MS.ID-IJPBS-8499
International Journal of Pharmacy and Biological Sciences-IJPBS® (2023) 13 (3): 191-200
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

# High Performance Thin Layer Chromatography: Method Development and Validation for Estimating the Gallic Acid from *Syzygium Cumini* Seeds

**T. S. Kamble*, M. S. Patil, A. S. Jain and S. B. Bangar**
**Department of Quality Assurance, Shri D. D. Vispute College of Pharmacy and Research Centre, Devad-Vichumbe, Panvel 410221, Maharashtra, India.**

Received: 13 Mar 2023 / Accepted: 7 Apr 2023 / Published online: 1 Jul 2023
***Corresponding Author Email:** tanmaykamble29@gmail.com

## Abstract

In the current research work, an effective, efficient, exact, easy-to-follow, and trustworthy method was developed for the estimation of gallic acid content from Jamun seed powder. In this research, HPTLC technique is used for analysis along with other quality control test parameters. The preliminary studies by virtue of solubility studies, FT-IR spectroscopy, melting point determination, etc. Working wavelength was determined by UV-Visible spectroscopy. The analytical method development was done for gallic acid estimation in Jamun seed powder, using HPTLC following its validation using ICH Q2(R1) guidelines. Methanol was chosen as the diluent. FT-IR scanning and melting point determination identified the drug component. The working wavelength was reported to be 256nm. HPTLC was used to develop the analytical method, and validation was done. With a correlation coefficient of 0.9957, the calibration curve was plotted over the linearity range of 2-12 µl/band. The mean recovery was found to be 97.82694%. The method was discovered to be precise and within acceptable limits. Also, the method was found to be robust. High-performance thin layer chromatography was used to estimate gallic acid in powdered jamun seed, and the analytical method was validated.

**Keywords**
*Syzygium cumini* seed powder; Gallic acid; HPTLC; Analytical method development and validation; ICH Q2(R1) guidelines.

*****

## INTRODUCTION:

### Pharmaceutical analysis:

Pharmaceutical analysis is a subfield of practical chemistry that entails a number of procedures for the identification, quantification, and purification of substances as well as for the separation of the constituents of solutions and mixtures and the determination of the chemical composition of compounds. Its primary focus is on the development and validation of analytical methods as well as quality control [1].

### Chromatography:

Chromatography is one of an analytical technique that can be used for qualitative and quantitative purpose. Based on the idea that molecules in mixtures applied to surfaces or placed into solid and liquid stationary phases (stable phase) separate while moving with the aid of the mobile phase, chromatography was developed. The separation process depends on a variety of molecular characteristics, including adsorption (liquid-solid), partition (liquid-solid), affinity, and variations in their molecular weight. In addition to separation, the

DOI: https://doi.org/10.5281/10.5281/zenodo.10648678

objective of using chromatography as a technique for quantitative analysis is to achieve an acceptable separation in a reasonable period.

The goal of using chromatography, which is also used for quantitative analysis, is to achieve a satisfactory separation within a reasonable amount of time. To that end, numerous chromatography techniques have been created. Some of them are affinity chromatography, thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), column chromatography, paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography [2,3].

**HPTLC:** One of the most important identity tests in the majority of pharmacopoeial monographs is thin layer chromatography [4]. High-performance thin layer chromatography (HPTLC), an improvement of TLC, is a reliable, straightforward, quick, and effective technique for quantitative compound analysis. Based on TLC, HPTLC is an analytical method that has been improved to allow for quantitative analysis of the compounds and to improve the resolution of the compounds that need to be separated [5,6].

**Jamun:** The poly-embryonic species Jamun, also called Malabar plum, Java plum, black plum, Jaman, Jambul, or Jambolana, is a member of the Myrtaceae family, also known as the Indian blackberry. The *Syzygium cumini* tree is a tropical, evergreen native to the Indian subcontinent that has since become a naturalized species in America, Africa, and Australia. There are numerous uses for the tree's leaves, seeds, and roots. The seeds are brown and have a sweet or bitter flavour, whereas the fruit is violet-dark blue in colour. The fruit is oval, 1-2 cm long, 0.5-1 cm wide, and tapering at the apex. It has a faint aroma [7,8].

Being an herbal remedy, many phytochemicals are present. Flavonoids, phenolic acids, terpenes, tannins, and anthocyanins are a few of the constituents. Methyl--orsellinate, -sitosterol, and 7-hydroxycalamenene 5, 6, H pyran Additionally, it demonstrates the presence of ellagic acid, quercetin, myricetin, tannic acid, jamboline, and gallic acid [9].

In conclusion, jamun is one of the best medicinal agents that can be used for its wide range of therapeutic outcomes. With the review of the historical context of the plant, jamun seeds can be one of the major subjects for the research due to presence of various constituents.

## MATERIALS AND METHODS:

**Chemicals and reagents:** Pure Jamun seed powder (*Syzygium cumini* seed powder) was procured from Attar Ayurveda, India. Analytical grade solvents were used. Standard Gallic acid was obtained from Research-Lab Fine Chem Industries, Mumbai, India.

**Instruments:** The instruments that were used for carrying out the research operations can be seed in Table 1 and Table 2.

**Table 1 Instruments Used in Research Work**

| Name of Instrument | Make/ Model | Use |
|---|---|---|
| UV-Visible spectrophotometer | Shimadzu UV- 1800<br>Software: UV probe | For selection of analytical wavelength |
| Electronic Weighing Balance | Shimadzu (Sensitivity 0.001 gm) | Weighing of the components |
| Ultra sonicator | LABLINE | For improving solubility |
| FT-IR spectrophotometer | Shimadzu | Identification of the sample |

**Table 2 Overview of The HPTLC Set-Up Used for Research Work**

| HPTLC system | Application |
|---|---|
| CAMAG TLC Autosampler Linomat 5 | Application of samples |
| CAMAG TLC Visualizer | Scanning at R white, 254, 366 nm |
| TLC Scanner 4 | Scanning at 254 nm |
| CAMAG Twin-Trough Chamber | Development of Plate |

**Preliminary Studies:**

**Solubility Studies:** By testing various solvents, the drug's solubility was determined. Solvents such as n-hexane, acetone, methanol, ethanol, and water were used to carry out the solubility operations.

**FTIR scanning:**

FTIR scanning of the drug was done to identify the drug.

Melting Point: The melting point is determined in a capillary tube in the Thiele tube setup with paraffin oil. The temperature at which the substance is completely melted as indicated by the disappearance of the solid, will be in the range of ±4°C from the stated value, unless otherwise indicated [10].

**UV-Visible spectroscopy:**

**Selection of working wavelength:**

Powdered *Syzygium cumini* seeds were dissolved in the solvent discovered through solubility studies to create the standard solution. With a wavelength range of 200-800 nm, the concentrations of 1000, 100, and 10 µg/mL were prepared and scanned against the blank for obtaining the spectra using a UV-visible spectrophotometer [11].

**HPTLC:**

**Analytical Method Development:**

**Selection of diluent:** The diluent was selected based on solubility studies.

**Preparation of standard solution:** An accurately weighed quantity of gallic acid was dissolved in methanol to make 1000 µg/ml. The stock solution of standard gallic acid was prepared by diluting this solution (1000 µg/ml) with methanol to give 100 µg/ml concentration of gallic acid.

**Preparation of sample solution:** Sample solution or sample solution of gallic acid was prepared by adding specific amount of jamun seed powder in methanol to give 1000 µg/ml concentration.

Mobile phase composition trials for HPTLC method development: The trials were conducted in-order to know the working mobile phase composition [12,13]. The trials can be seen in Table 3.

**Table 3 Trials of Mobile Phase Compositions**

| Sr. no. | Mobile Phase Composition | Ratio |
|---|---|---|
| 1 | ACN: Methanol | 8: 2 (%v/v) |
| 2 | ACN: Water | 8: 2 (%v/v) |
| 3 | Chloroform: Ethyl acetate: Formic acid | 5: 4: 1 (%v/v/v) |
| 4 | ACN: Chloroform: Ethyl acetate: Formic acid | 2: 5: 2: 1 (%v/v/v/v) |

**Validation of developed analytical method:**

The developed method was validated in accordance with ICH Q2(R1) guidelines. The optimized method was validated in terms of specificity, linearity, accuracy, precision & robustness [14,15].

**Specificity:**

Screened for Blank and placebo interference at the main drug peaks. The use of a reference standard ensured specificity, which is shown by the absence of interference from other components in the matrix. By examining the standard drugs, sample, diluent, and solvents used, the specificity of the method was determined. By comparing the spot's Rf and spectrum with those of standards, the presence of gallic acid was identified in the sample.

**Linearity:**

By preparing a standard gallic acid solution at six different concentration levels, linearity was tested. A calibration curve between concentrations and peak area was created after recording the average peak area. By using a microliter syringe and an automatic sample applicator to apply 2 - 12 µl/band (2, 4, 6, 8, 10, 12) of the gallic acid standard solution from a 100 µg/ml stock solution on the TLC plate, linearity was demonstrated. The plates underwent development, drying, and scanning. For each level of drug concentration, the drug peak area was determined, and a graph of drug concentration versus peak area was created.

**Accuracy:**

By performing recovery studies in accordance with ICH guidelines at three different concentration levels (80%, 100%, and 120%), i.e., by multiple level recovery studies by replicate analysis (n=3), the accuracy of the developed method was confirmed. Different levels were used to calculate the percent recovery.

**Precision:**

The system reproducibility was analysed by adding 6 bands of 6µl/band concentration of sample solution of gallic acid on the TLC plate. This was followed by the development, drying, and scanning of the plate. For performing the method precision, inter-day and intra-day analysis was done by applying 600 ng/band concentration of the sample solution onto the TLC plate respectively.

Limit of Detection: The lowest amount in the analyte in the sample which can be determined but cannot be qualified is known as limit of detection.

**Limit of Quantitation:**

The lowest amount of analyte in the sample that can be quantitatively determined is known as limit of quantification.

**Robustness:**

Robustness was analysed by making the minute changes in few parameters of the analytical method. Parameters such as concentration of mobile phase composition and saturation time. 4µl/band of concentration was added on the TLC plate and the effects of changes were examined on the results which were generated in form of peak areas and Rf value.

**RESULTS:**

**Preliminary studies:**

Solubility studies: Solubility studies were done from which it was observed that the drug shows solubility in alcoholic solvents from which Methanol was the best fit.

FTIR Scanning: The FTIR spectrum estimation was done with Jamun seed powder which can be seen in Figure 1.

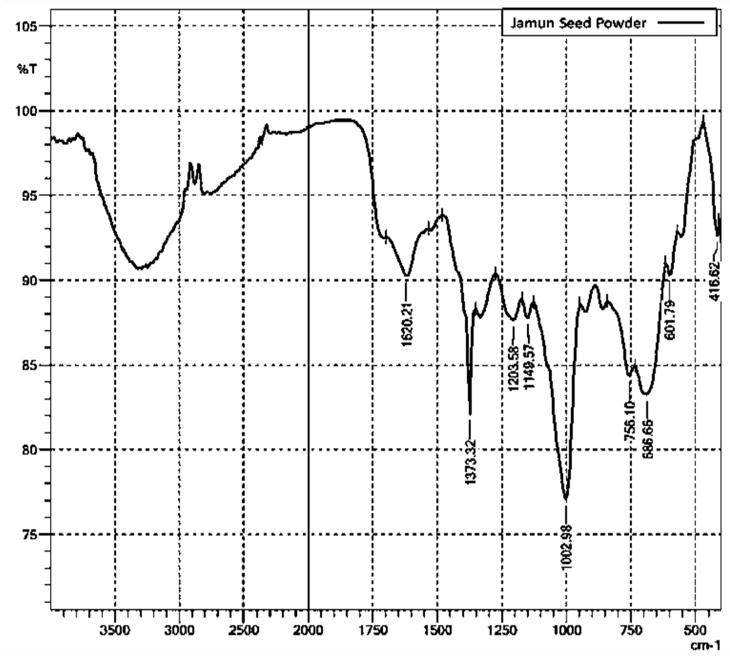
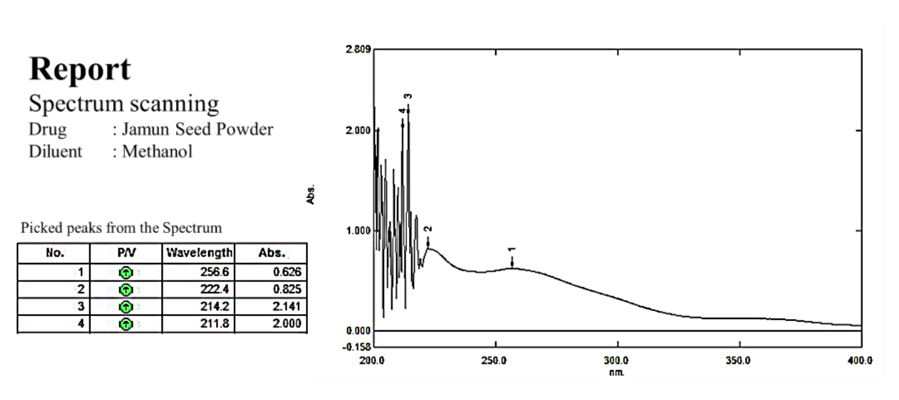


**Figure 1 FTIR Spectrum for *Syzygium cumini* seed powder.**



Report

Spectrum scanning

Drug : Jamun Seed Powder

Diluent : Methanol

Picked peaks from the Spectrum

| No. | P/V | Wavelength | Abs. |
|---|---|---|---|
| 1 | ● | 256.6 | 0.626 |
| 2 | ● | 222.4 | 0.825 |
| 3 | ● | 214.2 | 2.141 |
| 4 | ● | 211.8 | 2.000 |

**Figure 2 UV Spectrum of *Syzygium cumini* seed powder for gallic acid.**

**Melting Point:** The melting point was determined and found to be in range of 260°-270° C.

**UV-Visible Spectroscopy:**

UV Working wavelength determination: Working wavelength was found to be 256 nm which can be seen in Figure 2.

**HPTLC:**

**Analytical method development:**

Mobile phase optimization: The results and the observations of the trials of mobile phase composition can be seen in Table 4.

Optimized attributes of HPTLC: The optimized attributes for analytical method development on HPTLC can be given in Table 5. Also, the chromatogram for optimized method of gallic acid estimation in *Syzygium cumini* seed powder by HPTLC analysis can be referred from Figure 3.

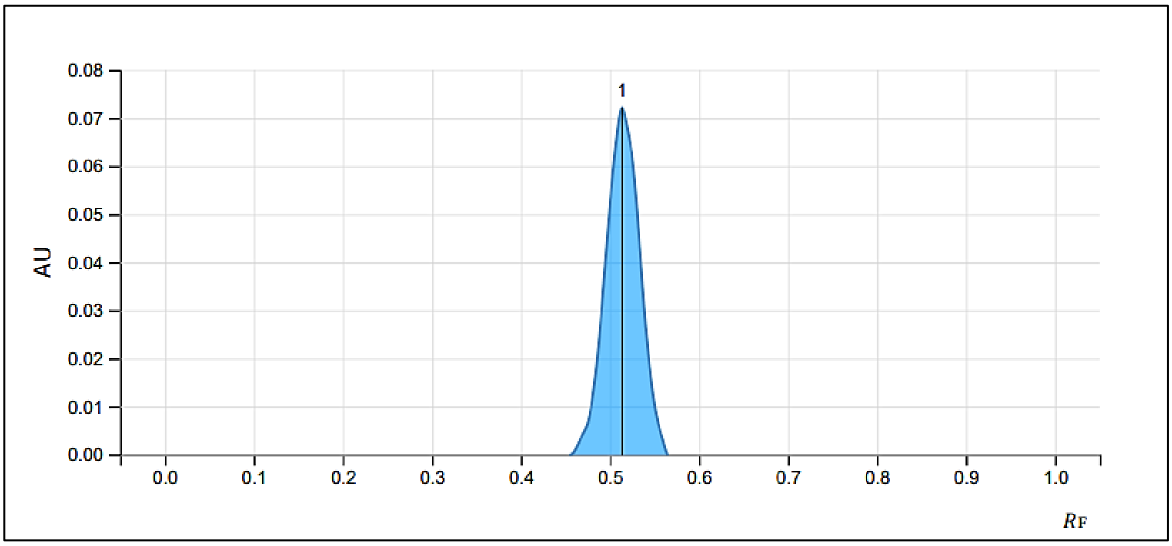
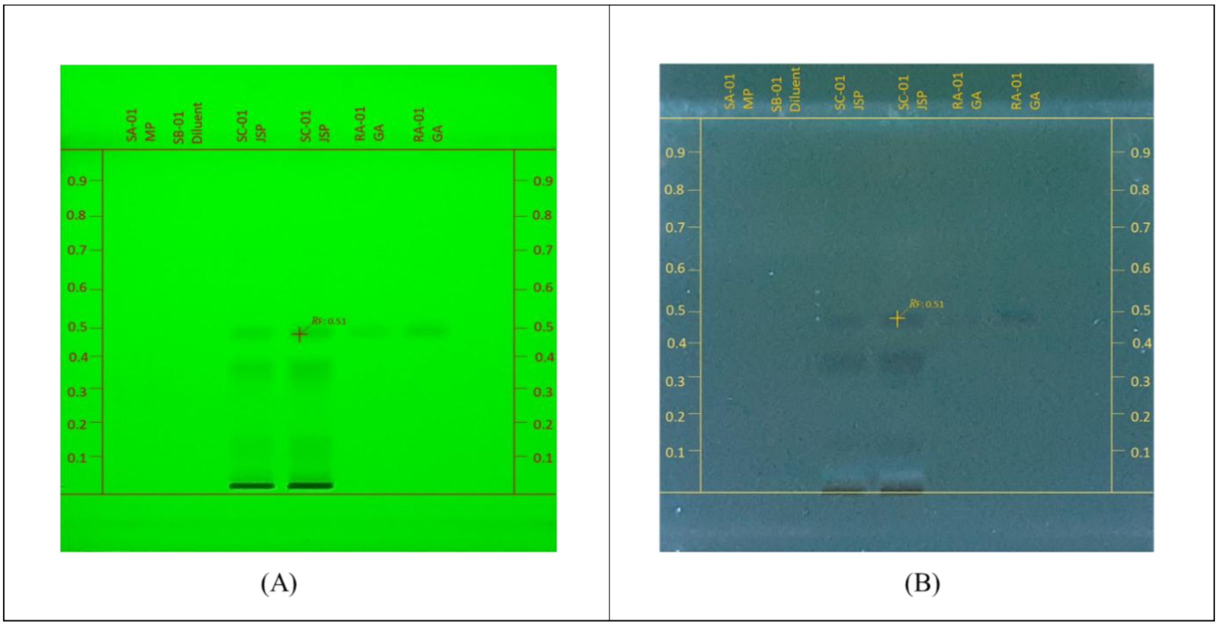


**Figure 3 Optimized method for gallic acid estimation in *Syzygium cumini* seed powder by HPTLC analysis.**



(A)

(B)

**Figure 4 Overview of Specificity at (A) 254nm and (B) 366nm**

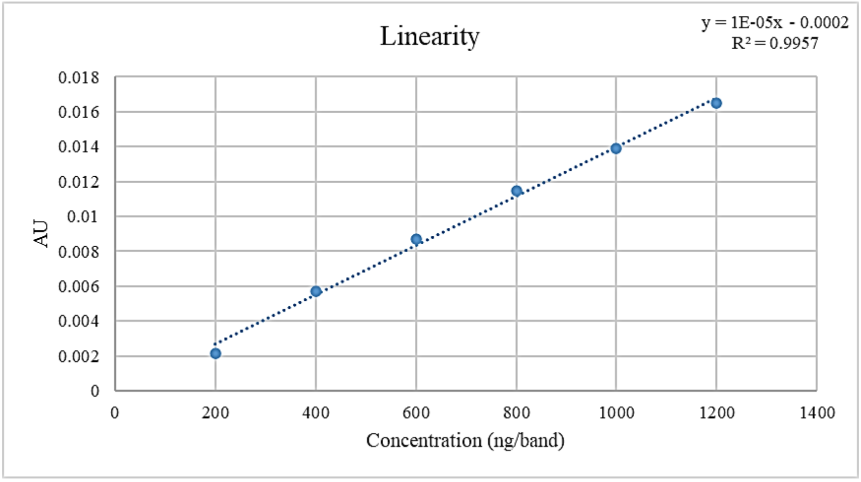


**Figure 5 Calibration curve of Gallic acid**

**Validation of developed analytical method:**

**Specificity:** The overview of applications for specificity can be seen in table 6. While Figure 4 shows the scanned plates at 254nm and 366nm. Here, no interference was observed when specificity was examined with diluent, mobile phase solvents, standard and sample.

**Linearity:** The data for linearity is given in Table 7. Calibration curve of Gallic acid is given in figure 5. From this, the linear correlation coefficient for concentrations vs. area for conventional gallic acid was determined to be 0.9957. The drug's concentration was linear over the range that was considered.

**Accuracy (Recovery):** Table 8 shows the data for recovery studies. Three recovery investigations were performed; the chromatograms were recorded, and the percentage recovery and mean percentage recovery were computed as shown in Table 9.

Precision: The overview of results seen in precision test can be referred from Table 10.

Limit of detection and Limit of quantification: Results for LOD and LOQ are given in Table 11.

**Robustness:** The robustness was performed for mobile phase concentration alteration and saturation time for which results can be seen in Table 12 and 13 respectively.

**Table 4 Results and Observations of The Trails of Mobile Phase Composition**

| Sr. no. | Mobile Phase Composition | Observation |
|---|---|---|
| 1 | ACN: Methanol (8: 2) | No proper separation was observed. |
| 2 | ACN: Water (8: 2) | Proper separation wasn't seen. |
| 3 | Chloroform: Ethyl acetate: Formic acid (5: 4: 1) | Bands ran close till solvent front. |
| 4 | ACN: Chloroform: Ethyl acetate: Formic acid (2: 5: 2: 1) | Separation was observed and tailing was seen to be minimal. |

**Table 5 The Optimized Attributes for Analytical Method Development On HPTLC**

| Attributes | Description |
|---|---|
| Mobile Phase Composition | Acetonitrile: Chloroform: Ethyl Acetate: Formic acid (2:5:2:1% v/v/v/v). |
| Saturation time | 20 mins |
| Wavelength | 254nm |
| Diluent | Methanol |
| Stationary phase | TLC Silica gel 60 $F_{254}$ by Merck |
| Retention Factor | 0.51 |
| Developing distance | 70mm |
| Name of standard used | Gallic Acid |
| Name of sample used | Jamun Seed Powder |

**Table 6 Overview of Application on TLC Plate for Specificity**

| Track no. | Sample applied |
|---|---|
| 1 | Optimized mobile phase composition |
| 2 | Diluent |
| 3 | Jamun seed powder |
| 4 | Jamun seed powder |
| 5 | Gallic acid standard |
| 6 | Gallic acid standard |

**Table 7 Data for Linearity On HPTLC**

| Track No. | Concentration (ng/band) | Peak Area |
|---|---|---|
| 1 | 200 | 0.00218 |
| 2 | 400 | 0.00572 |
| 3 | 600 | 0.00869 |
| 4 | 800 | 0.01146 |
| 5 | 1000 | 0.01391 |
| 6 | 1200 | 0.01652 |

**Table 7 Data for Recovery Studies**

| Level % | Amount of Standard added (µl/band) | Amount of Sample added (µl/band) | Final concentration (µl/band) |
|---|---|---|---|
| 80 | 3.2 | 4 | 7.2 |
| 100 | 4 | 4 | 8 |
| 120 | 4.8 | 4 | 8.8 |

**Table 8 Recovery Results Observed for Recovery Studies**

| Track no. | Sample | Peak Area | Mean Peak Area | Expected Value | % Recovery | Mean % Recovery |
|---|---|---|---|---|---|---|
| 1 | 80% | 0.012395 | 0.012472 | 0.012632 | 98.73819364 | 97.82694 |
| 2 | | 0.012436 | | | | |
| 3 | | 0.012586 | | | | |
| 4 | 100% | 0.013281 | 0.013206 | 0.013606 | 97.05493518 | |
| 5 | | 0.013292 | | | | |
| 6 | | 0.013044 | | | | |
| 7 | 120% | 0.013523 | 0.013767 | 0.014093 | 97.68769931 | |
| 8 | | 0.013888 | | | | |
| 9 | | 0.013890 | | | | |

**Table 9 Overview of Results Seen in Precision Test**

| Sr. No. | Peak Area | | |
|---|---|---|---|
| | ***Intra Day*** | ***System*** | ***Inter Day*** |
| 1 | 0.00869 | 0.00878 | 0.00869 |
| 2 | 0.00862 | 0.00868 | 0.00862 |
| 3 | 0.0086 | 0.00886 | 0.00878 |
| 4 | 0.00867 | 0.00869 | 0.00881 |
| 5 | 0.00875 | 0.00875 | 0.00858 |
| 6 | 0.00853 | 0.00861 | 0.00875 |
| **Mean** | 0.008643333 | 0.008728333 | 0.008705 |
| **SD** | 0.000077 | 0.000087502 | 0.000091378 |
| **%RSD** | 0.889180581 | 1.002509615 | 1.049722394 |

Table 10 Results Observed for LOD And LOQ

| | |
|---|---|
| **LOD** | 66 ng/ band |
| **LOQ** | 200 ng/ band |

**Table 11 Robustness Results for Concentration of Mobile Phase Composition Parameter**

| Track No. | Volume (µl/ band) | Peak Area | Mean Peak Area | SD | %RSD | Mean % RSD |
|---|---|---|---|---|---|---|
| 1 | 4 | 0.0057 | 0.005617 | 0.0000971 | 1.729235 | 1.290517 |
| | | 0.00551 | | | | |
| | | 0.00564 | | | | |
| 2 | 6 | 0.00856 | 0.00853 | 0.0000889 | 1.041992 | |
| | | 0.00843 | | | | |
| | | 0.0086 | | | | |
| 3 | 8 | 0.01149 | 0.011363 | 0.0001250 | 1.100323 | |
| | | 0.01136 | | | | |
| | | 0.01124 | | | | |

**Table 12 Robustness Results for Saturation Time Parameter**

| Track No. | Volume (µl/ band) | Peak Area | Mean Peak Area | SD | %RSD | Mean % RSD |
|---|---|---|---|---|---|---|
| 1 | 4 | 0.0058 | 0.005753 | 0.0000451 | 0.783763 | 0.842166 |
| | | 0.00571 | | | | |
| | | 0.00575 | | | | |
| 2 | 6 | 0.00859 | 0.00864 | 0.0000557 | 0.644417 | |
| | | 0.00863 | | | | |
| | | 0.0087 | | | | |
| 3 | 8 | 0.01159 | 0.011457 | 0.0001258 | 1.098317 | |
| | | 0.01144 | | | | |
| | | 0.01134 | | | | |

## DISCUSSION:

Solubility studies were carried from which methanol was seen to give good result on basis of which methanol was selected to be the diluent for the further experimentation procedures. FT-IR scanning and melting point determination was done in-order to identify the drug component. This was followed by determining the working wavelength on UV-Visible spectroscopy from which the further project wavelength was confirmed which can used. Analytical method development was done HPTLC, by taking an account of various parameters concerned. Validation of developed method was carried in which no interference was seen with diluent, blank, standard and sample from which we can say that the method developed is specific and can be used further. This was followed by plotting the calibration curve over the linearity range of 2 to 12 µl/band where correlation coefficient was found to be 0.9957 which is under the acceptance limits of linearity. Percentage recovery was performed at concentration level of 80%, 100% & 120% where mean recovery was reported at 97.82694% which is under the limits of herbal drug quality control parameters. The precision test was done for system precision where RSD was observed to be 1.002509615%, that for inter-day precision which was performed on consecutive different days was found to be 1.049722394% while the precision performed on the same day i.e., intra-day precision was found to be 0.889180581% which all falls within the acceptable limits. For validating the method more test parameters were carried by performing robustness in-order to assure the tendency of the method to remain as it is by even slight changes. In this test, concentration of the mobile phase composition was altered and checked where percent RSD was found to be 1.290517% and percent RSD of test with altered saturation time was seen to be 0.842166% which both are within the acceptable limits. For developed analytical method the limit of detection was observed to be 66 ng/band while 200 ng/band for quantitation limits.

## CONCLUSION:

To develop a more effective, efficient, exact, easy-to-follow, and trustworthy method for estimating the amount of gallic acid in the powdered jamun seed, high-performance thin layer chromatography was applied. Additionally, the devised analytical method's validation was carried out in accordance with ICH Q2 (R1) requirements, and all of the parameters were acquired within acceptable bounds.

## ACKNOWLEDGEMENTS:

We are thankful to Management, Principal SRSPM's Shri D.D. Vispute College of Pharmacy and Research Centre, New Panvel for providing all necessary facility required for this research work.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE:

The writers of this article did not engage in any animal-related activities.

## CONSENT FOR PUBLICATION:

As a corresponding author, I give my consent for publication of my review paper to your reputed journal.

## DECLARATION OF CONFLICTING INTERESTS:

There are no conflicts of interest to declare.

## FUNDING:

There is no funding source.

## REFERENCES:

[1] Skoog, D. A. 2012. *Fundamentals of Analytical Chemistry*, 9th Ed.; Cengage - Brooks/Cole: Belmont, CA.

[2] Coskun, O.; et. al. 2016.Separation Techniques: Chromatography. *North. Clin. Istanb. 3* (2), 156–160. https://doi.org/10.14744/nci.2016.32757.

[3] Hibbert, D. B.; et. al. 2012. Experimental Design in Chromatography: A Tutorial Review. *J. Chromatogr. B*. *910*, 2–13. https://doi.org/10.1016/j.jchromb.2012.01.020.

[4] Attimarad, M.; Ahmed, K. K. M.; Aldhubaib, B. E.; Harsha, S. 2011. High-Performance Thin Layer Chromatography: A Powerful Analytical Technique in Pharmaceutical Drug Discovery. *Pharm. Methods*. *2* (2), 71–75. https://doi.org/10.4103/2229-4708.84436.

[5] Schibli, A.; Reich, E. 2005. Stationary Phases for Planar Separations — Plates for Modern TLC. *LCGC N. Am. 23* (5), 458–469.

[6] Sonia, K.; Lakshmi, K. S. 2017. HPTLC Method Development and Validation: An Overview. *J. Pharm. Sci. Res. 9* (5), 652–657.

[7] Ramteke, V.; Kurrey, V.; Kar, S. 2015. Jamun A Traditional Fruit and Medicine. *Pop. Kheti. 3*, 188–190.

[8] Sahu, P. P.; Behera, L.; Nayak, S.; Samal, K. C. 2020. Health Benefits of Jamun (Syzygium Cumini) an Underutilised Fruit: A Ray in Nanotechnology Field. *Journal of Pharmacognosy and Phytochemistry*. *Sp 9*(5), 74-80.

[9] Kamble, T. S.; Shirke, K. S.; Uppar, K. B.; Bangar, S. B.; Naware, N. S.; Ambatkar, S. S.; Patil, M.; Jain, A. 2023. An Overview of the Historical Context for Jamun's Diverse Medicinal Properties. *Sci. Phytochem. 2* (1), 42–55. https://doi.org/10.58920/sciphy02010042.

[10] Blank, E. W. 1933. Micromelting-Point Determination with the Thiele Tube. *Ind. Eng. Chem. Anal. Ed. 5* (1), 74–75. https://doi.org/10.1021/ac50081a040.

[11] Mansuri, A.; Desai, S. 2019. 32 Factorial Design for Optimization of HPLC-UV Method for Quantification of Gallic Acid in Lohasava and Pippalyasava. *Indian J. Pharm. Educ. Res. 53*, s347–s355. https://doi.org/10.5530/ijper.53.3s.105.

[12] Dhalwal, K.; Biradar, Y. S.; Rajani, M. 2006. High-Performance Thin-Layer Chromatography Densitometric Method for Simultaneous Quantitation of Phyllanthin, Hypophyllanthin, Gallic Acid, and Ellagic Acid in Phyllanthus Amarus. *J. AOAC Int. 89* (3), 619–623. https://doi.org/10.1093/jaoac/89.3.619.

[13] Dalavi, N.; Gawali, V.; Bhalsing, M. 2018. Comparative HPTLC Estimation and Antibactrial Effect of Ellagic Acid, Gallic Acid and Ethanolic Extract of *Syzygium cumini* Seeds Under Accelerated Storage Condition. *Int. J. Pharmacogn. Phytochem. Res. 9*. https://doi.org/10.25258/phyto.v9i07.11164.

[14] International Council of Harmonization (ICH). 2005. ICHQ2(R1): Validation of the analytical procedures: Text and Methodology. https://www.ich.org/page/quality-guidelines (Accessed 2023-06-01).

[15] Halse, P.; Jadhav, V.; Jain, A.; Kachare, S.; Khude, H. 2022. HPTLC Chromatographic Studies and Evaluation of Ayurvedic Formulation. *International Journal of Pharmacy & Pharmaceutical Research. 25*(3), 248-269.