Development and validation of a HPTLC/densitometry method for the quantitative determination of flavanone glycoside (naringin) in *Drynaria quercifolia* fronds

Pradeep Kamboj*, Vinod Gauttam, A.N. Kalia

Department of Pharmacognosy, ISF College of Pharmacy,
Firozepur GT Road, Moga-142001, Punjab, India
email: dr.pradeepkamboj@gmail.com

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Plant profile

Botanical name: *Drynaria quercifolia*
Family: Polypodiaceae

SYNONYMS

Sanskrit: Aswakatri
English: Oak-leaf Fern

Distribution – Throughout India growing on old trees, rocks and walls

MEDICINAL PROPERTIES

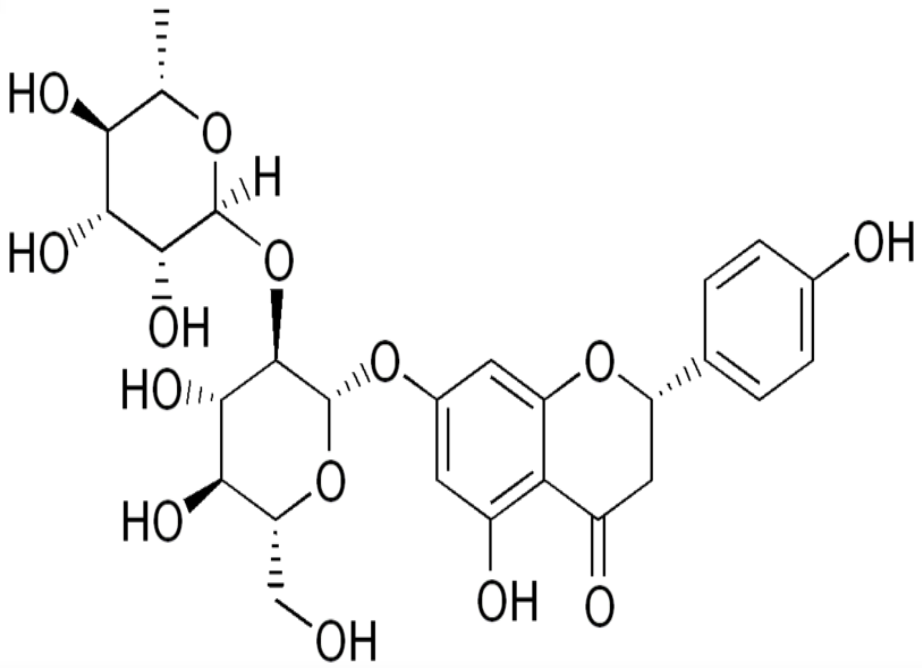
Inflammation, chronic jaundice, infection, typhoid, arthritis, headache, diarrhoea, and ulcers

Useful parts: Fronds & whole plant

(Ramesh et al., 2001; Bhattacharya et al., 1990; Kirtikar & Basu 1994; Bhattacharya et al., 1990)

Phytochemical profile

- Catechin,
- Coumarins,
- Flavonoids (naringin),
- Phenolics,
- Saponin,
- Triterpenes,
- Steroids,
- β-Amyrin,
- β-Sitisterol



Structure of flavanone glycoside (naringin)

(Anuja et al., 2010; Ramesh et al., 2001; Khan et al.,2007)

Experimental

Chromatographic conditions

Stationary phase	:	Aluminum pre-coated silica gel 60F_{254} plates (20 × 10 cm) :
Thickness	:	200 μm
Sample application	:	Linomat V
Solvent system	:	Ethyl acetate-chloroform-methanol-formic acid (7:3:2:0.1, v/v/v/v)
Band width	:	6 mm
Development chamber	:	Twin trough glass chamber
Chamber saturation time	:	15 min
Migration distance	:	80 mm
Wavelength	:	286 nm
Scanning	:	(*CAMAG SCANNER* III)
Mode	:	Absorbance/ reflectance
Slit dimensions	:	4 ×0.3 mm

Sample preparation: 0.5 g/10 ml solution of *Drynaria quercifolia* fronds powder was prepared by refluxing in methanol for 45 min

Validation of Method

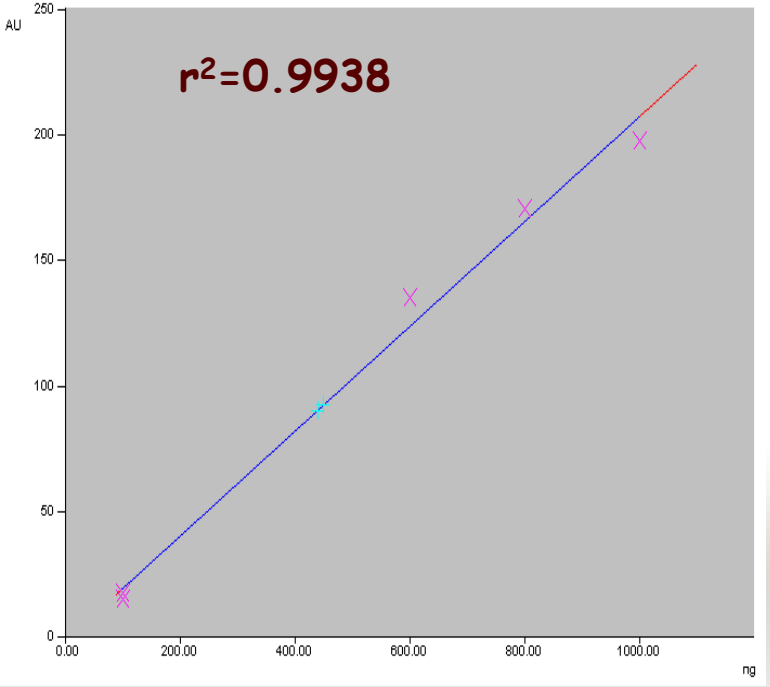
1. Linearity
2. Precision
3. Recovery studies
4. Specificity
5. Limit of detection and quantification
6. Robustness

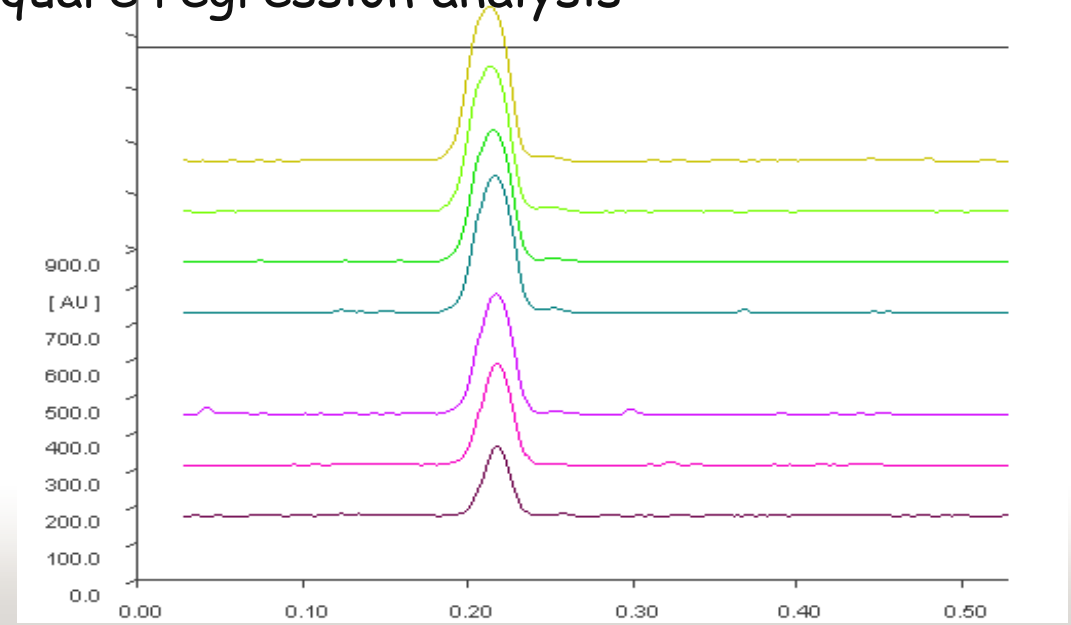
ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures, 1994

Results

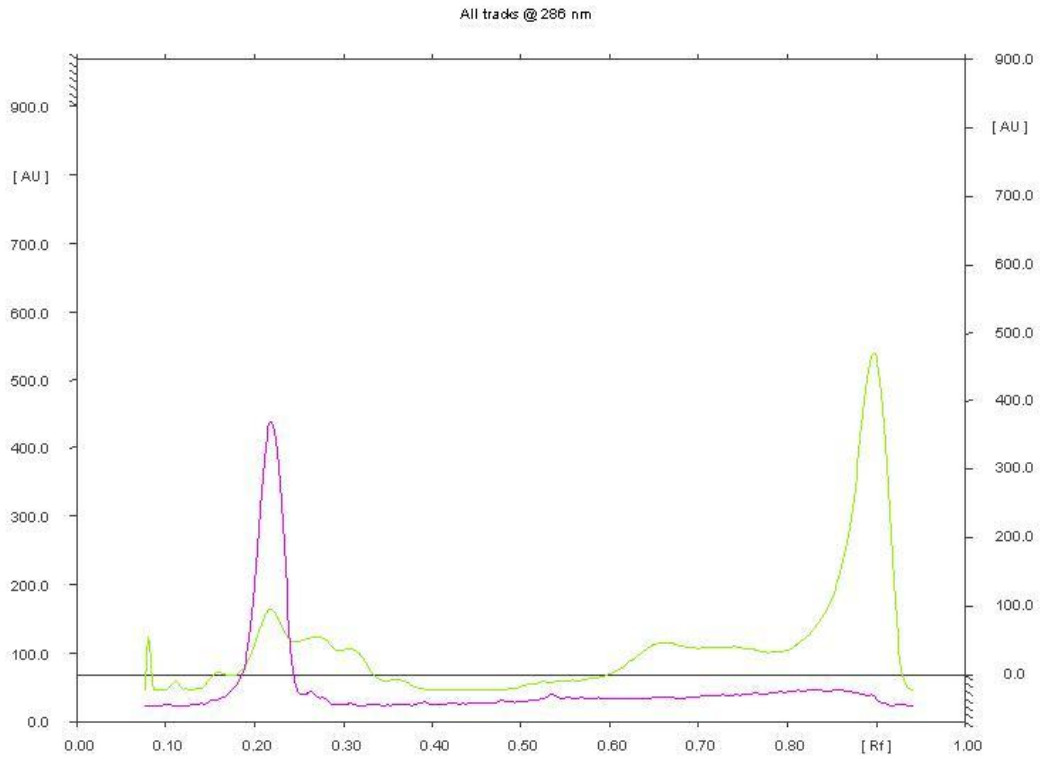
Calibration curve (naringin)

Different volumes of stock solution were spotted to obtain the concentration range 200-1200 ng of naringin, respectively. The data of peak areas were plotted against concentrations and were treated by least-square regression analysis

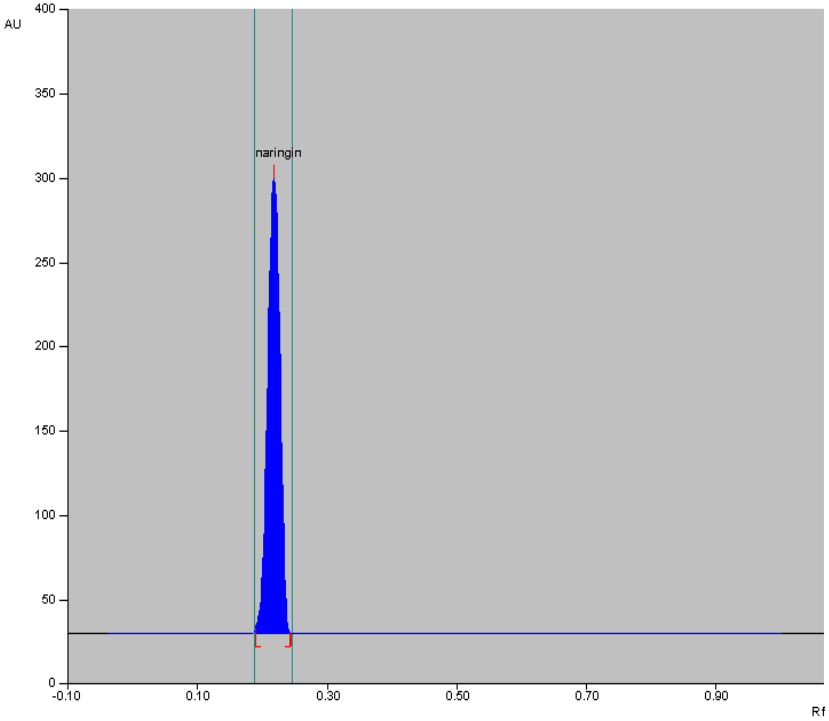




Two dimensional pattern for peaks of standard reference naringin



Two dimensional chromatogram of standard naringin & sample



Peak of standard naringin
Rf= 0.22 ± 0.03

Quantity of naringin

The quantity of naringin found in *Drynaria quercifolia* (fronds) was 0.067%

Validation of Method

1. **Linearity**

The correlation coefficient for the used method was found to be 0.9938 and thus exhibits good linearity between concentration and area.

2. **Precision**

Repeatability

Repeatability of the sample application and measurement of peak area were carried out using six replicates of the same spot (800 ng of naringin) and was expressed in terms of (%R.S.D) and (S.E)

S. No	Sample (ng/spot)	Area (AUC)
1	800	7518.53
2	800	7603.72
3	800	7496.12
4	800	7464.82
5	800	7328.93
6	800	7148.31

%SD = 1.08
%RSD=0.012
SE = 0.4409

Intra-day & inter-day precision

The intra-day and inter-day variation for the determination of naringin was carried at three different concentration levels of 400, 600 and 800 ng/spot

	Intra Day precision				Inter Day precision			
Amount of reference standard (ng)	Mean area	%SD	%RSD	S.E.	Mean area	%S.D	%R.S.D	S.E
400	3690.77	3.38	0.09	1.38	3762.64	3.11	0.08	1.09
600	6321.51	3.91	0.06	0.02	6503.20	3.85	0.06	1.36
800	7742.8	2.06	0.02	0.84	7708.6	2.21	0.02	0.78

3. Recovery studies

The pre-analyzed samples were spiked with extra 50, 100 and 150% of the standard naringin and the mixtures were reanalyzed.

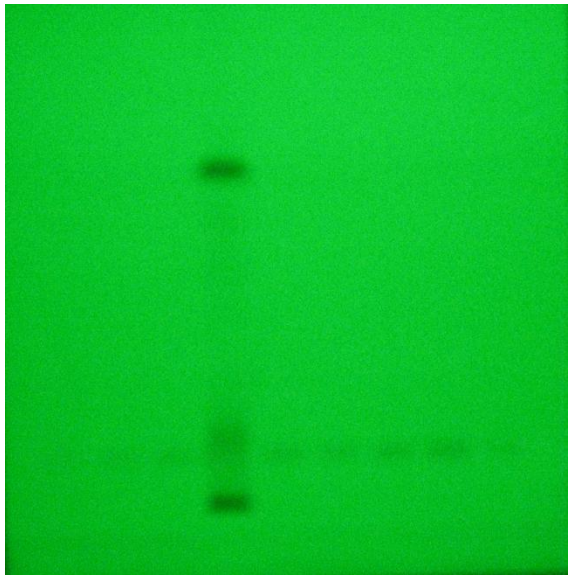
The experiment was conducted three time (n=3). This was done to check for the recovery of the naringin at different levels in the extract.

Amt. of extract taken 'A' (ng)	Amt. of naringin spiked 'B' (ng)	Amt of A +B (ng)	Theoretical area (AUC) 'C'	Practical area (Mean Area) (ng) 'D'	Recovery D/C×100 (%)
400	200	6	6668.27	6977.01	104.63
400	400	8	7911.01	8316.84	105.13
400	600	10	9328.39	9144.62	98.03

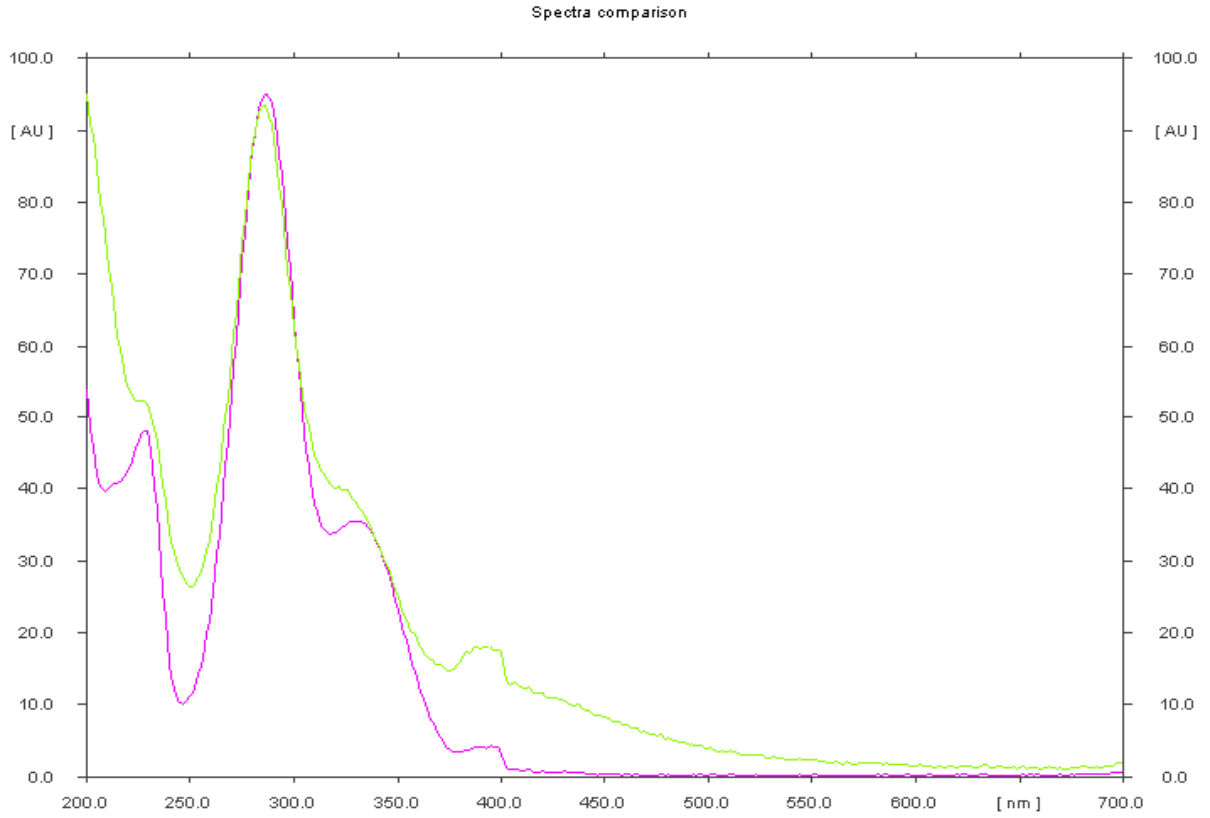
The percentage recovery of naringin was found to be 98.03-105.13%

4. Specificity

The spectrum of sample naringin was found to be similar and overlap with that of the reference standard spectrum & a good correlation (0.9982) overlain of spectra was obtained between sample and standard



HPTLC of standard naringin & sample



Spectral comparison of standard and samples

5. Limit of detection and limit of quantification (LOD & LOQ)

LOD was calculated by making the dilutions of stock solution and determining the minimum amount which could be detected densitometrically

Minimum detectable limit **(LOD) = 60 ng/spot**

Limit of quantification **(LOQ) = 180 ng/spot**

6. Robustness

Robustness of the method was done in triplicate at a concentration level of 800 ng/spot and the %R.S.D and S.E. of peak areas were calculated

Small changes (Parameters)	S.D. of Peak Area	% RSD	S.E.
Mobile Phase composition	2.18	0.01	0.72
Mobile phase volume	2.67	0.01	1.08
Duration of saturation	2.68	0.01	1.09

Conclusion

- The developed HPTLC method is simple, precise, robust and specific for qualitative and quantitative estimation of naringin in herbal(s) extract

- The result of recovery studies of naringin shows the reliability and suitability of the method

- Further, this method can be used as marker compound to standardization the plant (***Drynaria quercifolia***) and its marketed formulations