy

### Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of GM. Beer-Lambert’s concentration range was found to be 50 - 110 µg/ml with respect to the peak and r$^2$ value 0.999.

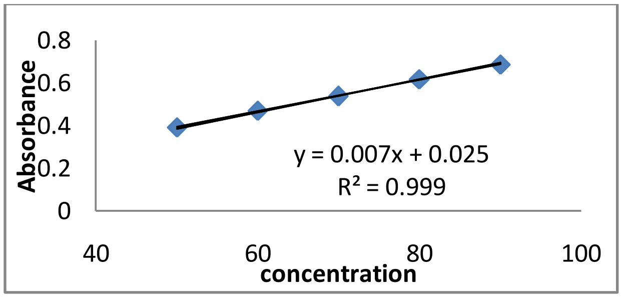

**Figure 1: calibration curve of Glucomannan by UV/Visible spectroscopic method**

### Summary of validation of UV/Visible spectroscopic method

The results of the reproducibility, shows the standard deviation of spectra in the range of 0.0004 – 0.0012, avg. % R.S.D was 0.146 %. The instrument was precised for its repeatability and intra and inter day variance in the results. For this, GM concentrations (70, 80 and 90 µg-ml$^{-1}$) were analyzed at different time intervals of 0, 4, 8 and 12 hr. in a day (intra-day precision) and for three continuous days (inter-day precision). The results are expressed as % R.S.D. The results of the % recovery of glucomannan, shows in Table 2. Recovery range was found to be 100.04 – 102.66 % (Table 2).

**Table 2: Results of validation using UV/Visible spectroscopic method**

| Sr. No. | Parameters | Observations |
|---|---|---|
| 1 | Absorption Maxima (nm) | 488 |
| 2 | Linearity range (µg/ml) | 50 – 90 |
| 3 | Regression equation (y) | y = 0.007x + 0.025 |
| 4 | Slope (m) | 0.007 |
| 4.1 | t-test | 0.006669 |
| 4.2 | C.I. | ±0.000976 |
| 7 | Intercept (c) | 0.025 |
| 7.1 | t-test | 0.006271 |
| 7.2 | C.I. | ±0.046231 |
| 10 | Correlation coefficient ($r^2$) | 0.999 |
| 11 | LOD (µg/ml) | 1.041 |
| 12 | LOQ (µg/ml) | 3.156 |
| 13 | Reproducibility avg. (RSD, [%], mean) | 0.146 |
| 14 | Intra-day precision (RSD, [%], n = 3) | 0.401 |
| 15 | Inter-day precision (RSD, [%], n = 3) | 0.365 |
| 16 | Accuracy (mean, [%], n = 3) | 101.4 |

### Specificity

The peak for GM in the samples was confirmed by spectral overlain of the sample with that of the standard GM (Figure. 2)

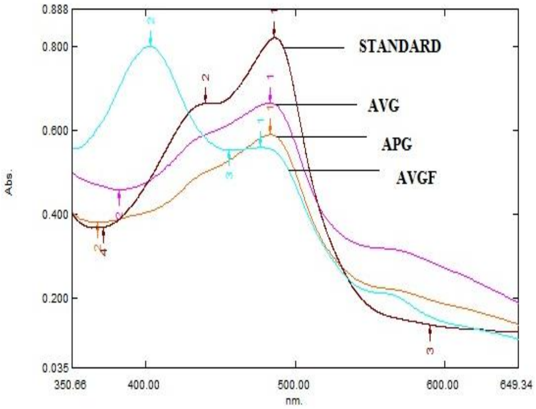

**Figure 2: Overlain of Chromatograms of standard GM and samples, 1st track from the top (std. glucomannan), 2nd from *Aloe vera* gel, 3rd from *Aloe perryi* gel and 4th spectrum is of marketed formulation.**

### Quantitative Estimation of glucomannan by UV/Visible spectroscopic method

Glucomannan content of different *Aloe* gels and its marketed formulations was estimated. In Dec. 2011 the % conc. of GM in AVG and APG was found to be 0.22 and 0.12 % w/w respectively. Where as in May 2012 the % conc. of GM in AVG and APG was found to be 0.53 and 0.25 % w/w. In marketed herbal formulations of *Aloe vera* the conc. of GM was found to be more in AVJF (0.23 % w/w) than AVGF (0.16 % w/w) (Table 3).The glucomannan content of marketed formulation AVJF were higher than AVGF while the rest parameters have comparatively lower values.

**Table 3: Con. of Glucomannan at different season by UV/Visible spectroscopic (n=3)**

| S. No. | Sample | Season | |
|---|---|---|---|
| | | **Dec. 2011** | **May-12** |
| **1** | **AVG (%w/w)** | 0.22 | 0.53 |
| **2** | **APG (%w/w)** | 0.12 | 0.25 |
| **3** | **(A) AVJF (%w/w)** | 0.23 | |
| **4** | **(B) AVGF (%w/w)** | 0.16 | |

### Estimation of Total Sugar content using UV/Visible spectroscopic method

The total sugar content of different *Aloe* gels was estimated. In Dec. 2011 the conc. of sugar in AVG and APG was found to be 0.023 and 0.0035 % w/w respectively. and in May 2012 the conc. of sugar in AVG and APG was found to be 0.042 and 0.0199 % w/w (Table 4).

**Table 4: Con. of Sugar and Phenolic content at different season by UV/Visible spectroscopic method (n=3)**

| S.No. | Parameters | Season | | | |
|---|---|---|---|---|---|
| | | **Dec.- 11** | | **12-May** | |
| | | **AVG** | **APG** | **AVG** | **APG** |
| 1 | Sugar content (% w/w) | 0.023 | 0.0035 | 0.042 | 0.0199 |
| 2 | Phenolic content (% w/w) | 0.0019 | 0.0027 | 0.0076 | 0.013 |

### Estimation of Total Phenolic content using UV/Visible spectroscopic method

The total phenolic content of different *Aloe* gels was estimated. In Dec. 2011 the conc. of phenol in AVG and APG was found to be 0.0019 and 0.0027 % w/w respectively. and in May 2012 the conc. of phenol in AVG and APG was found to be 0.0076 and 0.013 % w/w (Table 4). The concentration of all the chemical constituents except phenolic content of were on higher side in the *Aloe* gel samples collected in May season. The glucomannan content of AVG were higher than APG and vice-versa for the sugar content. The phenolic content of APG were higher than AVG in Dec. season, while were lower than AVG in May season.

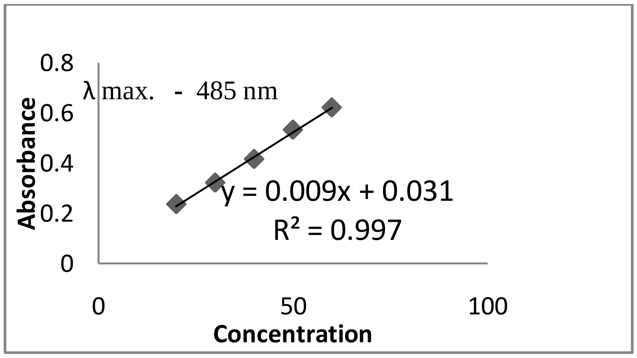

**Figure 3: Standard calibration curve of Glucose using UV/Visible spectroscopic method**

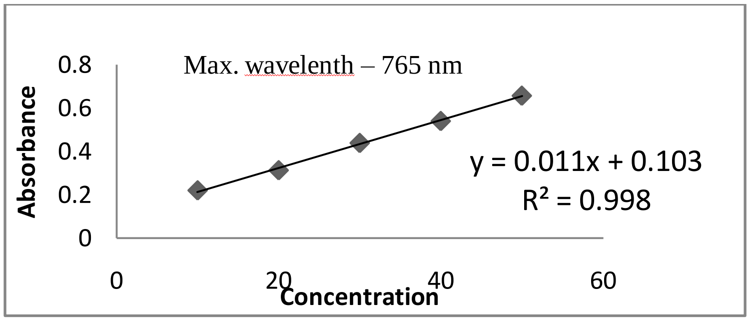

**Figure 4: Calibration curve of Gallic acid using UV/Visible spectroscopic method**

**Results of Method Validation of Glucomannan using HPTLC method**

The mobile phase containing n-butanol : ethanol : water : acetic acid in ratio of (2 : 4 : 4 : 0.05 v/v/v) was found optimum for the analysis which gave a sharp and well defined peak of glucomannan at Rf 0.69 after 20 minutes of chamber saturation. The chromatogram obtained from the optimized solvent system was illustrated in Figure. 5.

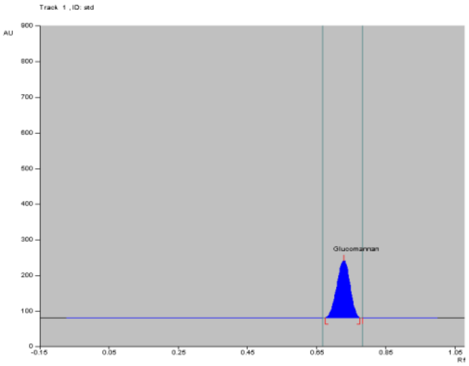

**Figure 5: Typical densitogram of standard glucomannan at *Rf* = 0.69**

**Linearity**

Standard glucomannan showed good linearity between concentration ranges of 400 - 1400 ng band$^{-1}$. The developed HPTLC method for estimation of glucomannan showed a good correlation coefficient ($r^2$ = 0.995) in concentration range of 400 – 1400 ng band$^{-1}$ with respect to the peak area (Figure. 6)

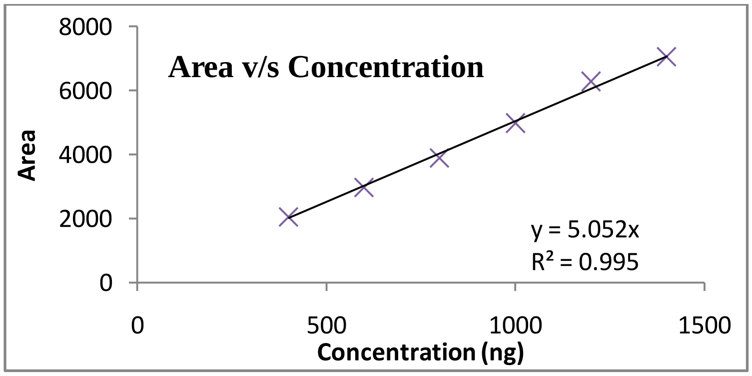

**Figure 6: Calibration curve of standard glucomannan by HPTLC method**

## Summary of validation data of HPTLC method

A band of 1000 ng was spotted in nine replicates. The % S.D. was found to be 1.37 for Repeatability. The measurement of the peak area at three different concentration (600, 800, 1000 ng band $^{-1}$) levels showed low values of % S.D. for inter- and intra-day variation. Slightl variation, in the chromatographic condition were done, shows the standard deviation of peak area in the range of 1.76 - 2.25. Detection limit and limit of quantification was found to be 60 and 180 ng band$^{-1}$ respectively. The proposed method afforded % recovery in the range of 98.89–99.77 % and mean of % recovery was found to be 99.24 % (Table 5).

**Table 5 : Results of validation of HPTLC method**

| Sr. No. | Parameters | Observations |
|---|---|---|
| 1 | Linearity range (ng band$^{-1}$) | 400 – 1400 |
| 2 | Rf | 0.69 |
| 3 | Regression equation (y) | y = 5.052x |
| 4 | Slope (m) | 5.052 |
| 5 | Intercept (c) | 0.0 |
| 6 | Correlation coefficient ($r^2$) | 0.995 |
| 7 | LOD (ng band$^{-1}$) | 60 |
| 8 | LOQ (ng band$^{-1}$) | 180 |
| 9 | Repeatability avg. (SD, [%], mean) | 1.37 |
| 10 | Intra-day precision (SD, [%], n = 3) | 1.31 |
| 11 | Inter-day precision (SD, [%], n = 3) | 1.26 |
| 12 | Accuracy (mean,[%], n = 3) | 99.24 |
| 13 | Robustness | Robust |
| 14 | Specificity | Specific |

## Specificity

The peak shown by glucomannan marker and its corresponding in the sample were analyzed for spectral details. The peak for glucomannan in the samples was confirmed by comparing the Rf value = 0.69 and spectra of the peak with that of the standard glucomannan (Figure. 7 and 8).

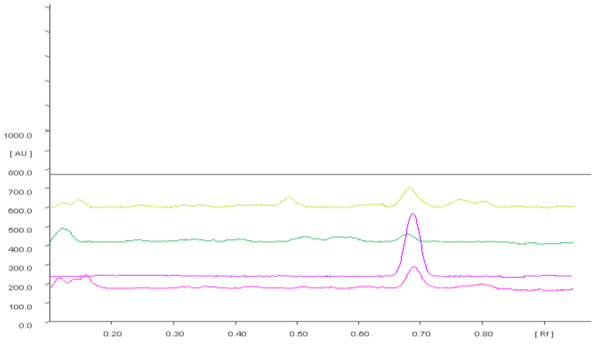

**Figure. 7: Overlain of Chromatograms of standard GM and samples, 3rd track from the top (std. glucomannan), 1st from *Aloe vera* gel, 4th from *Aloe perryi* gel and 2nd spectrum is of marketed formulation.**

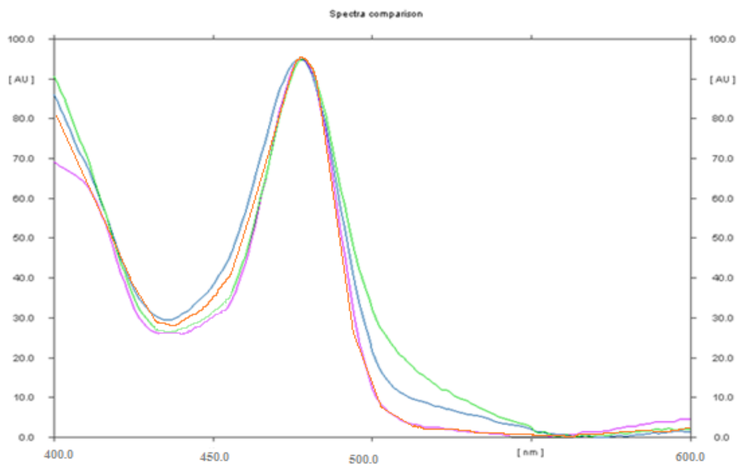

**Figure. 8: Spectra comparison between standard glucomannan and glucomannan in samples**

## Quantitative Estimation of glucomannan in *Aloe* gels and marketed formulations by HPTLC

Glucomannan content was determined in the of *Aloe* gels of both the varieties (AVG and APG) and its marketed formulations. In Dec. 2011 the conc. of GM in AVG and APG was found to be 0.31 and 0.24 % w/w respectively. and in May 2012 the conc. of GM in AVG and APG was found to be 0.67 and 0.43 % w/w. In marketed herbal formulations the conc. of GM was found to be more in AVJF (0.23 % w/w) than AVGF (0.16 % w/w) (Table 6).

Glucomannan, sugar and phenolic content (% w/w) of the above samples were determined. These parameters have high contribution in May season, except the phenolic contents of APG. The glucomannan content of AVG were higher than APG and vice-versa for the sugar contents. The phenolic content of APG were higher than AVG in Dec. season, while were lower than AVG in May season.

**Table 6: Content of glucomannan in *Aloe* gels and its formulations by HPTLC method**

| S. No. | Sample | Season | |
|---|---|---|---|
| | | **Dec. 2011** | **May-12** |
| 1 | AVG (%w/w) | 0.31 | 0.67 |
| 2 | APG (%w/w) | 0.24 | 0.43 |
| 3 | (A) AVJF (%w/w) | 0.39 | |
| 4 | (B) AVGF (%w/w) | 0.28 | |

## CONCLUSION

The quantitative, Colorimetric and HPTLC method was developed for estimation of GM in *Aloe* gels and formulation. The developed method was found to be simple, sensitive, accurate, precised, and economic and can be used for routine quality control analysis of GM in bulk as well as in pharmaceutical dosage form. *Aloe* gels (*Aloe vera* and *Aloe perryi*) can be differentiated on the basis of chemical properties. *Aloe* gel obtained from *Aloe vera* is of better quality than *Aloe perryi*. The Best harvesting time is summer season (May) for both the spp. Hence it is concluded that the purpose of proposed project has been achieved, moreever the developed methods meeting all validation parameters requirements of ICH guidelines.

## ACKNOWLEDGMENT

We express our sincere thanks to Management and Shri Parveen Garg, Honarable Chairman, ISF College of Pharmacy, Moga, India, for providing necessary facilities and financial support for this study.

## REFERENCES

1. Alexis R. Eberendu, Gabriela Luta, Joshua A. Edwards, Bill H. Mcanalley, and Brice Davis. Quantitative Colorimetric Analysis of Aloe Polysaccharides as a Measure of Aloe Vera Quality in Commercial Products. Journal of AOAC International 2005; 3: 684-691.
2. Bozzi A, Perrin C, Austin S, Vera FA. Quality and authenticity of aloe vera gel powders. Food and Bioproducts Processing 2006; 3: 7-10.
3. Canga A Gonzalez, Fernandez Martinez N, Sahagun AM, Garcia Vieitez JJ, Diez Liebana MJ, Calle Pardo AP, Castro Robles LJ, Sierra Vega M. Glucomannan: properties and therapeutic applications. Nutr Hosp 2004; 19, 1: 45-50.
4. DuBois M, Gilles KA, Hamilton JK, Rebers PA, & Smith F. Anal. Biochem 1956; 28: 350–358.
5. Dutt Bharvi. A Study of Patenting Activity in Aloe vera. Journal of Intellectual Property Rights 2002; 7: 330-341.
6. Femenia Antoni, Emma S Sanchez, Susana Simal, Carmen Rossello, Compositional features of polysaccharides from Aloe vera (Aloe barbadensis Miller) plant tissues. Carbohydrate Polymers 1999; 39: 109–117.
7. Hamman Josias H, Composition and Applications of Aloe vera Leaf Gel. Molecules 2008; 13: 1599-1616.
8. ICH, Q2A (R1). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization, Geneva 2005.
9. Loots Du Toit, Francois H vander Westhuizen, Lisa Botes. Aloe ferox Leaf Gel Phytochemical Content. Antioxidant Capacity and Possible Health Benefits. J Agric Food Chem 2007; 55, 17: 6891-6896.

10. Olennikov Daniil N. Densitometric HPTLC Analysis of Aloenin in Aloe Pharmaceuticals. Journal of Planar Chromatography 2009; 22, 5: 359–362.
11. Ramachandra CT, Rao PS, Processing of Aloe Vera Leaf Gel: A Review**.** American Journal of Agricultural and Biological Sciences 2008; 3: 502-510.
12. Reynolds T, Dweck AC. Aloe vera leaf gel: a review update. Journal of Ethnopharmacology 1999; 68: 3–37.
13. Sande M Alonso, Teijeiro-Osorio D, Remuñán-López C, Alonso MJ. Glucomannan a promising polysaccharide for biopharmaceutical purposes. European Journal of Pharmaceutics and Biopharmaceutics 2009; 72: 453–462.
14. Singh R B, Shelley. Polysaccharide structure of degraded glucomannan from Abrus precatorius Linn. Seeds. Journal of Environmental Biology 2007; 28, 2: 461-464.
15. Singleton V L, Rossi J A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J of Enology Viticulture 1965; 16: 144–158.
16. Steenkamp V, Stewart M J. Medicinal Applications and Toxicological Activities of Aloe Products. Pharmaceutical Biology 2007; 45, 5: 411–420.
17. WHO. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva 1999.