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Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF ELLAGIC ACID IN ANTIDIABETIC HERBAL FORMULATION

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ABSTRACT

A simple, sensitive, precise, rapid and reliable HPTLC method for the estimation of ellagic acid in marketed herbal formulation, Glucomap tablet was developed. In this method, precoated Silica Gel F₂₅₄ Plates were used as stationary phase and Toluene: ethyle acetate: formic acid: methanol (3:3:8:2 v/v) as mobile phase. Developed chromatogram was scanned at 280 nm, the wavelength of maximum absorption for ellagic acid. The aptness of developed HPTLC method for estimation of ellagic acid was established by validating it as per the ICH guidelines. The content of ellagic acid in crude drug *Terminalia arjuna* and polyherbal formulation was also studied. The developed method has been successfully applied for the determination of ellagic acid in polyherbal formulation.

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INTRODUCTION:

Standardization is necessary to make sure the availability of a uniform product in all parts of the world¹. Standardization assures a consistently stronger product with guaranteed constituents. WHO collaborates and assists health ministries in establishing mechanisms for the introduction of traditional plant medicines into primary healthcare programs, in assessing safety and efficacy, in ensuring adequate supplies, and in the quality control of raw and processed materials².

High-performance thin-layer chromatography (HPTLC) is still increasingly finding its way in pharmaceutical analysis in some parts of the world. The technique achieves for given applications a precision and trueness comparable to high performance liquid chromatography (HPLC). It also allows simultaneous estimation of several samples utilizing only a small quantity of a mobile phase, hence minimizing the analysis time and cost³. On the basis of review of literature a few analytical techniques have been reported for the analysis of ellagic acid is available for its estimation in polyherbal formulations. The main aim of this study was to develop and validate an accurate and reproducible HPTLC method for estimation of ellagic acid in Glucomap tablet as an ingredient. The validation was done as per ICH guidelines⁴.

MATERIALS AND METHODS:

Calibration curve of ellagic acid

The fingerprint method for Glucomap tablet was developed by high-performance thin layer chromatography determination using ellagic acid as a standard. The fingerprinting method was developed for selected components of GT (*Terminalia arjuna*), laboratory batches (GT-I, GT-II, GT-III) & marketed formulation (GTM) via estimation of ellagic acid by using following experimental techniques. All chemicals and reagents used were of analytical grade and were purchased from Hi-Media, India.

Table 1: Instrumentation and chromatographic conditions

| Stationary phase | Precoated Silica Gel F ₂₅₄ Plates (Merck) |
|----------------------|---|
| Mobile phase | Toluene: ethyle acetate: formic acid: methanol (3: 3: 8: 2 v/v) |
| Saturation | 40 mins |
| Temperature | 25 ± 2 °C |
| Development chamber | Glass twin trough development chamber |
| Applicator | CAMAG Linomat IV applicator |
| Scanner | CAMAG Scanner III Win Cats (4.06), Switzerland |
| Mode of scanning | Absorption (deuterium) |
| Detection wavelength | 280 nm |
| Scanning Speed | 20 mm/s |

The stock solution of ellagic acid was prepared by dissolving 10 mg of ellagic acid in 100 ml of methanol. This solution was diluted as needed to prepare different concentrations of standard solutions. A stock solution of ellagic acid (100 $\mu\text{g mL}^{-1}$) was prepared in methanol. Different concentrations of stock solution were prepared & spotted on the TLC plate to obtain a 100 - 600 ng spot⁻¹ of ellagic acid, respectively. The data of peak areas plotted against the corresponding concentrations were treated by least-square regression analysis method validation.

Method validation

The method was validated for precision, accuracy, limit of detection & limit of quantification, robustness, ruggedness & specificity of sample application.

Precision and accuracy

Repeatability of the sample application and measurement of peak area were carried out using six replicates of the same spot (600 ng spot⁻¹ for ellagic acid) was expressed in terms of percent relative standard deviation (%RSD). The intra- and inter-day variation for the determination of ellagic acid was carried at three different concentration levels of 100, 300, 600 ng spot⁻¹.

Limit of detection (LOD) & Limit of quantification (LOQ)

LOD was determined based on the lowest concentration detected by instrument in the sample. LOQ was determined based on the lowest concentration quantified by the instrument in the sample (LOQ = 5 x LOD). In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted six times LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting the known concentrations of ellagic acid until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Robustness By introducing small changes in the mobile phase composition, mobile phase volume, duration of mobile phase saturation and activation of pre-washed TLC plates with water; the effects on the results were examined. Robustness of the method was done in triplicate at a concentration level of 600 ng spot⁻¹ for ellagic acid and the % R.S.D of peak areas was calculated.

Ruggedness A solution of concentration 600 ng spot⁻¹ was prepared and analyzed on day 0 and after 6, 12, 24, 48 and 72 h. Data were treated for % RSD to assess ruggedness of the method for ellagic acid.

Specificity The specificity of the method was confirmed by analyzing the standard drugs and samples. The spot for ellagic acid in the sample was confirmed by comparing the Rf values and spectra of the spot with that of the standard. The peak purity of the ellagic acid was assessed by comparing the spectra at three different levels.

Recovery The recovery was determined by the standard addition technique. The pre-analyzed samples were spiked with extra 50, 100 and 150 % of the standard ellagic acid and the mixtures were reanalyzed by the proposed method. The experiment was conducted six times. This was done to check for the recovery of the ellagic acid at different levels in the formulations.

RESULTS AND DISCUSSION:

The method was validated for precision, accuracy, limit of detection & limit of quantification, robustness, ruggedness & specificity of sample application.

Precision and accuracy

The intra- and inter-day variation for the determination of ellagic acid was carried at three different concentration levels of 100, 300, 600 ng spot⁻¹ were calculated and depicted in table 1.

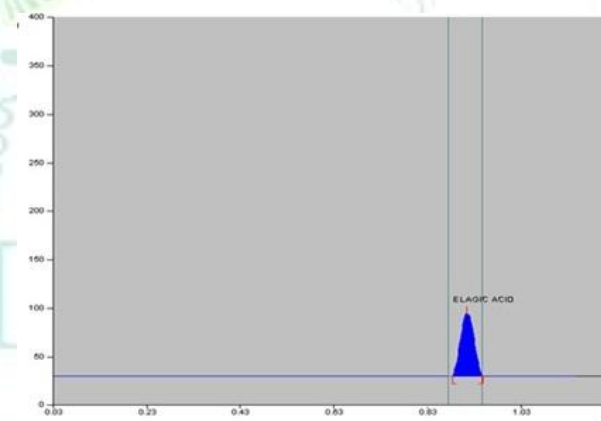


Figure 1: HPTLC chromatogram of ellagic acid

Table 2: Intra and inter-day precision of HPTLC method

| Amount of ellagic acid (ng/spot) | Intra -day precision | | Inter-day precision | |
|-------------------------------------|----------------------|--------------|---------------------|--------------|
| | Area \pm SD | RSD % | Area \pm SD | RSD % |
| 100 | 985.65 \pm 0.571 | 0.058 | 1148.42 \pm 0.387 | 0.034 |
| 300 | 2052.88 \pm 0.482 | 0.023 | 2275.17 \pm 0.474 | 0.021 |
| 600 | 3984.26 \pm 0.623 | 0.016 | 4159.34 \pm 0.691 | 0.017 |
| Mean | | 0.032 | | 0.024 |

Limit of detection (LOD) & Limit of quantification (LOQ)

LOD and LOQ were experimentally verified by diluting the known concentrations of ellagic acid and found to be 71.85 and 222.73 respectively (Table 2).

Table 3: Summary of validation parameters of HPTLC (ellagic acid)

| S. N. | Parameters | Data of ellagic acid |
|-------|------------------------------------|----------------------|
| 1 | Retention Factor (Rf) | 0.90 |
| 2 | Beer's law limit (ng/spot) | 100-600 |
| 3 | Correlation coefficients (r^2) | 0.996 |
| 4 | LOD (ng/spot) | 71.85 |
| 5 | LOQ (ng/spot) | 222.735 |
| 6 | Precision (% RSD) | |
| | Repeatability | 0.325 |
| | Intraday | 0.032 |
| | Interday | 0.024 |
| 7 | Recovery Studies | |
| | Accuracy (% RSD) | 0.285 |
| | SE | 0.236 |
| | Recovery % | 99.34 |
| 8 | Robustness | Robust |
| 9 | Specificity | Specific |

(Mean value, $n=6$)

Robustness

The proposed method was found to be robust (Table 2).

Recovery

The recovery studies were done and determined by the standard addition technique. The %RSD was found to be 0.285.

Estimation of ellagic acid in crude drug and formulations

The sample of selected components of GT (*Terminalia arjuna*), laboratory batches (GT-I, GT-II, GT-III) & marketed preparation (GTM) were prepared separately by weigh sample accurately 200 mg and extracted in methanol by heating and make up 100 ml volume with methanol. The appropriate aliquots from prepared above samples extract were withdrawn in 10 ml volumetric flask separately. The sample solution was spotted on TLC plate & after that development & scanning of TLC plate. A single spot at $R_f = 0.90$ was observed in the sample chromatogram of the ellagic acid with some other components. No interference was found in analysis from the some other components present in the samples. The result of ellagic acid content was illustrated in table 3.

Table 4: Content of ellagic acid in crude drug and formulations

| S. No. | Name | Ellagic acid content (% w/w) | Standard error |
|--------|--------------------------|------------------------------|--------------------|
| 1 | <i>Terminalia arjuna</i> | 1.110 ± 0.135 | 0.104 |
| 2 | Glucomap | GT-I | 0.0255 ± 0.134 |
| | | GT-II | 0.0280 ± 0.451 |
| | | GT-III | 0.0310 ± 0.362 |
| | | GTM | 0.0220 ± 0.235 |

Mean \pm SD of 6 determinations

CONCLUSION:

The developed HPTLC technique is precise, accurate, and robust for the determination of ellagic acid in Glucomap tablet. Statistical analysis proves that the method is reproducible for the analysis of ellagic acid. The content of ellagic acid in marketed polyherbal formulation is comparable to laboratory formulations. Therefore, this method can be successfully used for the routine analysis of ellagic acid for standardization and quality control of pharmaceutical products containing ellagic acid as an ingredient.

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