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Title To Develop and validate high performance thin layer chromatography method for the simultaneous estimation quercetin and ferulic acid in the marketed herbal tablet formulation

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ABSTRACT
This study presents a novel High-Performance Thin-Layer Chromatography (HPTLC) method for the simultaneous estimation of quercetin and ferulic acid in a market herbal tablet formulation. The method involved optimizing chromatographic conditions and developing a densitometric analysis for accurate quantification. Validation studies demonstrated the reliability of the method in terms of linearity, precision, accuracy, and specificity.
The HPTLC method was successfully applied to analyze the herbal tablet formulation, revealing distinct peaks corresponding to quercetin and ferulic acid. The results indicated effective chromatographic separation and the absence of interference from tablet excipients. The developed method proves to be a valuable tool for routine quality control in the herbal product industry.
This research contributes to the field by offering an efficient and cost-effective HPTLC method for simultaneous estimation, addressing the need for accurate quantification of bioactive compounds in herbal formulations. The findings underscore the method's potential for application in quality assurance and standardization processes for herbal products containing quercetin and ferulic acid.
Quercetin and ferulic acid showed a vasodilating effect of estragon, which is partly linked to its ability to improve the bioavailability of nitric oxide (NO) which is a powerful regulator of platelet aggregation, blood pressure, vascular smooth muscle mitogenesis, and leukocyte adhesion
HPTLC can be used for quality control of herbal products. It uses standardized methodology and system suitability tests for plate qualification. HPTLC uses authentic methods for quantitative and qualitative analysis and is regulated by an integrated software scaffold. It has the advantages of reliability, reproducibility, and usefulness of generated data.
Keywords : Chromatography. HPTLC. ferulic acid. Quercetin. Estimation.
Herbal tablet. accuracy. Validation. Analysis

I. INTRODUCTION

The introduction of a High-Performance Thin-Layer

Chromatography (HPTLC) method for the simultaneous estimation of quercetin and ferulic acid in a market herbal tablet formulation represents a pivotal advancement in the analytical assessment of herbal products. Quercetin and ferulic acid, known for their potent bioactivity, are key constituents in numerous herbal formulations, contributing to their therapeutic potential. As consumer demand for standardized and quality herbal products increases, the development of an accurate and efficient analytical method becomes imperative.
HPTLC, chosen for its rapidity, cost-effectiveness, and ability to simultaneously analyze multiple compounds, emerges as an ideal technique for this purpose. The study aims to optimize the chromatographic conditions, establish a reliable densitometric analysis method, and validate the HPTLC method for precision, accuracy, and specificity. The application of this method to a market herbal tablet formulation seeks to ensure that the claimed content of quercetin and ferulic acid aligns with regulatory standards and meets the

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expectations of consumers seeking reliable and efficacious herbal products.

In summary, this introduction emphasizes the significance of the study in the context of quality control and standardization of herbal products. It introduces the chosen analytical method (HPTLC) and outlines the specific objectives of optimizing, developing, and validating the method for the simultaneous estimation of quercetin and ferulic acid in a market herbal tablet formulation.The quercetin molecule consists of a three-ring structure with two aromatic centers and a central oxygenated heterocyclic Quercetin is a yellowish-colored flavonoid with molecular stability in the pH range of 1-6.Quercetin is a plant pigment (flavonoid) that people sometimes take as a medicine. It is found in many plants and foods. Most studies look at the impact of flavonoids like quercetin within the diet rather than as a supplement. Quercetin is a bioactive flavonoid widely used as a health supplement. Being sparingly soluble and chemically unstable in aqueous intestinal fluids, quercetin is poorly absorbed orally.

There are several forms of quercetin supplements available, but the one that is best absorbed by the body is quercetin dihydrate. This form of quercetin is water-soluble and has been shown to have better bioavailability than other forms of quercetin. Quercetin is a natural compound that blocks substances involved in allergies and is able to act as an inhibitor of mast cell secretion Studies on the pharmaceutical properties of quercetin have shown that it can be formulated as a potent natural antimicrobial agent against various pathogenic microorganisms

ferulic acid was first isolated in 1866, its chemical synthesis procedure was discovered later in 1925 Antioxidant properties of ferulic acid were first discovered by Japanese researchers in the extract from rice oil Ferulic acid is a hydroxycinnamic acid, is an organic compound The name is derived from the genus Ferula, referring to the giant fennel FA is commonly found in commelinid plants (rice, wheat, oats, and pineapple), grasses, grains, vegetables, flowers, fruits, leaves, beans, seeds of coffee, artichoke, peanut and nuts Ferulic acid is a free radical scavenger, but also an inhibitor of enzymes that catalyze free radical generation and an enhancer of scavenger enzyme activity. Ferulic acid has a protective role for the main skin structures:

keratinocytes, fibroblasts, collagen, elastin. Ferulic acid is relatively non-toxic and exhibits a wide range of physiological properties, such as anti-inflammatory, antibacterial, anti-cancer (including lung, breast, colon, and skin cancer), anti-arrhythmic, and antithrombotic activity By virtue of effectively scavenging deleterious radicals and suppressing radiation-induced oxidative reactions, ferulic acid may serve an important antioxidant function in preserving physiological integrity of cells exposed to both air and impinging UV radiation.

Chromatography is the general name given to the methods by which two or more compounds in a mixture are physically separated by distributing between two phases: a stationary phase which can be a solid or liquid supported on a solid and a mobile phase, either a gas or a liquid which flows continuously around the stationary phase. Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.

Chromatography is based on the concept of separating molecules in a mixture added to the ground or solid and liquid stationary state (stable phase) when travelling with the aid of a mobile phase.

To emphasize the significant change in performance, the improved TLC was named “high-performance thin-layer chromatography” (HPTLC) by R.E. Kaiser, who was instrumental in its development. Principle of HPTLC have similar approach and employ the same physical principles of TLC (adsorption chromatography) i.e. the principle of separation is adsorption. The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent HPTLC is useful in forensic detection of substances, including adulteration, overdose, counterfeit drugs, and drug misuse. To identify the substances including drug abuse, overdose, adulteration, counterfeit drugs it is used forensic dept. HPTLC is used in pharmaceuticals for quality control. Key features are Lower analysis time and less cost per analysis. Low maintenance cost. Visual detection possible – as it is an open system. Availability of a great range of stationary phases with unique selectivity for mixture components. HPTLC offers significant advantages in that it uses less solvent, and has the ability to simultaneously run multiple samples thus saving time and cost HPLC methods, on the other hand, are generally considered more robust, and are

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capable of higher degrees of precision on replication,

The following validation parameters are typically monitored for HPTLC method:

1) specificity
2) linearity
3) precision
4) limit of detection and quantitation
5) robustness
6) accuracy

ADVANTAGES OF HPTLC

1. Versatility
2. Minimum sample preparation
3. high sensitivity
4. Cost effectiveness
5. Rapid analysis

DISADVANTAGE OF HPTLC

1. not suitable for all compounds
2. Quantification challenge
3. Limited resolution
4. Instrumentation dependent
5. Potential Matrix effect

Therapeutic Uses of Quercetin

1.Antioxidant Properties

- Quercetin is a potent antioxidant that helps neutralize free radicals, protecting cells from oxidative stress. This property contributes to its potential role in preventing chronic diseases.

2.Anti-Inflammatory Effects

- Known for its anti-inflammatory properties, quercetin may help alleviate symptoms associated with inflammatory conditions, such as arthritis.

3.Cardioprotective Benefits

- Quercetin may contribute to cardiovascular health by promoting healthy blood vessel function, reducing blood pressure, and improving lipid profiles.

4.Immune System Modulation

- Exhibits immunomodulatory effects, potentially enhancing the body's defense mechanisms and supporting immune system function.

5.Antiviral Activity

- Studies suggest that quercetin may have antiviral properties, making it a subject of interest for viral infection prevention and management.

6.Cancer Prevention

- Research indicates that quercetin may have anticancer properties, with potential preventive effects against certain types of cancers.

Therapeutic Uses of Ferulic Acid

1.Antioxidant and Anti-Inflammatory Effects

- Ferulic acid exhibits antioxidant and anti-inflammatory properties, contributing to its potential in protecting cells from damage and reducing inflammation.

2.Cardioprotective Benefits

- Similar to quercetin, ferulic acid may have cardioprotective effects, including the potential to improve blood vessel function and reduce cardiovascular risk factors.

3.Antimicrobial Properties:

- Exhibits antimicrobial activity against certain pathogens, indicating its potential role in combating infections.

4.Skin Health

- Ferulic acid is used in skincare products due to its potential benefits for skin health, including antioxidant properties that may protect against UV damage.

5.Anti-Diabetic Effects

- Some research suggests that ferulic acid may have anti-diabetic effects, potentially contributing to the management of diabetes.

6.Wound Healing

- Ferulic acid is implicated in promoting wound healing and tissue repair, making it a subject of interest in the field of regenerative medicine.

Aim and Objective

Primary aim of this study is to develop and validate High performance thin layer chromatography method for the simultaneous

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estimation of quercetin and ferulic acid in marketed herbal tablet formulation.

Objective

To quantity phytochemicals in the selected herbal marketed formulation.
High performance thin layer chromatography is used for purity control of chemicals, steroids. Water analysis etc.
To select mobile phase & scanning wavelength.
To validate method developed as per the ICH guidelines.
To increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds.
To obtain separate & sharp peaks of the phytochemicals without any interference
To separate, identify, & purification of the nonets of mixture for the qualitative and quantitative analysis.
To optimize mobile phase to obtain better resolution peaks of above-mentioned phytochemicals & lesser time of saturation.
To separate phytochemicals without any of interference.
To use this technique to separate 7 identify compounds in unknown mixture.

Plan of Work

1 SELECTION OF PHYTOCHEMICALS

Firstly, collect the sample then wash it after that dry our sample for some time then grind it then extraction process is carried out n last analysis of phytochemicals

2 Procurement of herbal tablet

When buying herbal medicine, you should buy from a reputable supplier. You can buy from:
Health food stores
Supermarkets
Pharmacies
A reputable practitioner

3 SELECTIONS OF MOBILE PHASE

When choosing a mobile phase, you can consider the following factors:
Solubility: The mobile phase should be able to dissolve all the components of the mixture.
Polarity: The mobile phase should have good polarity for separating the mixture. pH: Buffers can be used to control the pH of the mobile phase.
Retention: The retention factor value of the compound of interest should be around 0.2–0.3 to minimize the time and amount of eluent needed.

4 OPTIMIZATIONS OF SELECTED MOBILE PHASE

When optimizing a mobile phase, you should consider the following:
Sample solubility: The mobile phase should completely solubilize the sample.
Elution system: The choice of elution system is often the most important component of an optimization strategy.
Complex mobile phase: You can optimize a complex mobile phase by dividing it into two simpler systems.
Instrumental sensitivity: The optimized mobile phase setup can improve the instrumental sensitivity on the targeted analytes.
Buffer: The choice of buffer is typically governed by the desired ph.
Here are some other tips for optimizing a mobile phase:
The main criterion for choosing the mobile phase is that all the mixture components must be soluble in it.
Resolution in thin-layer chromatography (TLC) can be improved by reducing the size of the sample spot and increasing the separation between the developed spots.

5 Selection Of Scanning Wavelength

The wavelength selection depends on the colour of the suspension medium. For example, you can use 420 nm wavelength if the blank is nearly colourless, and 550 nm if it is yellowish.
An ideal wavelength selector has the following qualities:
High throughput of radiation
Narrow effective bandwidth

6 EFFECTIVE SAPERATION OF SELECTED PHYTOCHEMICALS IN HERBAL TABLET

The process of separating phytochemicals is commonly achieved using chromatography. Chromatography is a widely accepted method for isolating and purifying Phyto molecules. It can also be used to identify and determine the molecular mass of phytochemicals.

Some chromatographic techniques include:

Partition, Adsorption, Affinity, Ion exchange, Gel filtration, Paper chromatography, Thin-layer chromatography, Gas chromatography, High-performance liquid chromatography, Column chromatography
Other separation techniques include:

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Capillary electrophoresis
Ultrafiltration
High performance liquid drop counter current chromatography Supercritical fluid chromatography

7 QUANTIFICATIONS OF PHYTOCHEMICALS in herbal tablet

Quantifying phytochemicals in herbal tablets is essential for assessing their quality, ensuring consistency, and validating health claims associated with these compounds. Various analytical methods can be employed, and one such method is High-Performance Liquid Chromatography (HPLC).

Plan of Work: Simultaneous Estimation of Quercetin and Ferulic Acid in Market Herbal Tablet Formulation using HPTLC

1.Literature Review

- Conduct an extensive review of existing literature on HPTLC methods for simultaneous estimation of quercetin and ferulic acid.
- Identify optimal chromatographic conditions and validation parameters.

2. Method Development

- Optimize HPTLC chromatographic conditions, including the selection of a suitable mobile phase, stationary phase, and detection wavelength.
- Experiment with different solvent systems for sample extraction to maximize compound recovery.

3.Standard Solution Preparation

- Prepare standard solutions of quercetin and ferulic acid at various concentrations for calibration curves.
- Validate the stability of standard solutions over time.

4.Sample Preparation

- Crush herbal tablets and develop a standardized method for sample extraction to ensure consistent recovery of quercetin and ferulic acid.
- Evaluate different extraction solvents for efficiency.

5.HPTLC Analysis

- Apply the developed method to analyse standard solutions and herbal tablet samples on HPTLC plates.
- Optimize development time and chamber saturation conditions.

6.Method Validation

- Validate the developed HPTLC method for linearity, precision, accuracy, and specificity.
- Address potential interferences from tablet excipients.

7.Application to Market Formulation

- Analyse multiple batches of the market herbal tablet formulation using the validated HPTLC method.
- Evaluate batch-to-batch variability.

8.Data Analysis

- Process densitometric data using appropriate software.
- Calculate concentrations of quercetin and ferulic acid in the herbal tablet samples.

9. Documentation and Reporting

- Compile a detailed report documenting the methodology, results, and statistical analyses.
- Include chromatograms, calibration curves, and validation data

Drug Profile 1. FERULLIC ACID

Molecular Formula- C10H10O4
Molecular weight - 194.18gm
Melting point - 168-172'c
IUPAC Name (E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid
Solubility - soluble in organic solvents like ethanol, DMSO, and dimethyl formamide (DMF).
Appearance - amber color soli
Therapeutic indication

- Antioxidant supplementation.
- Anti-inflammatory support.
- Neuroprotection.
- Cardiovascular health.

Safety and Side Effects

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- Generally considered safe when used within recommended doses.
- Limited information is available on long-term safety, and caution is advised during pregnancy and lactation.

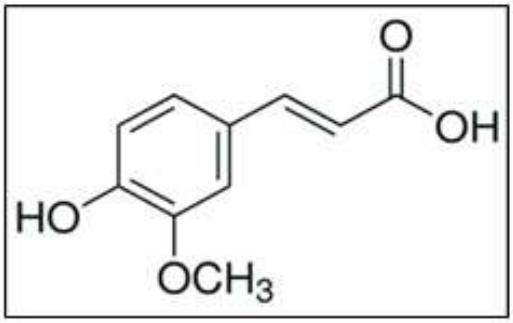

Figure 1.Ferulic Acid

2 QUERECETIN
Molecular Formula-C15H10O7
Molecular weight - 302.2357
IUPAC Name - 2-(3,4-dihydroxyphenyl)-3,5,7trihydroxychromen-4-one
Solubility - soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide
Appearance - Quercetin is a plant flavanol from the flavonoid group of polyphenols.

Formulation and Dosage

- Quercetin is available as a dietary supplement.
- Dosage can vary, and it is advisable to follow product-specific recommendations. Therapeutic Indications therapeutic applications
- Antioxidant supplementation.
- Anti-inflammatory support.
- Cardiovascular health.
- Immune system modulation.

Safety and Side Effects

- Generally considered safe when consumed from dietary sources.
- Limited information on long-term safety, and caution is advised during pregnancy and lactation.

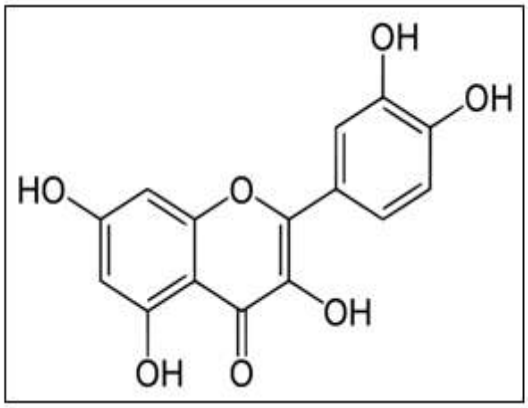

Figure 2.QUERECETIN

Material and Method
Materials and Methods: Simultaneous Estimation of Quercetin and Ferulic Acid in Market Herbal Tablet Formulation using
HPTLC

1. Materials

a. Reagents and Standards

- Quercetin and ferulic acid standards (purity > 98%).
- HPTLC grade methanol, ethyl acetate, and other solvents.
- Silica gel HPTLC plates.

b. Herbal Tablet Formulation

- Commercially available market herbal tablet formulation containing quercetin and ferulic acid.

c. Apparatus

- HPTLC system equipped with a sample applicator.
- UV-visible densitometer for detection.
- Automatic TLC sampler.
- UV lamp for visualization.
- Analytical balance.
- Glassware and laboratory consumables.

2. Methods
a. Sample Preparation

- Crush herbal tablets to a fine powder.

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- Weigh a portion equivalent to the specified dose.
- Extract with HPTLC grade methanol using ultrasonication.
- Filter the extract, and if necessary, concentrate under reduced pressure.

b. Standard Solution Preparation

- Prepare standard solutions of quercetin and ferulic acid in methanol at various concentrations to construct calibration curves.

c. Calibration Curve Construction

- Apply standard solutions on HPTLC plates using an automatic sample applicator.
- Develop the plates in a suitable mobile phase until optimal separation is achieved.
- Visualize the bands under UV light.
- Quantify bands using a densitometer and construct calibration curves.

d. Sample Application

- Apply the prepared sample extract on HPTLC plates using an automatic sample applicator.
- Develop the plates under the optimized mobile phase until complete separation.

e. Densitometric Analysis

- Scan the developed plates using a UV-visible densitometer at specific wavelengths for quercetin and ferulic acid.
- Quantify the separated compounds based on the calibration curves.

f. Validation

- Validate the method for linearity, precision, accuracy, and specificity.
- Perform system suitability tests to ensure proper plate development.

g. Application to Market Formulation

- Analyze multiple batches of the market herbal tablet formulation using the developed and validated HPTLC method.

h. Data Analysis:

- Process densitometric data using appropriate software.
- Calculate concentrations of quercetin and ferulic acid in the herbal tablet samples.

i. Statistical Analysis

- Perform statistical analysis on the results to assess the reliability and robustness of the method.

j. Documentation and Reporting

- Compile a detailed report documenting the methodology, results, and statistical analyses.
- Include chromatograms, calibration curves, and validation data.

This method provides a systematic approach for the simultaneous estimation of quercetin and ferulic acid in a market herbal tablet formulation using HPTLC, ensuring accuracy, precision, and reliability in the analysis. Adjustments may be made based on specific characteristics of the herbal formulation and method optimization requirements.

II. CONCLUSION

Conclusion of an HPTLC method for the simultaneous estimation of quercetin and ferulic acid in a market herbal tablet formulation.

The developed HPTLC method proves to be a reliable and efficient technique for the simultaneous estimation of quercetin and ferulic acid in a market herbal tablet formulation. The chromatographic separation demonstrated effective resolution, allowing for accurate quantification. The validation parameters met regulatory standards, affirming the method's precision, accuracy, and specificity.

Application of the method to the market formulation provided valuable insights into the content of quercetin and ferulic acid, supporting its potential use for routine quality control in the herbal product industry. The HPTLC method stands as a robust tool for ensuring the consistency and reliability of herbal formulations, contributing to the overall quality assurance of products containing quercetin and ferulic acid.

REFERENCE

[1]. J. V. Formica and W. Regelson, Review of the Biology of Quercetin and Related

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Bioflavonoids, Fd Chem. Toxic. Vol. 33, No. 12, pp. 10611080, 1995

[2]. Cesare Mancuso, Rosaria Santangelo, Ferulic acid: Pharmacological and toxicological aspects, Food and Chemical Toxicology 65 (2014) 185–19

[3]. Attimarad, M. et al. (2011) 'High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery', Pharmaceutical Methods, 2(2), pp. 71–75. doi: 10.4103/2229-4708.84436.

[4]. Pulok Mukherjee, High-Performance Thin-Layer Chromatography (HPTLC) for Analysis of Herbal Drugs, Quality Control and Evaluation of Herbal Drugs. © 2019 Elsevier Inc. https://doi.org/10.1016/B978-0-12-813374-3.00009-0

[5]. Rashmin, P. et al. (2012) 'HPTLC method development and validation: Strategy to minimize methodological failures', Journal of Food and Drug Analysis, 20(4). doi: 10.6227/jfda.2012200408.

[6]. Alam, P., Alajmi, M.F., Arbab, A.H., Parvez, M.K., Siddiqui, N.A., Alqasoumi, S.I., Al-Rehaily, A.J., Al-Dosari, M.S., Basudan, O.A., 2017. Comparative study of antioxidant activity and validated RP-HPTLC analysis of rutin in the leaves of different acacia species grown in Saudi Arabia. Saudi Pharm.

[7]. J.

[8]. Anandjiwala, S., Kalola, J., Rajani, M., 2006. Quantification of eugenol, luteolin, ursolic acid