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Original Research Article

Spectrophotometric method development and validation for estimation of rutin in some herbal formulation

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Abstract

Three simple, rapid, accurate precise and economic spectrophotometric methods (A, B and C) have been developed for estimation of rutin in several herbal formulations. Method A and B are based on the complexation of rutin with cobalt (II) nitrate and nickel (II) chloride to give colored complexes. The absorption maxima, λ_{max}, are at 359.70 nm for method A, 347.85 nm for method B and 340.90 nm for method C respectively. Beer's law was obeyed in the concentration range of 0.004-0.04 mg mL^{-1} for all methods. Developed methods are validated as per ICH guidelines and could be successfully adopted for the routine estimation of rutin.

Key Words: Herbal formulations, ICH guidelines, rutin, spectrophotometric.

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INTRODUCTION

Rutin (3,3′,4′,5,7-pentahydroxy flavones-3-rutinoside) (Fig. 1), which was also named vitamin P, eldrin, melin, sophorin, and violaquercitrin, is a typical glycoside of the natural flavonoids widely distributed in plants[1]. Rutin is slightly soluble in water and has a higher solubility in organic solvent such as methanol[2]. Rutin, a flavonoid glycoside, found in vegetables, fruits, tea and herbs[3]. Moreover, rutin possess different protective effects including antioxidant, anti-cancer and anti-inflammatory properties[2]. Also, rutin has a protective effect against doxorubicin-induced memory deficits and has neuroprotective effects[4,5]. In addition, it has a protective function in ischemic organs including the heart and brain[6]. Several methods have been developed for the determination of rutin in different plant extracts; these include HPLC[7,8], capillary electrophoresis[9-11] and spectrophotometry[12-14]. Recently, HPTLC has been applied for the determination of flavonoids[15–18]. The present work deals with the development of three simple and sensitive spectrophotometric methods for the quantitative estimation of rutin in herbal formulations. To our knowledge, there is no pharmacopeial method or any validated method that quantifies rutin in its herbal formulation. The objective of this study is to develop simple spectrophotometric method for quantification of rutin in its formulations and compare its quality with what is available in the local and internationalmarket. These methods are validated according to the international standards[19,20]. The developed analytical method will be applied in quantification of rutin in its final herbal dosage form.

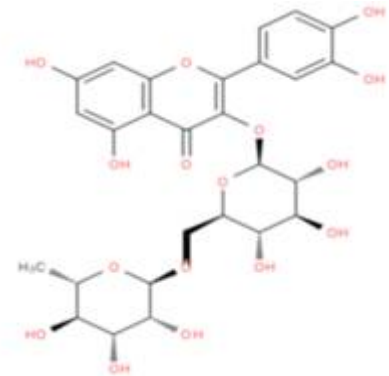


Figure 1: Chemical structure of rutin

MATERIALS AND METHODS

Reagents and chemicals: Standard rutin powder 95% was purchased from Sigma Aldrich, St. Louis, USA. Five samples of herbal formulations namely Dr's best extra strength Ginkgo, Goonj, Bilovas, Health Vit Ginkgo Biloba and Ginkgo Vital were purchased from the local drugstores and via internet. As a solvent HPLC grade methanol and water were used and purchased from SRL chemicals, India. Co (II) nitrate and Ni (II) choride used was analytical grade and were purchased from Loba chemie, India.

Apparatus: Absorbance was measured using Thermo Scientific (Evolution 201) Sr.no-5A3O253001, MA 02454 ultraviolet-visible spectrophotometer supplied by Thermo Fisher Scientific Inc, Waltham, the accuracy of this instrument is ± 0.080nm. A digital pH meter Systronics model µ pH system 361 supplied by Systronics Pvt. Ltd Ahmadabad, India with the accuracy of ± 0.01 is used. For accurate Weighing purpose, A Reptech Precision Balance, having accuracy of ± 0.0001 gm supplied by Reptech. Pvt. Ltd. Ahmadabad, India is used. For sonication the Life-care ultrasonicator Sr. no-2K1204011 with operating Frequency of 33 KHz ± 3 KHz. It has digital temperature controller up to 60°C was used.

Preparation of standard and sample solution: A stock solution of rutin (0.1 mg mL^{-1}) was prepared by dissolving 10 mg compound in 100 ml methanol: water (70:30 v/v). Five different herbal formulations were analysed by the given methods. Ten tablets or capsules of each formulation were taken, and the average weight was calculated. For the preparation of sample solutions, the weighed content of single dosage of each herbal formulation was crushed in mortar. These powdered samples were placed in 100ml volumetric flask and dissolved in methanol: water (70:30 v/v) up to mark and sonicated for 30 min. After sonication sample solutions were filtered through Whatman filter paper No. 41 these solutions were used for spectrophotometric study.

Method Development Method A: For the assay of rutin in five herbal formulations in first method 1-10 ml from the stock solution (0.1 mg mL^{-1}) transferred into a series of 25 ml volumetric flasks and the final volume brought to 25 ml with the solvent methanol: water (70:30 v/v). The absorbance of these all sets are measured at 359.7 nm spectra show in Fig 2. The amount of rutin present in the sample was calculated from the calibration curve.

Method B: In this method for the quantitative determination of rutin the samples were prepared by taking the rutin solution as made in method A. 2 mL solution of rutin ranging from 0.004-0.04 mg mL^{-1} were transferred in to a series of 10 mL volumetric flasks. To each flask, 2 mL of Co (II) nitrate was added at room temperature and formation of complexes was seen at pH 7.60. After 30 min, the absorbance of complex was measured at 347.80 nm and spectra for it showed in Fig. 3. The amount of rutin present in the formulations was computed from a calibration curve.

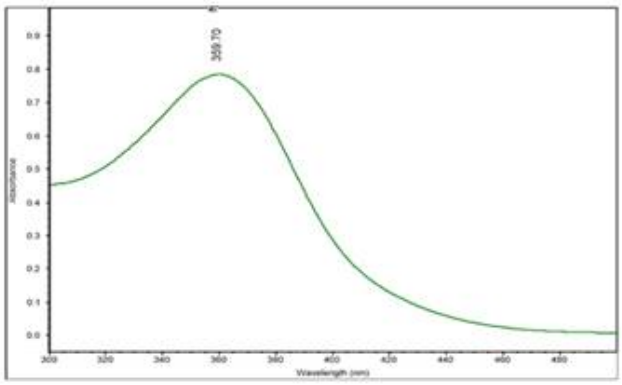


Figure 2: Spectra of Rutin

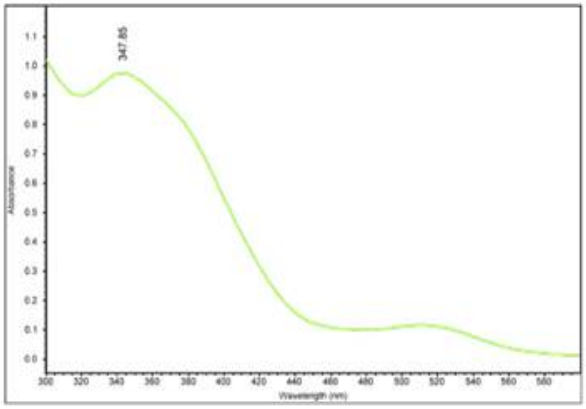


Figure 3: Spectra of Rutin- Co (II) complex

Method C- 2mL of rutin ranging from 0.004-0.04 mg mL^{-1} were transferred into a series of 10 mL volumetric flasks. To each flask, 2 mL of Ni (II) chloride was added

at room temperature formation of complexes occur at pH 7.28. After 30 min, the absorbance of complex was measured at 340.90 nm as showed in Fig. 4. The amount of rutin present in the formulations was computed from a calibration curve.

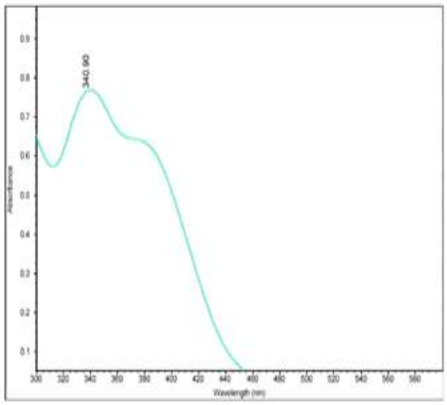


Figure 4: Spectra of Rutin- Ni (II) complex

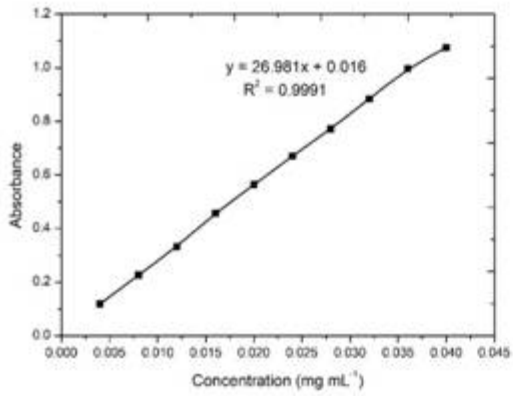
RESULTS AND DISCUSSION

Method validation: All the methods were validated as per ICH guidelines [21] for Linearity, Precision, limit of detection, limit of quantification and accuracy.

Linearity: Linearity of the methods A B and C were performed by analysing standard solution of rutin by the proposed method in concentration range 0.004-0.04 mg mL^{-1}. Characteristic parameters for regression equation and correlation are given in table 1. The linearity of the calibration graphs was validated by the high value of correlation coefficients of the regression (Fig. 5-7).

Table 1: Regression parameters for the analysis of rutin

Parameter	Values		
	Method A	Method B	Method C
Range	0.004-0.04 mg mL^{-1}	0.004-0.04 mg mL^{-1}	0.004-0.04 mg mL^{-1}
Slope	26.981	18.469	21.212
Intercept	0.016	0.364	-0.0214
Regression coefficient	0.9991	0.9990	0.9996
Regression Equation	Y = 26.981x + 0.016	Y = 18.469 x + 0.0364	Y = 21.212 x – 0.0214



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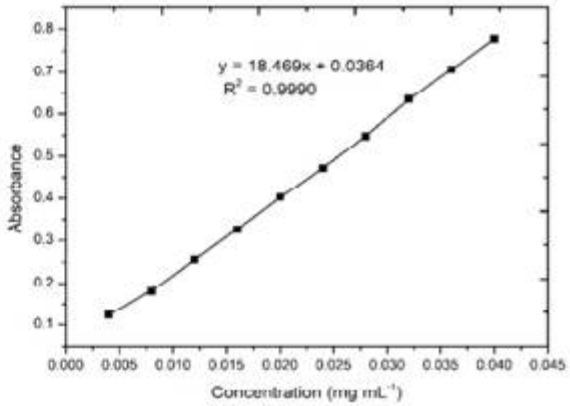

Figure 6:

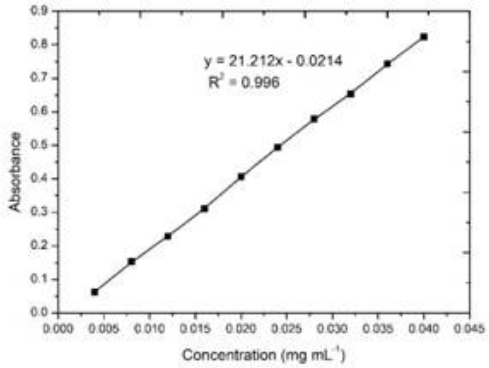

Figure 7:

Figure 5: Calibration curve for method A
Figure 6: Calibration curve for method B
Figure 7: Calibration curve for method C

Precision Method precision: Precision of the method was checked by repeatedly applying six different solutions containing rutin 0.020 mg mL^{-1} without changing the parameters of the proposed method. Result for the method precision was showed in table 2.

Table 2: Method precision data

Rutin (0.020mg mL^{-1})	Absorbance Method A	Absorbance Method B	Absorbance Method C
1	0.562	0.405	0.409
2	0.565	0.401	0.407
3	0.568	0.408	0.406
4	0.559	0398	0.399
5	0.560	0.402	0.410
6	0.569	0.403	0.408
Mean	0.563	0.4028	0.406
SD	0.0042	0.00343	0.00394
RSD (% CV)	0.7391	0.8515	0.9685

Intraday and Interday precision: The results of intraday and interday precision was studied by taking three different concentrations and repeated three times. The result of intraday and interday precision was given in table 3. The developed method was found to be precise as the % RSD values for system precision, method precision and intermediate precision studies were less than 2%, as recommended by ICH guideline.

Table 3: Intraday and inerday precision data for method A, B and C

Rutin (mg mL^{-1})	Intraday		Interday	
	Mean ± S.D	% C.V	Mean ± S.D	% C.V
	Method A			
0.004	0.118 ±0.001	0.847	0.109 ± 0.002	1.392
0.020	0.557 ±0.004	0.809	0.542 ± 0.007	1.292
0.036	0.989 ±0.008	0.811	0.952 ± 0.011	1.204
	Method B			
0.004	0.124 ±0.001	1.228	0.116 ± 0.002	1.724
0.020	0.400 ±0.004	1.126	0.381 ± 0.006	1.444
0.036	0.699 ±0.008	1.155	0.678 ± 0.009	1.407
	Method C			
0.004	0.063 ±0.0005	0.907	0.058 ± 0.001	1.724
0.020	0.404 ±0.004	0.998	0.389 ± 0.007	1.803
0.036	0.739 ±0.007	0.947	0.715 ± 0.012	1.678

Accuracy (% recovery): The accuracy of the proposed methods was determined by recovery study of rutin using standard addition method. Known amounts of standard solutions of rutin was added at 80, 100 and 120 % level to prequantified sample solution of rutin. The amount of rutin was estimated by applying obtained values to the regression line equation. The results of recovery study are given in table 4. The recoveries of the standard addition method in the range from 98.509 to 100.059 % for method A, 99.007 to 100.073 % for method B and 98.711 to 99.591 for method C suggest good accuracy of the proposed method.

Table 4: Data of recovery study of rutin by method A, B and C

Accuracy level (%)	% Recovery ± S.D. (n=3)				
	Forhbmulation 1	Formulation 2	Formulation 3	Formulation 4	Formulation 5
			Method A		
80	99.125 ±0.220	98.979 ±0.129	99.271 ±0.334	99.344 ±0.219	99.417 ±0.703
100	98.935 ±0.355	99.289 ±0.308	99.053 ±0.272	98.994 ±0.269	100.059 ±0.369
120	98.509 ±0.515	99.055 ±0.0875	99.702 ±0.149	99.354 ±0.228	99.353 ±0.228
			Method B		
80	99.490 ±0.176	99.184 ±0.176	99.185 ±0.467	99.285 ±0.180	99.184 ±0.467
100	99.258 ±0.858	99.007 ±0.250	99.339 ±0.378	99.921 ±1.370	99.256 ±0.656
120	99.151 ±0.004	99.009 ±0.127	99.434 ±0.122	100.073 ±0.801	99.364 ±0.208
			Method C		
80	99.033 ±0.321	98.926 ±0.492	99.034 ±0.322	99.465 ±0.743	98.710 ±0.559

100	99.508 ±0.247	99.262 ±0.247	99.181 ±0.138	98.426 ±0.144	99.592 ±0.509
120	99.326 ±0.515	98.988 ±0.204	99.258 ±0.115	99.189 ±0.205	99.189 ±0.205

Limit of detection (LOD) and limit of quantification (LOQ): LOD and the LOQ of the drug were calculated using the following equations as per (ICH) guidelines using following equations, and the results are showed in table 5.

LOD = 3.3 s/S

LOQ = 10 s/S

Where, s = Standard deviation of intercept calibration curves,

S = Mean slope of calibration curve.

Table 5: LOD and LOQ for method A, B and C

Method	LOD (mg mL^{-1})	LOQ (mg mL^{-1})
A	0.859	2.605
B	0.311	0.942
C	0.607	1.839

Analysis of marketed formulations: Analysis of samples of marketed formulations were carried out by applying the method as given above. For the quantification of rutin in five formulations, the prepared sample solutions were taken and method A, B and C were applied. The amount of rutin present in the sample solution was determined by fitting absorbance values corresponding to rutin into calibration curve. The results obtained for rutin in all the five formulations were compared with the corresponding labelled amount showed in table 6.

Table 6: Assay of formulation by proposed method

Formulation	Label claim (mg)	Amount of rutin found (%w/w) by method A	Amount of rutin found (%w/w) by method B	Amount of rutin found (%w/w) by method C
1	0.0200	99.35	98.50	99.52
2	0.0085	97.64	99.76	98.94
3	0.0350	98.29	98.85	99.14
4	0.0300	98.00	97.67	97.33
5	0.0250	99.60	98.00	98.40

CONCLUSION

The developed spectrophotometric methods are simple, sensitive, accurate, precise, economical and successfully applied for routine estimations of rutin in herbal formulations. The most striking feature of the spectrometric methods is their simplicity and rapidity. For spectroscopic methods there was no need for time-consuming sample preparation steps. The developed method was validated as per ICH Guidelines. The values of the standard deviation were satisfactorily low, and the recovery was close to 100%, which indicates the reproducibility and accuracy of the three methods.

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