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Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Morin in Bulk and Formulation

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A B S T R A C T

Aim: To develop and validate a simple, precise and cost-effective UV- visible spectrophotometric method for the estimation of morinin proliposomeformulation according to the ICH Q2 (R1) guideline. **Methods:** Spiked Morin solution was scanned over UV-visible range for its wavelength of maximum absorbance. Various calibration standards of Morin were prepared and absorbance was recorded at wavelength of maximum absorbance. Calibration curve of concentration vs. absorbance was plotted and linearity and range was calculated. Various analytical method validation parameters viz. accuracy, precision, LOD, LOQ, robustness and ruggedness were calculated using QC standards. **Results:** The maximum wavelength of Morin was found to be 250 nm. The correlation coefficient over the concentration range of 1-12 µg/ml was found to be 0.9993s. Developed UV method was found to be precise during the intra-day and inter-day study and shows percent relative standard deviation in the range of 100.41 to 99.16 & 100.4 respectively. The total percent recovery of Morin was found to be 99.45to 99.77 %. Developed method was found to be robust and rugged for the intended use. Morin content of proliposome formulation was successfully calculated using developed UV-Visible method. **Conclusion:** A simple, precise and cost-effective UV- visible spectrometry method for the estimation of Morin was developed. The said method was developed using economical percentage of organic phase in aqueous media as solvent. Said validated UV- visible method can be efficiently used for the estimation of morin in homemade proliposome formulation

Keywords: UV- visible spectrometry, Morin, Validation

1.INTRODUCTION

Morin is a kind of flavonoid belonging to flavanol group. It is abundantly found in different species of Moraceae family, which are used as dietary supplements and herbal medicines. Besides this, morin is also available in tea, coffee, red wine and active component of guava).The chemical formula of morin is 2-(2, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-l-benzopyran- 4-one having molecular weight of 302.24 g/mol. Hydroxyl groups present in the structure plays an important role in its antioxidant, estrogenic and antiperoxidative property.Till date, there are very few reports demonstrating the UV visible spectrophotometric method for estimation of morin. Earlier reported UV visible spectrophotometric methods were found to contain either higher percentage of organic solvents or the use of costly solvents. Therefore, considering the therapeutic importance of the morin and the need of simple yet precise and robust analytical methodology for the same, it was envisaged that development of UV-Visible spectrophotometric method for the determination of morin in homemade proliposome formulation by using co-solvent system consisting economic percentage of organic solvent will be worth.

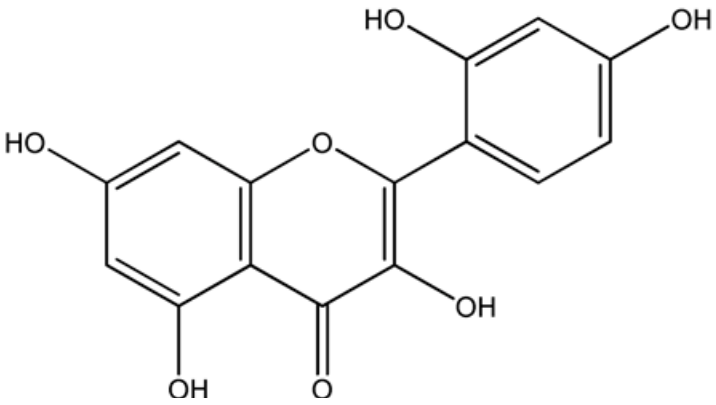

Fig. 1: Chemical structure of Morin

2.MATERIALS AND METHODS

Morin was purchased from TCI Chemicals (India) Pvt. Ltd. All other chemicals of analytical grade were used for study.

Instruments Used

A double beam UV-visible spectrometer (UV-530, Jasco) with spectra manager software was used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Vibra HT, Essae) with internal calibration mode was used for the accurate weighing purpose.

Preparation of standard stock solution

Accurately weighed 1 mg of morin was transferred into the calibrated volumetric flask and dissolved in 5 ml mixture of Methanol : 0.1mM Phosphate buffer (40:60 v/v) to achieve a stock solution of 1000 µg/ml (Stock-I). Stock- I solution was suitably diluted with co-solvent system of Methanol: 0.1mM Phosphate buffer to achieve a solution of 100 µg/ml (Stock-II).

Determination of wavelength of maximum absorbance (λmax)

Stock-II solution was scanned using full scan mode with medium scanning speed for the entire range of UV and visible i.e. 800 to 200 nm with co-solvent system as a blank. After obtaining the spectrum, λmax was identified with the help of software. In order to achieve reproducible results, above method was repeated five times.

Preparation of calibration curve

Calibration curve was prepared by diluting the stock-II solution to achieve the Sevan different calibration standards representing 1, 2, 4, 6, 8, 10, 12, 16 µg/ml strength. Absorbance of each calibration standard was measured at pre-identified λmax; 250 nm using fixed wavelength measurement mode. The calibration curve representing concentration vs. absorbance was plotted using Excel program of Microsoft Office 2010. Above mentioned procedure was repeated five times so that reproducible results can be obtained.

Method Validation

Developed UV method for the estimation of Morin was validated as per the ICH guidelines. Different parameters like linearity and range, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ) were calculated using predefined calibration standards or quality control standards as described below [9-10].

Linearity and Range

Linearity of the proposed UV method was established using seven different calibration standards. After analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy

The accuracy of the proposed UV method was evaluated using recovery studies after standard addition of analyte of interest. Three different solutions of Morin were prepared in pentaplicate at level of 80%, 100% and 120% of its predefined concentrations (1.5, 6 and 11µg/ml). To the predefined concentrations, different amounts of Morin were added (standard addition method) and the accuracy was calculated on the basis of percent recovery. For calculating the percent recovery, following formula was used

%RC= (SPS-S/SP) × 100

Where,

SPS = Amount found in the spiked sample

S = Amount found in the sample

SP = Amount added to the sample

% RC = Percent recovery

Precision

The precision of the proposed UV method was established by performing intra- and inter-day UV analysis of quality control samples (1.5, 6 and 11 µg/ml). Morin solutions of 1.5, 6 and 11 µg/ml strength (n=5 for each concentration) were analyzed at morning, afternoon and evening time of three consecutive days. Deviation in the results was calculated in terms of % relative standard deviation (% RSD).

Robustness

Robustness of the developed UV method was established using different percentage of methanol in co-solvent system. Methanol percentage in co-solvent system was intentionally adjusted to 37 and 63 % and middle level quality control sample (6 µg/ml) of morin was prepared using above mentioned co-solvent system separately. Samples (n=5) were analyzed at 250 nm for morin content. The results were calculated in terms of % RSD.

Ruggedness

Ruggedness study of the method was carried out by analyzing triplicate samples of Morin solution (6µg/ml) using two different instruments (V-530, Jasco and BA-UV-2600, Bioage). Results were expressed in terms of % RSD.

Limit of Detection (LOD)

The LOD of the developed UV method was calculated by using following formula

LOD=3.3×SD/S

Where, SD= Standard deviation of Y-intercepts

S= Slope

Limit of Quantitation (LOQ)

The LOQ of the developed UV method was calculated by using following formula

LOQ= 10×SD/S

Where, SD= Standard deviation of Y-intercepts

S= Slope

Estimation of Morin content in Proliposome Formulation

In-house Proliposome of Morin was prepared using solvent evaporation technique. Accurately weighed amounts of lipid mixture comprising of phosphatidyl choline (soya lecithin) and cholesterol and dissolved in 20 ml of solvents mixture containing chloroform and methanol in the ratio 1:1. The suspension containing phosphatidyl choline, Morin, cholesterol, and mannitol was transferred in to round bottom flask. All material got dissolved in solvent mixture, except carrier material; hence resultant suspension was obtained like slurry due to the addition of mannitol act as base carrier. The organic solvent mixture was evaporated with the help of rotary vacuum evaporator under the reduce pressure 50mbar at the temperature of 40 ± 2 °C to 55 ± 2 °C. After ensuring the removal of solvent, the resultant powder was further dried in a vacuum desiccator at room temperature so as to obtain dry, free flowing powder. This powder was stored in the tightly closed container.

Five hundred mg proliposomes powder (equivalent to 1 mg Morin) containing morin were dissolved in 5 ml methanol using ultrasonication and the solution was filtered using 0.22 µm filter. Filtered solution was suitably diluted and subjected to spectrofluorimetric analysis using prevalidated method.

3.RESULTS AND DISCUSSION

Determination of wavelength of maximum absorbance

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for Morin solution (6 µg/ml) was carried out using full scan mode of UV-Visible spectrophotometer (figure 2). Full scan was processed using Jasco UV software and the λ_{max} was identified with the help of software. The λ_{max} was found to be 250 nm for Morin.

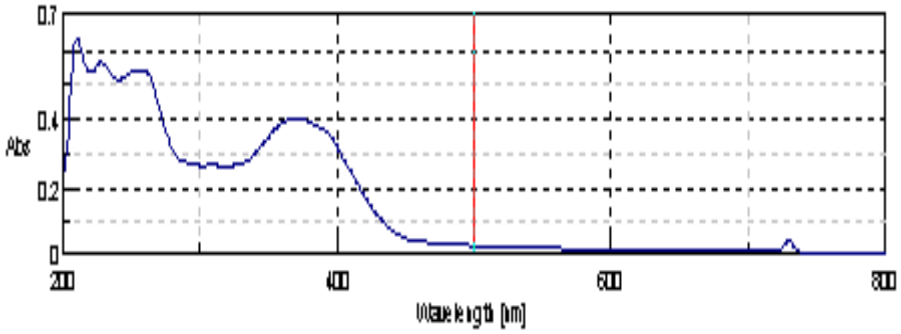

Fig. 2: UV-visible spectra of Morin

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and a mathematical equation stating correlation between concentration and the response. As compare to graphical method, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of Morin, calibration curve for Morin was developed using seven different calibration standards. The absorbance of different calibration standards at 250 nm was recorded using fixed wavelength mode. Calibration curve was repeated five times and the mean values ± standard deviation was reported as shown in Table 1.

Table No 1: Calibration standard data for Morin

S. NO.	Concentration (µg/ml)	Absorbance
1	1	0.0764±0.0025
2	2	0.1564±0.0038
3	4	0.3012±0.0048
4	6	0.4528±0.0055
5	8	0.6124±0.0062
6	10	0.7845±0.0066
7	12	0.9145+0.0068

Method validation

Linearity and Range

Linearity and range are the key parameters of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, seven point calibration curve of Morin covering a range of 1-12 µg/ml was plotted. Details of concentrations and the respective mean absorbance values are depicted in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; y = 0.077x+0.0019 with correlation coefficient 0.9993 as shown in Figure 3. From the linearity study, it was revealed that, developed UV method was linear over the concentration range of 1 to 12 µg/ml.

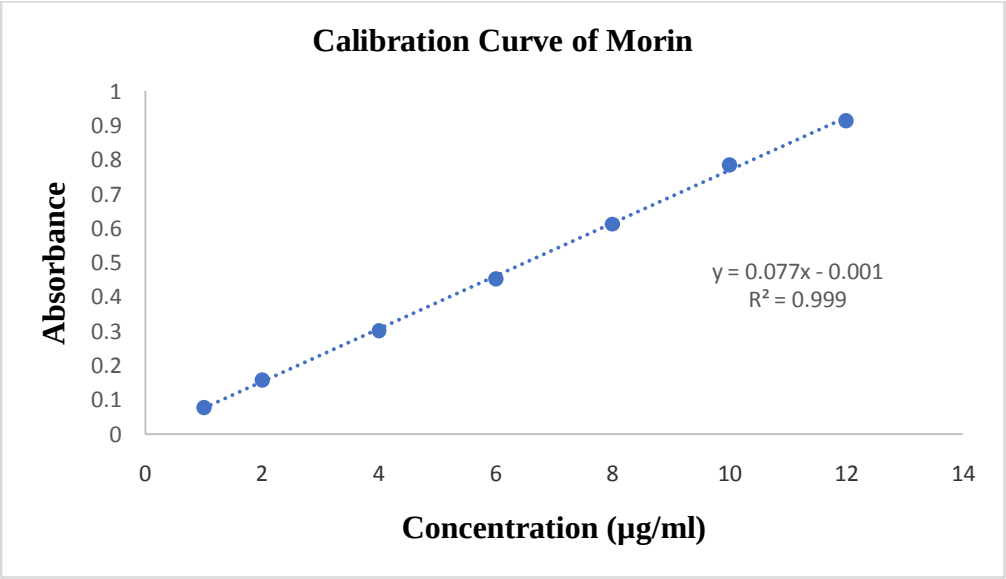

Fig. 3: Calibration curve for Morin

Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. In case of UV method for Morin, accuracy was established using recovery studies. At 80 % standard addition, mean recovery of Morin was found to be 100.41% whereas at 100 and 120 % standard addition, it was found to be 99.83 and 100.44% respectively. % RSD was found to be less than 2 for the morin recovery studies as shown in Table 2. From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 98 to 100% and the % RSD was well below 2%.

Table No 2: Accuracy data of UV method for Morin

S No.	Concentration (%)	Origin level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	% RSD
1	80	1.2	1.205	100.41	100.21	99.45
2	80	1.2	1.201	100.08		
3	80	1.2	1.202	100.16		
4	100	6	5.99	99.83	99.83	99.16
5	100	6	6.01	100.16		
6	100	6	5.97	99.5		
7	120	13.2	13.25	100.37	100.44	99.77
8	120	13.2	13.18	99.84		
9	120	13.2	13.35	101.13		

Precision

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at 1.5, 6 and 11 µg/ml levels of Morin. The results in terms of mean absorbance values, percent assay and % RSD for the intra- and inter-day precision study are demonstrated in Table 3 and Table 4 respectively. % RSD values of intra-day precision study were found to be in between 99.45 and 99.77 whereas those of inter-day precision study were in between 1.5 and 11. Overall, % RSD values of less than 2 demonstrated the precision of developed UV method.

Table No 3: Intra-day precision data of UV method for Morin

		Morning			Afternoon			Evening		
S. NO	**Concentration Range (µg/ml)**	**Mean Amount**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1	1.5	1.501	100.06	99.65	1.506	100.4	100.1	1.496	99.77	99.45
2	6	5.998	99.96	99.78	5.890	98.16	99.99	6.005	100.15	99.66
3	11	10.98	99.81	99.45	10.95	99.81	99.95	10.98	99.81	99.77

Table No 4: Inter-day precision data of UV method for Morin

		Day 1			Day 2			Day 3		
S. NO	**Concentration Range (µg/ml)**	**Mean Amount**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1	1.5	1.499	99.93	99.65	1.506	100.4	99.25	1.509	100.6	99.16
2	6	5.995	99.91	99.45	6.008	100.13	99.66	5.995	99.91	99.45
3	11	10.98	99.81	99.20	10.97	99.72	99.58	11.01	100.09	99.2

Robustness

Robustness of analytical method is the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It is an important parameter of analytical method as a small, un-intentional change in method parameters like solvent composition, buffer strength, pH etc. may occur during routine use and may hamper the performance of said method. It is expected that such change should not alter the performance of the analytical method. Therefore, robust analytical method is preferred. Robustness of proposed UV method was established by modifying the composition of co-solvent system. Change in methanol percentage (57 to 63 %) in co-solvent system did not affect the method performance. % RSD values were found to be in between 99.97 and 99.82 as shown in Table 5. % RSD values below 2 depicted that proposed UV method is robust in nature.

Table 5: Robustness data of UV method for Morin

S. NO	Concentration (µg/ml)	% Methanol	Absorbance	% RSD
1	6	57	0.4525	99.97
2	6	63	0.4530	99.82

Ruggedness

Ruggedness of analytical method is the ability of a method to resist the change in its performance in spite of influential environmental factors like temperature. Rugged analytical methods are preferred as these methods are free from impact of environmental/external factors. In order to establish the ruggedness of proposed UV method, Morin solution was analyzed using two different UV-Visible spectrophotometers of two different labs. Sample analysis and data processing resulted into % RSD values between 99.77 and 99.45 Results revealed that proposed UV method was rugged as it showed % RSD values less than 2 as shown in Table 6.

Table 6: Ruggedness data of UV method for Morin

S. No.	Concentration (µg/ml)	Instrument	Absorbance	% RSD
1	6	Jasco	0.4533	99.77
2	6	Bio-age	0.4524	99.45

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of proposed UV method was found to be 0.854 and 5.281 µg/ml respectively as shown in Table 7. Lower LOQ value indicated that proposed method would be sensitive enough to quantify the Morin content of samples at its lower level.

Table No 7: LOD & LOQ data for UV method for Morin

1	LOD	0.854 µg/ml
2	LOQ	5.281 µg/ml

Estimation of Morin content in marketed formulation

The developed UV method was successfully applied for estimation of Morin content in proliposome powder. By proposed UV method, Morin content in the proliposome powder was found to be 100.89 ± %.

CONCLUSION

A simple, accurate and precise UV-Visible spectrophotometric method for the estimation of Morin was developed and validated. The Proposed method was found to be robust and rugged in nature and was successfully used for the estimation of Morin.

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