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Simultaneous Estimation of Berberine and Quercetin in Pathydi Kada Formulation by Hptlc

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Abstract

A sensitive, selective and precise thin-layer chromatographic method has been developed and validated for the analysis of Berberine and Quercetin in Pathydi Kada laboratory prepared and Market formulation. Separation and quantification were achieved by TLC using mobile phase of Ethyl acetate: Methanol: Glacial acetic acid (6: 4: 0.5) v/v/v (Rf 0.2 & 0.84 for Berberine and Quercetin respectively) on precoated silica gel 60F254 aluminum plates and determination was carried out at 254 and 366 nm for berberine and quercetin respectively. The calibration curve was linear in the concentration range of 4-12 µg spot-1. The method was validated for precision, repeatability and accuracy. The proposed method was found to be simple, precise, specific, sensitive and accurate for the quantification of Berberine and Quercetin. This is the first TLC report for the simultaneous estimation of Berberine and Quercetin in Pathydi Kada formulation and may be useful for the routine quality control.

Keywords: *Pathydi Kada, Berberine, Quercetin*

1. Introduction

Pathyadi decoction is mentioned in Sharangdhara Samhita, especially in the management of Shiroroga. Pathyadi kada, the poly-herbal Ayurvedic decoction containing *Terminalia chebula (fruit), erminalia bellirica (fruit), Embelica officinalis (fruit), Andrographis paniculata (whole plant), Curcuma longa (rhizome), Azadiracta indica (stem bark) and Tinospora cardifolia (stem). This polyherbal preparation is* extremely effective for all types of headaches. Pathyadi kada also reduces the intensity and frequency of migraine attacks.

Apart from headache, Pathyadi kada is found to be beneficial for Earache, Toothache and Night blindness, Eye strain or other eye disorders, Inflammations related to ear and eyes, Sinusitis.

Stabbing pain in the cheek, lips, gums, or chin or one side of the face and effectively reverses acute dilation of blood vessels and aids in maintaining blood pressure.

Considering wide therapeutic applications of Pathyadi kada, and presence of the marker constituents, to ensure identity and quality of Pathyadi kada a simple, sensitive, specific and reproducible HPTLC method is developed for the quantification of Berberine and Quercetin markers in the Pathyadi kada formulation.

2. Materials And Methods

The selected formulation of Pathyadi kada was procured from the Ayurvedic Pharmacy in the local Market from Pune, the crude drugs required for the preparation of lab formulation were procured from Soham Ayurved Rasashala, Solapur, Maharashtra.

Solvents and chemicals:

Standard Berberine and Quercetin markers were procured from Yucca enterprises. All chemicals and reagents used were of analytical grade and purchased from Rankem and S. D. Fine chemicals, India. Silica gel 60F HPTLC pre-coated plates were purchased from Merck.

Preparation of standard solution:

A stock solution of Berberine and Quercetin (1 mg/ml) were prepared by dissolving 10 mg of accurately weighed Berberine and Quercetin in methanol and making up the volume to 10 ml with methanol. This concentration was used as the working standard for the HPTLC method.

Preparation of sample solution:

100mg extracted with 100 ml of acetone for 1 hr on sonicator with heat. the extract was filtered and allowed to dry completely. The residue was weighed to 50 mg and dissolved in 10 ml of acetone the spotting volume was 50µl. Thus, the spotting concentration was 250µg.

HPTLC Instrumentation and Method Development:

The stationary phase used was precoated silica gel aluminium plate 60F254 (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat V (Switzerland) on to which the test solutions were spotted in the form of bands of width 6 mm with a Camag microlitre syringe. The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The slit dimension was kept at 5mm × 0.45 mm, bandwidth was set at 20 nm, each track and 10 mm/s scanning speed was employed.

The composition of the mobile phase was Ethyl acetate:Methanol:Glacial acetic acid (6: 4: 0.5) v/v/v for Berberine and Quercetin was employed. The linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 minutes at room temperature (25 ± 2°C). The length of the chromatogram run was 80 mm. Subsequently, the plate was allowed to dry at room temperature. The separated bands on the HPTLC plates were scanned over the wavelength of 200 – 400 nm. The source of radiation utilized was the D2 lamp and fluorescent lamp for the detection and quantification was carried at 254 and 366 nm for berberine and quercetin respectively.

Method Validation:

The validation of the developed HPTLC method was carried out according to ICH guidelines.

Linearity:

The linearity was analyzed for different concentration ranging from 1- 100 µg/spot were spotted. The data of the peak areas plotted against the corresponding concentrations were treated by least-square regression analysis.

LOD and LOQ were determined by using standard deviation method. Detection limit =3.3σ /S and quantification limit=10 σ /S where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve.

Precision studies:

Precision of the method was evaluated by repeatability (intra-day) and reproducibility (inter-day).

The triplicates of three different concentrations of standard mixture solution were spotted and analyzed on same day for intra-day study and two different days for inter-day study with respective chromatographic conditions.

Accuracy studies:

Recovery study method was employed to evaluate accuracy of the method. The samples were spiked with 80, 100 and 120 % of median concentrations of standards.

Accuracy was calculated from the following equation: [(spiked concentration − mean concentration)/spiked concentration] × 100.

Robustness:

For the determination of the robustness of method, chromatographic parameters, such as mobile phase composition and detection wavelength and saturation time were intentionally varied to determine their influence on the retention factor and quantitative analysis. The mobile phase composition was altered by ± 2 % changes in the composition of methanol. The chamber saturation time was altered from 15

min to 30 min. The method was analyzed using two altered wavelengths; 364 nm and 368 nm for berberine and 252 and 256 for quercetin respectively.

3. Results and Discussion

Method optimization:

The proposed method gave very good separation and resolution of the standard Berberine and Quercetin.

Method validation:

1. Linearity, limit of detection and quanfication

Under the above-described experimental conditions, linear correlation between the peak area and applied concentration was found to occur in the concentration range 2-12 µg/ spot for Berberine and Quercetin**.**

2. Precision studies:

Precision data on repeatability (intra-day) and instrumental variation was obtained for Berberine and Quercetin at three different concentration levels. Precision studies showed R.S.D. less than 2%, indicating a good precision.

3. Accuracy:

The sample containing 4µg Berberine and Quercetin was spiked with the known amount of standards, and the percent ratios between the recovered and expected concentrations were calculated. Recoveries were obtained in the range of 80-120%, depicting the HPTLC proposed method for estimation is accurate for the quantification of Berberine and Quercetin.

4. Robustness:

No changes were observed in retention time and peak shape of both the standards with the changes made with mobile phase and chamber saturation time. The resolution and the separation of markers were also unaltered.

Analysis of TGMF and TGLF formulations:

The developed method was able to be providing a well resolved chromatogram with no alterations in peaks of Berberine and Quercetin.

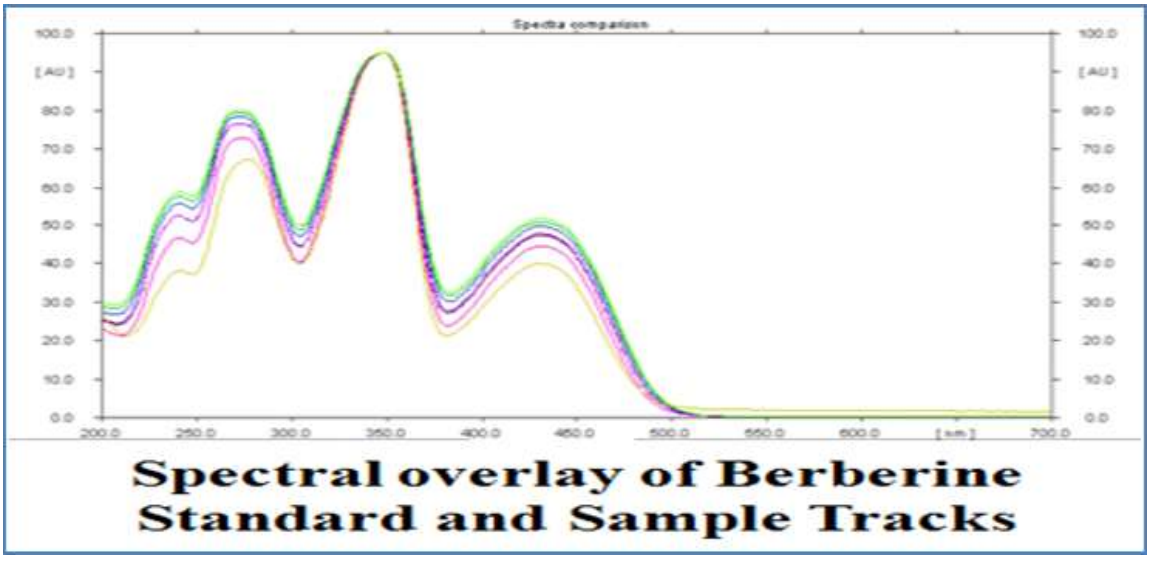

Figure 1: Spectral Overlay of Berberine Standard and Sample Tracks

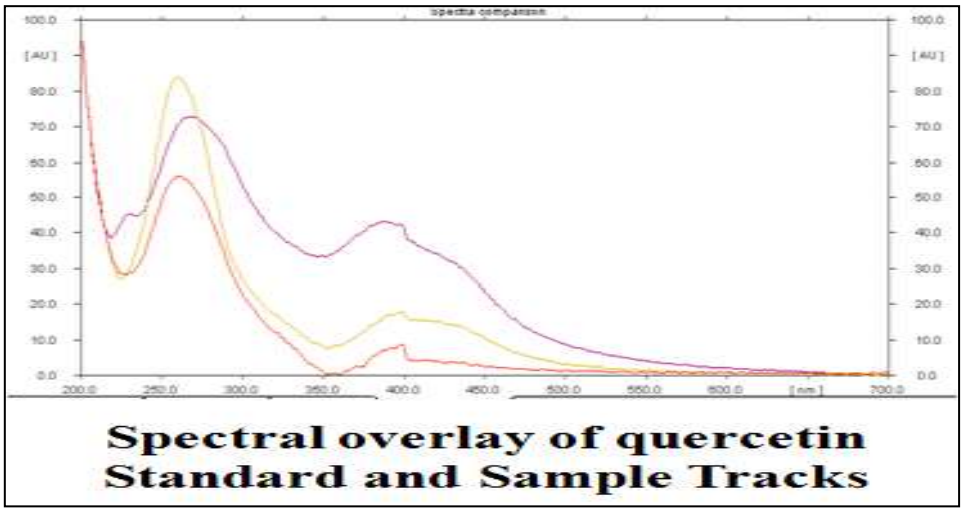

Figure 2: Spectral Overlay of Quercetin Standard and Sample Tracks

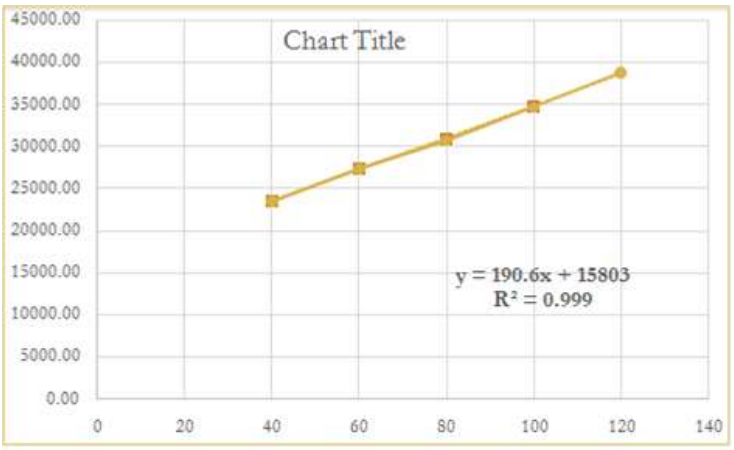

Figure 3: Calibration Plot of Berberine

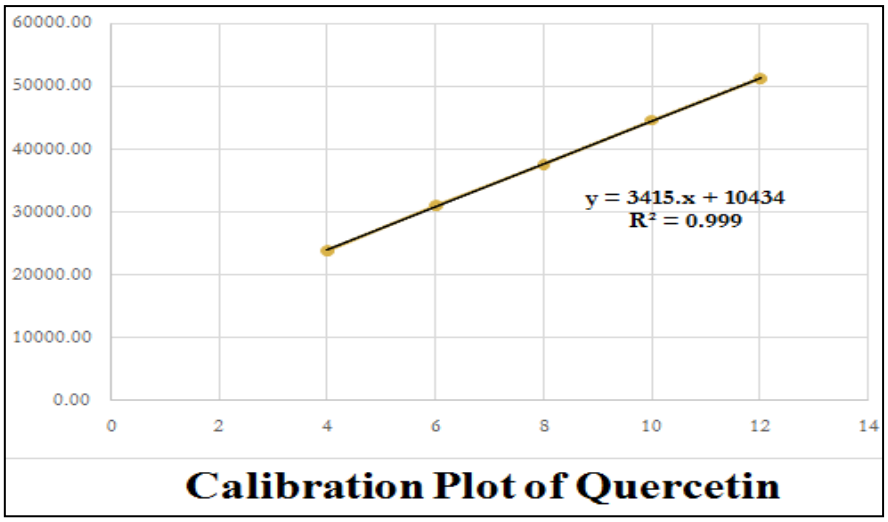

Figure 4: Calibration Plot of Quercetin

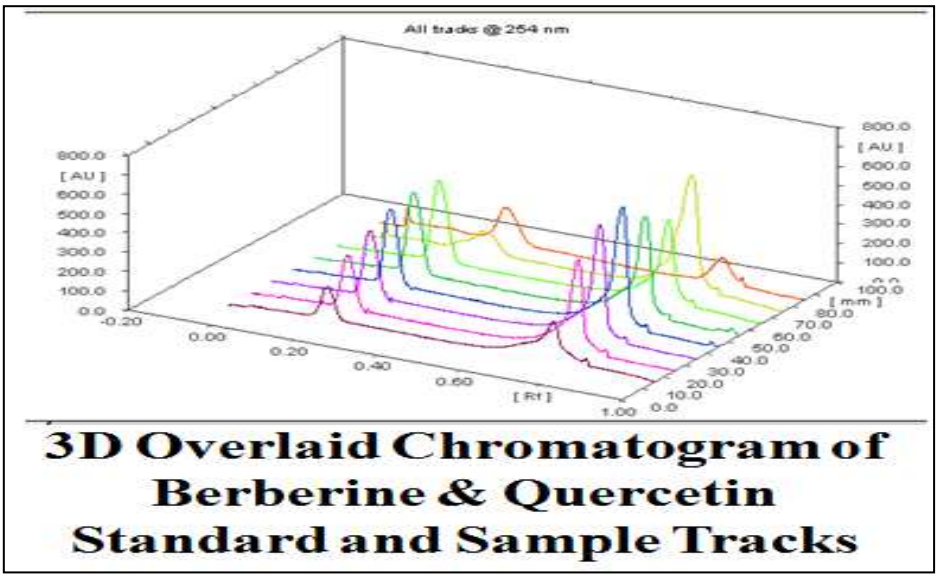

Figure 5: 3D Overlaid Chromatogram of Standard and Sample Tracks

a) Linearity regression Data of Berberine and Quercetin

Sl No.	Parameter	Results	
		Berberine	Quercetin
1	R_F	0.2	0.84
2	Dynamic range (µg spot^{-1})	1-15µg/µl	10-100µg/µl
3	Equation	y=3415.x + 10434	Y=190.6x +15803
4	Slope	3415	190.6
5	Intercept	10434	15803
6	Limit of Detection	0.448004	7.230246
7	Limit of Quantification	1.357589	21.90984
8	Linearity (Correlation coefficient)	0.999	0.999
9	Specificity	Specific	Specific

b) Precision studies data of PKLF and PKMF

Instrumental Precision (% RSD)	Concentration Conc (µg /spot)	Method Precision (% RSD)	Concentration Conc ((µg/spot)	Method Precision(% RSD)
	Berberine		Quercetin	
Intra day	6	1.21	60	0.99
	8	1.56	80	1.69
	10	1.11	100	1.39
Inter day	6	0.81	60	0.61
	8	0.17	80	0.60
	10	0.48	100	0.64

c) Recovery studies of Berberine

Sl No.	Amount of Berberine present in the sample(µg)	Amount of Berberine added (µg)	Amount of Berberine found (µg)	Recovery (%)
1	8	6.4	14.28	98.18
2	8	8	16.64	107.95
3	8	9.6	18.14	101.58

d) Recovery studies of Quercetin

Sl No.	Amount of Quercetin present in the sample(µg)	Amount of Quercetin added (µg)	Amount of Quercetin found (µg)	Recovery (%)
1	80	64	144.75	100.52
2	80	80	161.43	100.8

3	80	96	177.79	101.01

4. Conclusion

The TLC method developed here for the quantification of Berberine and quercetin in Pathyadi kada is simple, rapid, cost-effective and easily adaptable for the screening and quantitative determination.

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References:

1. Gopinathan G and Dhiman KS, Triphala in Eye Diseases: A Critical Review, J Homeop Ayurv Med 2013, 2:2 pg. 1-3.
2. Harding J J, Cataract: Biochemistry, Epidemiology and Pharmacology; 1st Ed.; Chapman & Hall: London, 1991, 83-124.
3. Tillotson A, Khalsa K and Caldecott T, Canadian Journal of Herbalism, 2001, 22, 216-44
4. Sabu M C and Kuttan R, J Ethnopharmacology, 2002, 81(2), 155-160.
5. Naik G H, Priyadarsini K I, Bhagirathi R G, Mishra B, Mishra K P, Banavalikar M M and Mohan H, Phytother Res., 2005, 19(7), 582-600.
6. Ughade S N, Zodpey S P and Khanolkar VA, Indian J Ophthalmol., 1998, 46(4), 221-227.
7. Baliga MS, Meera S, Mathai B, Rai MP, Pawar V, et al. (2012) Scientific validation of the ethno medicinal properties of the Ayurvedic drug Triphala: a review. Chin J Integr Med 18: 946-954.
8. Stahl E, 1969. Thin layer Chromatography; Spray reagents, 2nd ed. New York, Springer-Verlag, 854-904.
9. Wagner H, Baldt S (1996) Plant Drug Analysis. Springer-Verlag, Berlin. 359.
10. ICH guideline Q2R1, validation of analytical procedures: text and methodology (November 1996/2005) Geneva, Switzerland.
11. NS Jeganathan et al. HPTLC method for estimation of Ellagic acid and Gallic acid in Triphala churnam formulations. Research Journal of Phytochemistry, 2 (1):1-9, 2008
12. Prawez Alam et al, HPTLC method for simultaneous determination of ascorbic acid and gallic acid biomarker from freeze dry pomegranate juice and herbal formulation: Saudi Pharmaceutical Journal 27 (2019) 975–980.
13. Ajay Kumar Meena et al. Determination of Gallic Acid in Ayurvedic Polyherbal Formulation Triphala churna and its ingredients by HPLC and HPTLC Research J. Pharm. and Tech. 11(8): August 2018 3243-49
14. M.S. Kondawar et al. Quantitative estimation of Gallic acid and Ascorbic acid in a marketed herbal medicine: Triphala Churna by High Performance Thin Layer Chromatography. International Journal of Pharm Tech Research Vol.3, No.3, pp 1593-1599, July-Sept 2011