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"Analytical Method Development and Validation of Formulated Dosage Form"

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ABSTRACT:
Guava is a large meal crop additionally as restorative plant found tropical and subtropical areas. Psidium guajava linn. is an family from Myrtaceae family, which incorporates 133 genera and extra of 3,800 plant species. Guava is rich most of phenols, triterpenes, flavonoids, oils, saponins, lectins, vitamin-c and fiber. The leaf of guava is attain supply of flavonoids mainly quercetin, kamiferol, luteol. The flavonoids have pharmacological interest like antibacterial, antioxidant, hepatoprotective. A pattern reproducible and specific HPTLC analytical technique development & validation for estimation of antiulcer agent. The chromatographic parameters become completed on TLC aluminium plate precoated with silica gel 60 F254 through the use of toluene: ethyl acetate: methanol as mobile phase. Detection Wavelength 254 &366nm. Saturation time become 20 min and Band width 6mm in HPTLC system. The evolved technique became confirmed as per ICH guideline. Validation parameters like linearity, accuracy, precision, specificity & robustness turned into executed to validate the evolved technique. Quercetin turned into observed to be liner withinside the variety of 40-200ng/band. Recovery completed by using 3 concentration, recovery of the samples must be 104%. The LOD and LOQ was observed to be 9.56ng/band and 28.97ng/band respectively. All validation parameters have been located to in the best limit. This approach may be utilized in determination of quercetin through HPTLC approach.

Index terms - Psidium guajava, Flavonids, Quercetin, HPTLC , Method Development .

I. INTRODUCTION

Analytical chemistry is the analysis of separation, quantification and chemical additives identification of herbal and synthetic materials constituted with one or more compounds or factors. Analytical method development is defined as when there are no definitive techniques are present, new methodologies are being progressed for evaluation of the novel product. Method validation is a "technique of organising documented evidence" that gives a high level of assure that the product (equipment) will meet the necessities of the preferred analytical applications. (1)

Guava is a small tropical evergreen tree. It belongs to the phylum Magnoliophyta, class Magnoliopsida, and family Myrtaceae including approximately 133genera and about 3800 species.(2) Psidium guajava is specifically found in SouthAmerica, South Africa, West Africa, South East Asia, South India, Sri Lanka, Hawaii, Bangladesh, Pakistan, and Egypt, etc. This plant has now been grown by many other countries having a tropical and sub-tropical climate. Psidium guajava is used as a nutritional food worldwide. (3) Guava is wealthy in tannins, phenols, triterpenes, ß flavonoids, simple oils, saponins, carotenoids, lectins, nutrients and unsaturated fats. Psidium guajava includes various phytochemical Protein, minerals, polysaccharides, alkaloids, vitamins, steroids, essential oils, glycosides, minerals flavonoids, enzymes, tannins, sesquiterpenoid alcohols, saponins, and triterpenoid acids, glycoalkaloids, and carotenoids alkenyl phenols etc. Flavonoid is naturally occurring group of compounds contain quercetin which exhibit the various pharmacological activity, chemically it is flavones nucleous containing two benzene rings fused together with heterocyclic pyrone ring. (2)

The physical properties of Quercetin is a yellow, crystalline solid with a bitter taste powder, insoluble in water, slightly soluble in alcohol, soluble in glacial acetic acid and aqueous alkaline solution

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Quercetin(3,3′,4′,5,7‑ pentahydroxyflavone) a common flavonol, is present as a glycoside in high concentration in fruits and vegetables such as apples, berries, onions, and capers. Qu accounts for nearly 50% of the dietary intake of all flavonols, with a daily intake of about 10–20 mg/day.

II. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

It is a sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits. It is also known as High Pressure Thin Layer Chromatography/Planar chromatography or Flat-bed chromatography.

2.1 Principle:[4]

It is an analytical method suitable for qualitative and quantitative method. Separation due to adsorption or partition or by both phenomena, depending upon the nature of adsorbents used on plate. The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster.

2.2 Steps concerned in HPTLC and Instrumentation: [6]

- Selection of stationary phase
- Sample & standard preparation
- Activation of precoated plate
- Application of sample
- Selection of mobile phase
- Chromatographic development
- Detection
- Quantification
- Documentation

2.3 Application of HPTLC:[10,11]

1. Presence of impurities in the drug.
2. Stability testing and forced degradation studies.
3. Phytoconstituents in plant extract.
4. Phytochemical analysis of volatile oils.
5. Forensic applications
6. Cosmetic analysis
7. Food analysis
8. Environmental analysis
9. Industrial application

III. MATERIALS AND METHODS: [7,8]

3.1 Standards and chemicals

All chemicals and reagents were of AR grade. Ethanol and aluminium‑ backed TLC plates precoated with 0.2 mm layer of silica gel 60 F254 (20 cm × 10 cm) were purchased from E. Merck (Germany). All standard drugs quercetin was purchased from Yucca enterprise.

3.2 Plant collection and identification

The fresh plant materials of Psidium guajava were collected from local area (Panvel, Raigad district). Fresh plant leaves were washed under tap water and shade drying was carried out. Prepare mixture of powder & pass through sieve & used for the experimental purpose. The other chemical & excipients of analytical grade was used.

3.3 Sample preparation

The leaves of guava were washed under running tap water to remove dust particle and shade dried at room temperature for 3-4weeks. The dried plant parts were reduced to coarse powder with a mechanical grinder and passed through a sieve. Approximately 50 g of guava powder was loaded in a paper thimble in a glass container and extraction was carried out using 500 ml of ethanol under continuous heating and reflux. The procedure was monitored until the colourless liquid of the extractions faded after 5-6 days.

3.4 Standard preparation

A stock solution (1 mg/mL) of standard quercetin was prepared by dissolving 10 mg of accurately weighed drug in ethanol diluted to 10 mL in the standard volumetric flask. Working solution was prepared by mixing each stock solution working solution was filtered through a 0.45 µm membrane filter before application on a TLC plate.

3.5 Instrumentation and conditions

The sample and standards were separately spotted as bands of width 8 mm with Camag microliter syringe on precoated silica gel aluminium plate 60 F254 (10 cm ×10 cm with 0.2 mm thickness), using a Camag Linomat applicator . The enter commands regarding slit dimension (6mm × 0.45 mm) and scanning speed (20 mm/s) were described using server LABSERVER v (3.1.21109.3) software. The mobile phase consisted of toluene: ethyl acetate: methanol (4:4:1 v/v/v). The plates were developed up to 70 % of total TLC plate height in a horizontal Camag twin trough glass chamber (10 cm × 10 cm)

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which was saturated with the mobile phase (5 mL in each side) for 30 min at RT and relative humidity 60%. The TLC plate was dried in with the help of an air dryer. The densitometric scanning was carried out using Camag TLC scanner of IV in the absorbance mode at 254 & 366 nm using deuterium lamp source

IV. VALIDATION OF THE METHOD :[9]

The proposed method was validated as per ICH guidelines.

4.1 Specificity.

The number, colour, intensity, and function of the zones in the HPTLC fingerprint chromatogram was evaluated for the specifity. A method will be known as specific if the sample tested from different location growth gives comparable fingerprint profile, and the reference marker is also detected. The developed method gives comparable pattern in the HPTLC chromatogram of guava leaves from different location growth only differ in the intensity of the detected bands, but all of the samples contained quercetin reference marker.

4.2 Linearity

standard stock solution (1000 µg/ml) of quercetin solution was prepared containing 100 µg/ml of quercetin. This solution was further used for spotting. Six replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analysing six concentrations over the concentration range 40-200 ng/band to obtain calibration curve. The results found to be linear with regression equation of y = 4E – 05x +0.003 and ^{R2} =0.9914.

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

Formula for calculation of LOD & LOQ is

LOD = 3.3σ / S

LOQ = 10σ / S

Where, σ = Standard deviation of Y intercept

S= Average of slop of the calibration curve

4.4 Robustness

By introducing small changes in the mobile phase composition, mobile phase volume and duration of mobile phase saturation, the effects on the results were examined and the % R.S.D peak area was calculated.

4.5 Recovery

The accuracy of the method was decided by calculating recovery of the standard drugs (Qu) using the standard addition method. A recognized amount of standard solutions (Qu)were introduced in analyzed samples and estimated.

4.6 Reproducibility

The precision was determined by repeatability of sample application & measurement of peak areas for six replicates of each of three concentration of quercetin under prescribed situation on same day, interday & intraday method. prescion was calculated & expressed in %RSD.

V. RESULTS & DISCUSSION

5.1 Method Development

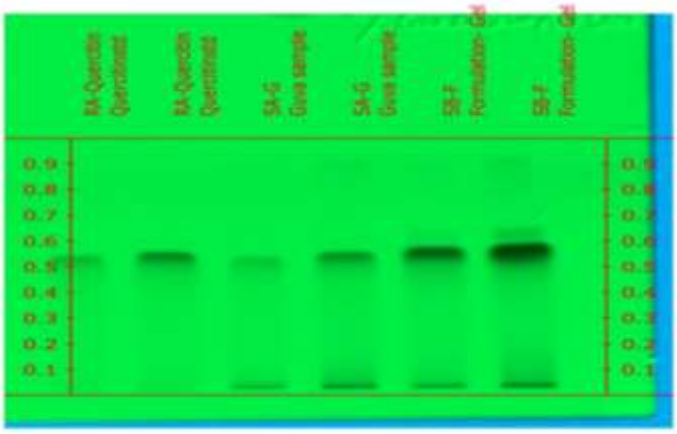

Fig 1:HPTLC of **Psidium guajava** at 254 nm

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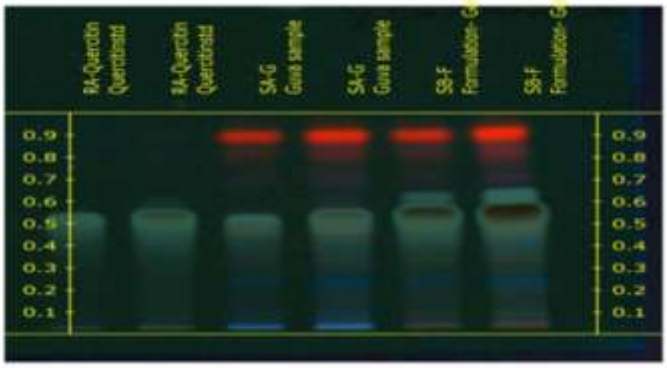

Fig 2:HPTLC of **Psidium guajava** at 366nm

5.2 Validation Parameters :

5.2.1 Specificity:

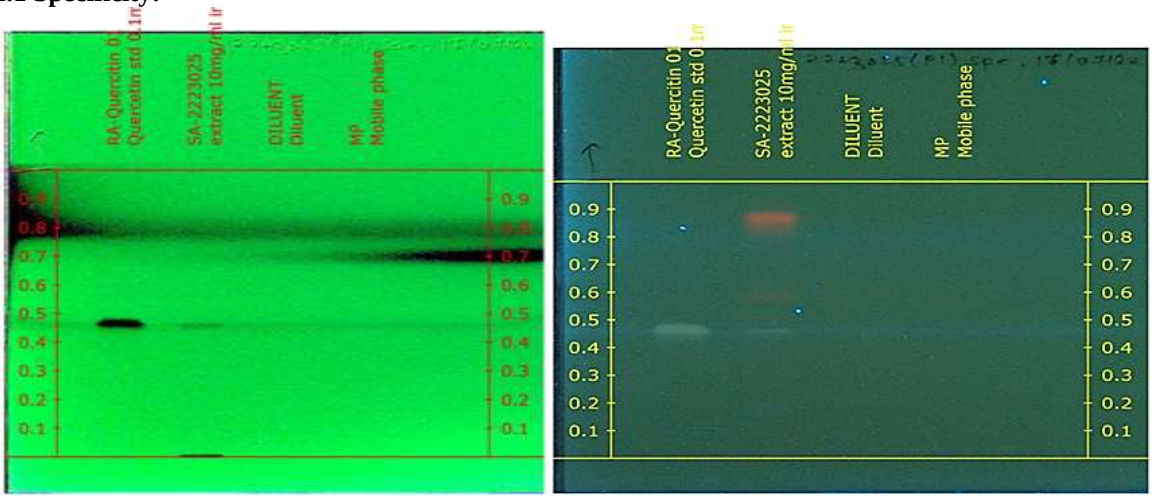

Fig No 3: Developed TLC plate image captured in TLC visualizer at 254 & 366nm for specificity

5.2.2. Linearity :

Table 1 : Linearity data of quercetin

Sr No	QUERCETIN	
	Concentration (ng /band)	Peak area response
1	0	0
2	40	0.00195
3	80	0.00402
4	120	0.00549
5	160	0.00694
6	200	0.00825
Correlation coefficient	0.9914	
Y intercept	4E-05x+0.003	

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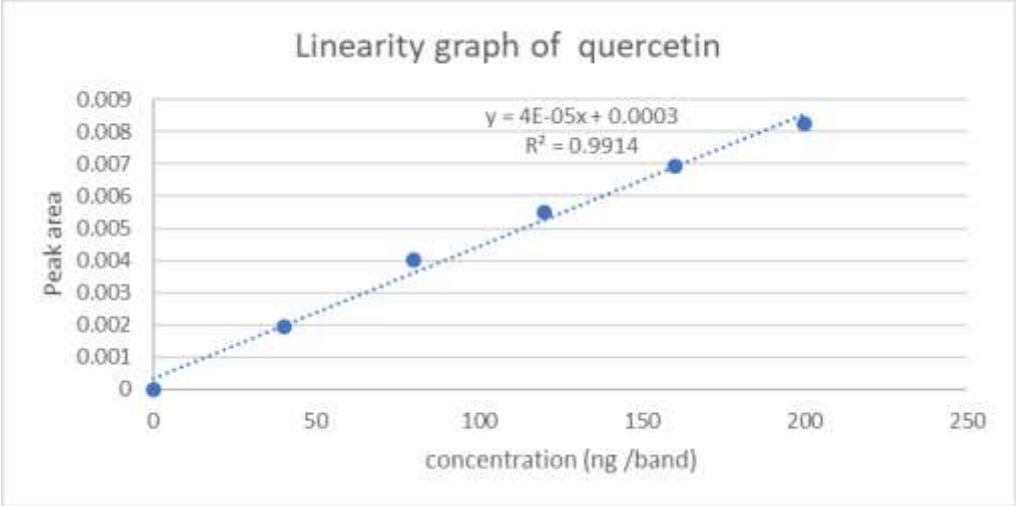

Fig 4 :Linearity graph of quercetin

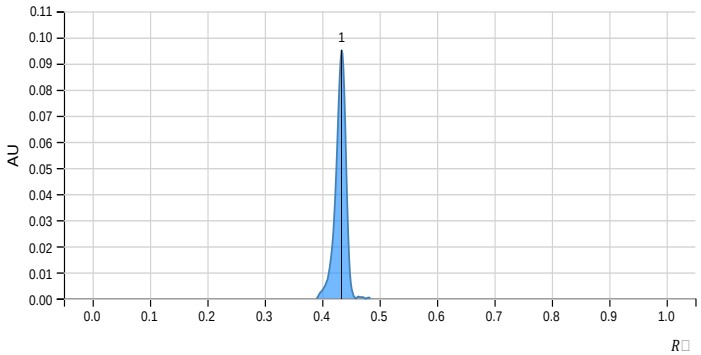

Fig 5: Chromatogram of 40 ng/band

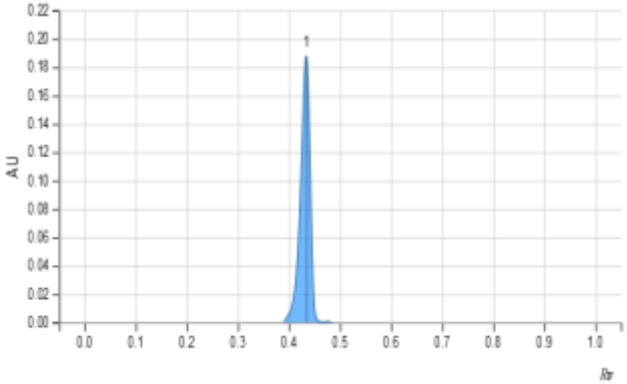

Fig 6: Chromatogram of 80 ng /band

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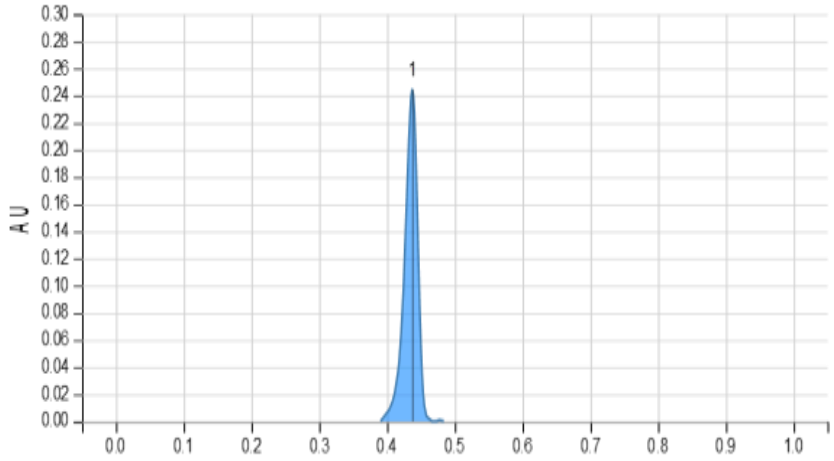

Fig No 7: Chromatogram of 120 ng/band

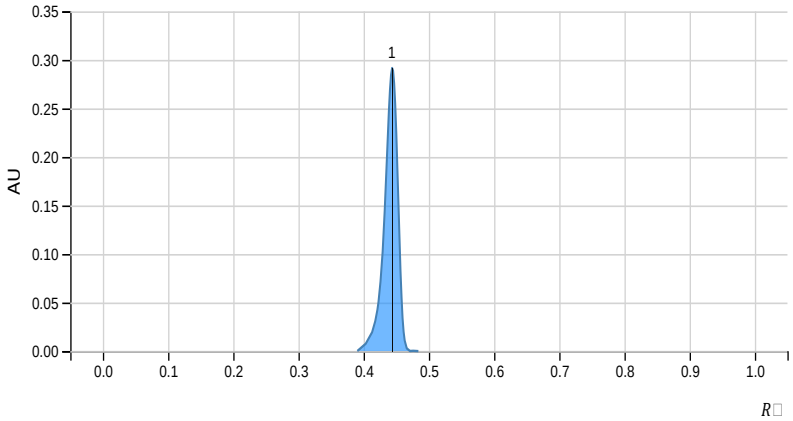

Fig No 8: Chromatogram of 160 ng/band

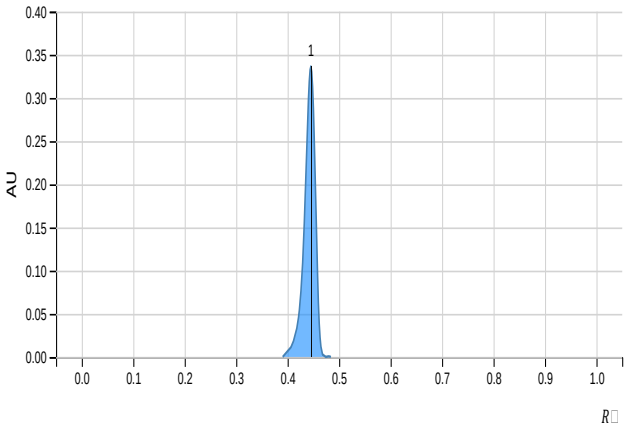

Fig No 9 : Chromatogram of 200 ng/band

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5.23 LOD &LOQ:

Table No 2 :LOD & LOQ data

LOD	LOQ
9.56 ng /band	28.97 ng /band

5.2.4. Robustness:

Table No 3:Robustness data

Parameter	S. D. of peak area	% RSD
Mobile phase composition (toluene: ethyl acetate: methanol)	1.27	0.20
Mobile phase volume (18, 20 and 22 mL)	1.24	0.19
Duration of saturation (20, 30 and 40 min)	1.18	0.18

5.Reproducibility:

Table 4 : Reproducibility data

Sr No	Interday prescion	Intraday prescion
1	0.009	0.00956
2	0.00898	0.00948
3	0.00889	0.00938
4	0.0088	0.00929
5	0.0087	0.00922
6	0.00863	0.00912
Average	0.008833	0.009342
Standard deviation	0.00015	0.000164
%RSD	0.015016	0.016425

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