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Development and Validation of High-Performance Liquid Chromatography Method for Estimation of Morin in Bulk and Formulation

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Abstract-- A new, simple, rapid, accurate and precise high-performance thin layer chromatography (HPLC) method has been developed and validated according to the guidelines of the International Conference on Harmonization (ICH Q2(R1) for the estimation of Morin in bulk and formulation. The chromatographic analysis were performed by an Waters 2998HPLC instrument using a X-Bridge, Waters C18, 250mmx4.6mm, 3.5 µm and mobile phase comprising0.1% acetic acid in water and Methanol (70:30 v/v)at flow rate of 1.0 ml/min. The eluent was monitored at 248 nm for determinationof Morin.The total run time was 10 min and the average retention time of Morin was found to be 4.611 min. The calibration curves were linear over the range of 1-12 ng/mL (R^2 = 0.999). The intra- and inter-day accuracy and precision values for all the analytes were within the acceptable range. The LOD and LOQ were 0.1176and 0.8618 ng/mL. The developed method was found to be robust. A simple, precise, accurate, linear and rapid RP-HPLC method was developed and validated as per ICH guidelines. The results suggest that the developed method was found to be robust and it can be applicable in routine analysis and efficiently used for the estimation of Morin in bulk as well as combined dosage form.

Keywords-- *Morin, HPLC method development, Validation*

I. INTRODUCTION

Morin is in a class of medications called a typical antipsychotics approved by the FDA for the treatment of schizophrenia in 2006. It works by changing the activity of certain natural substances in the brain[1]. It is used to treat the symptoms of schizophrenia (a mental illness that causes disturbed or unusual thinking, loss of interest in life, and strong or inappropriate emotions).Morin is the primary active metabolite of the older antipsychotic risperidone[2-3]. Morin has antagonist effect at ai and (X2 adrenergic receptors and at Hi histamine receptors. It does not bind to muscarinic acetylcholine receptors. In addition it binds with dopamine and serotonin receptors[4-5].

A literature survey on Morin revealed that, until now only few analytical methods were reported for its estimation of Morinsuch as UV-visible spectroscopy, HPLC method in bulk and API form[6-8]. However the reported RP-HPLC method utilizes complex mobile composition, so there is a need to develop an RPHPLCmethod having simple composition of mobile phase. Hence an attempt has been made to develop and validate a novel, simple and sensitive RP-HPLC method in accordance with ICHguidelines for the estimation of Morin in its bulk and formulation.

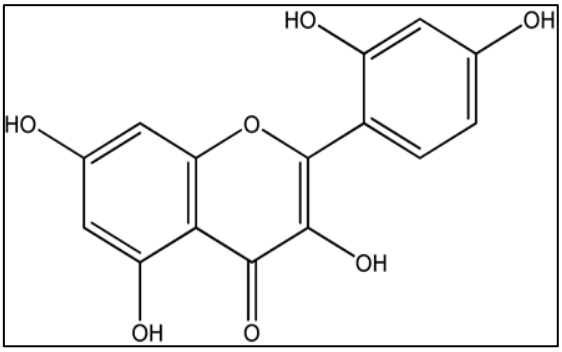

Figure 1: Chemical structure of Morin

II. MATERIALS AND METHODS

Chemicals and Reagents

Morin (purity 98% by HPLC)) was purchased from TCI Chemicals (India) Pvt. Ltd. All the other chemicals of analytical grade were used for the proposed study

Instruments

The chromatographic separation was performed on a Waters Binary gradient HPLC system, Japan, with the automatic injection facility was employed for this work. The detector consists of a Waters 2998 Photodiode Array Detector (PDA) and operated at 248 nm. The software used was Empower 2. The column used was C-18, 4.6x250mm 3.5 µm (XBridge). Chromatographic analysis was carried out at room temperature**.** Ultrasonicator (PCi Analyticals) is use for mobile phase degasing. Vibra HT (Essae) analytical balance was used for weighing of chemicals.

Preparation of Mobile Phase

The mobile phase 0.1% acetic acid in waterand Methanol were mixed in the ratio of 70:30 v/v and filtered through membrane filter (Millipore Nylon disc filter of 0.45 µ). This filtered mobile phase was sonicated for 15 min in ultrasonic bath.

Preparation of standard stock solution

Stock solutions (1 mg/mL) of morin prepared in HPLC grade methanol andfiltered through 0.45-m nylon membrane syringe filter.

Preparation of standard calibration curve

Calibration curve was prepared by diluting the stock-I solution to achieve the seven different calibration standards representing 1, 2,3, 4, 6, 10 and 12 ng/ml strength ofMorin. All these solutions were injected into HPLC column and the peak area of each solution was measured. The standard calibration curves of peak area Vs concentration (ng) were plotted.

Method Validation

The validation of pre-optimized chromatographic method was performed according to the Q2 (R1) guidelines of International Conference on Harmonization (ICH).Various analytical method validation parameters like system suitability, linearity, range, LOD, LOQ, accuracy, precision and stability were assessed [9-10].

System Suitability

Before performing the main analysis, the system suitability test was carried out using freshly prepared standard working solutions of 1.5 ng/mL of Morin.Standard working solution was repeated analyzed by using proposed HPLC conditions. During analysis, various parameters viz. retention time, peak area, and the number of theoretical plate were measured. Acceptable upper limit of % RSD for peak area and retention time was set at 2 whereas acceptable lower limit of number of theoretical plates was set at 2000. System was considered to be suitable only when obtained values were within the set limits.

Linearity& Range

Linearity of the proposed method was calculated by using seven different calibration standards of morin. The calibration curves were constructed using the Calibration Standards representing1, 2, 3, 4, 6, 8 and 12 ng/ml strength of Morin. Concentration vs. peak areas were plotted, subjected to linear regression analysis and linearity in terms of R-squared values and respective range were reported.

Accuracy (% Recovery):

Accuracy of pre-optimized HPLC method was assessed using recovery studies by standard addition method. To the solutions with predefined amount of Morin (1.5, 6 and 11.5 ng/mL), its 80, 100 and 120 % amount was added externally and the % recovery of the drugs was calculated.

Precision

The precision of the developed method was evaluated by performing Intra-day and Inter-day studies. Intra-day precision study was carried out by analyzing five replicates of three different concentrations (1.5, 12 and 11.5 ng/ml of Morin) at morning, afternoon and evening time of the same day. Similarly, inter-day precision study was carried out by analyzing the samples on three consecutive days. Intra- and inter-day precision results were expressed in terms of % RSD.

Robustness

Robustness of the proposed HPLC method was evaluated by making slight, deliberate change in chromatographic parameters viz. column temperature, flow rate of mobile phase and the mobile phase composition. Modified chromatographic conditions for the assessment of robustness were $\pm 1^{o}C$ deviation in column temperature,±1.0 ml/min deviation in flow rate of mobile phase and ±1 unit deviation in volume of methanol. For the robustness study, a solution (6 ng/ml) was repeatedly (n=5) analyzed for retention time and peak area of Morin using above mentioned modified chromatographic conditions. Results of the robustness study were expressed in terms of % RSD. Proposed method was considered to be robust only when the % RSD values for both retention time and peak areas were below 2.

Limit of detection (LOD) and Limit of quantification(LOQ)

LOD is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with acceptable accuracy and precision. LOD and LOQ were calculated using following formula.

$LOD = 3.3 \times SD/S$

$LOQ = 10 \times SD/S$

Where SD = standard deviation of response (peak area) and S = slope of the calibration curve.

Estimation of Morin content in pharmaceutical 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. Predefined volume of solution was analyzed using pre-optimized HPLC conditions. Contents of pharmaceutical formulation were calculated by comparing mean peak area of sample with that of the standard.

III. RESULTS AND DISCUSSION

Optimization of RP-HPLC Method

While developing HPLC method for estimation of morin, various mobile phase combinations and the stationary phases were tried. Selection of mobile phase composition and stationary phases was based on the solubility behavior, pKa values and the relative retention of morin was optimally resolved (Figure 2) over C-18 HPLC column using combination of 0.1% acetic acid in water and Methanol (70:30v/v) as a mobile phase. The details of optimized chromatographic conditions are shown in Table No. 1.

Table 1: The optimized chromatographic conditions

Separation variable	Optimized conditions
Chromatography	Waters Binary gradient
Column	C18-250 mm × 4.6 mm, 4.0 µ (Kromasil)
Mobile phase	0.1% acetic acid in waterand Methanol (70:30 v/v))
Flow rate	1 mL/min
Total Run Time	10 Min
Temperature	40^0C
Detection wavelength	250nm
Retention time	4.610 min

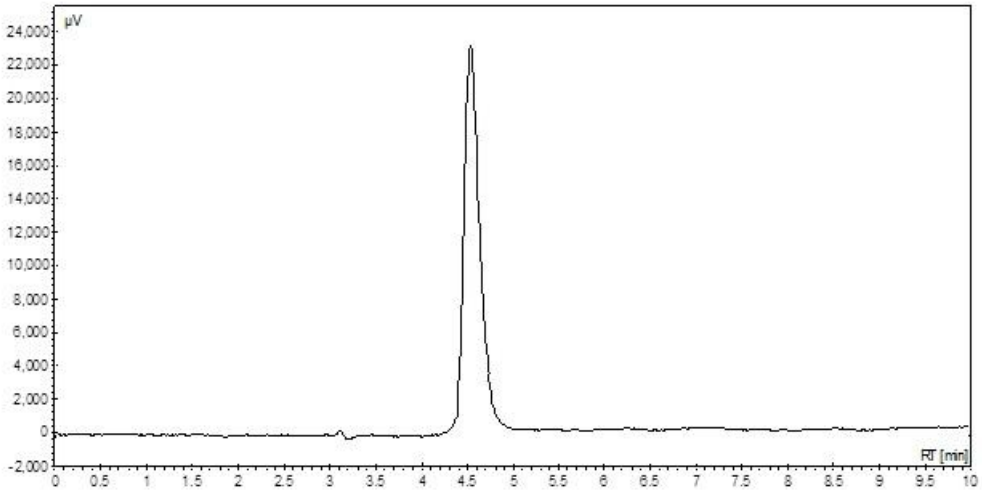

Figure 2: A typical RP-HPLC chromatogram of Morin

System suitability

During system suitability test, RSD of all parameter were calculated to evaluate the suitability of the developed method. From the results, it was found that %RSD for retention time and peak area was less than 2 and the number of theoretical plates were more than 2000 (Table 2). On the basis of obtained results, it was found that system is suitable for the analysis. The details of system suitability results are summarized in Table 2.

Table 2: System suitability parameters for Morin

Sr.No.	Parameter	Acceptance criteria	Results		
			Morin	%RSD	Status
1	Retention Time	%RSD ≤ 2%	4.611	0.057	Passed
2	Area	%RSD ≤ 2%	30159	0.8944	Passed
3	Theoretical	≥ 2000	4925	1.314	Passed

	plates				

Method validation

Linearity and Range

Linearity and range are the important 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(1-12 ng/ ml)were constructed. Different concentrations and peak area values are depicted in Table 3. Calibration curve when subjected to least square regression analysis yielded an equation; y = 20132x + 164.32 with correlation coefficient 0.9999 respectively (Fig. 4). From the linearity study, it was revealed that, there is a linear relationship between response and amount of drug within the range 1-12 ng/ml.

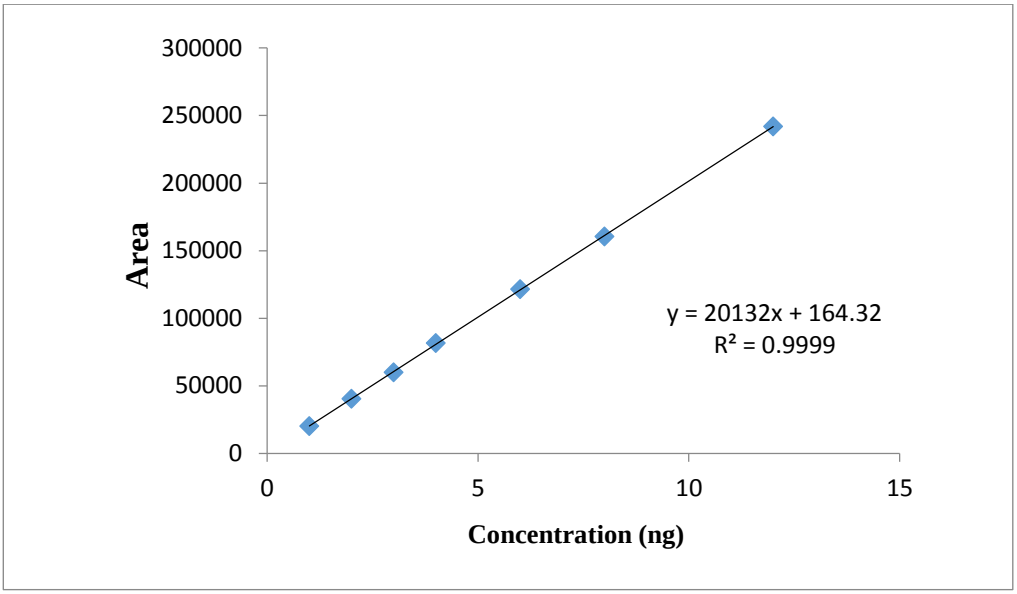

Figure 3: Calibration curve for Morin

Table 3: Calibration standard data for Morin

S. No	Conc. (ng/mL)	Peak Area
1	1	20154±0.85
2	2	40401±0.95
3	3	59955±0.0.62
4	4	81604±0.82
5	6	121475±0.42
6	8	160459±0.25
7	12	241871±0.16

Accuracy (percentage Recovery)

Accuracy is the closeness of test results to the true value obtained by proposed method. The accuracy of an analytical method should be established over its calibration range so that at any point of determination, results obtained would be accurate. For Morin, accuracy was determined using recovery studies. At 80, 100 and 120 % standard addition, mean recovery of morin was found to be in between99.91to 100.14 %. The relative standard deviation (% RSD) was found to be less than 2 (Table 4). From the results of accuracy studies, it was concluded that the proposed method is accurate.

Table 4: Recovery studies of Morin

Sr. No.	Sample	Spiked level	Theoretical Concentration (ng/mL)	Practical Concentration (ng/mL)	% Recovery	Mean % Recovery	% RSD
1	Morin	80%	1.2	1.199	99.91	100.07	1.08
		100%	6	6.011	100.18		
		120%	13.8	13.820	100.14		

Precision

Precision was studied by analysis LQC, MQC and HQC STDs containing both the drugs at concentrations covering the entire calibration range**.** The results expressed in terms of % RSD for the intra- and inter-day precision study (Table 4 and 5).Percent RSD values of intra-day precision study were found to be in between0.6475 to 1.0120, whereas inter-day precision was found to be in between 0.4875 to0.9738. It was concluded that the analytical technique showed good repeatability.

Table 5: Intra-day precision data for Morin

Sr.No.	Morin			
	Amount present (ng/ml)	Amount recovered (ng/ml)	% Assay	% RSD
1	1.5	1.498	99.86	1.0120
2	6	5.998	99.96	0.9490
3	11.5	11.495	99.95	0.6475

Table 6: Inter-day precision data for Morin

Sr.No.	Morin			
	Amount present (ng/ml)	Amount recovered (ng/ml)	% Assay	% RSD
1	1.5	1.495	99.66	0.9738
2	6	6.002	100.03	0.6444
3	11.5	11.499	99.99	0.4875

Robustness

An analytical method is considered to be robust when the small, internal changes in method parameters did not alter the final results significantly. Robustness of the proposed method was established by slightly changing the column temperature, mobile phase flow rate and mobile phase composition. It was found that, slight change in internal method parameters did not alter the final result (retention time and peak area) significantly. The % RSD values were found to be less than 2 (Table No.7). Thus, proposed method was found to be robust.

Table 7: Robustness study for Morin

Sr.	Parameter	Setting	Morin

			RT	% RSD	Peak Area	% RSD
1	Mobile phase flow rate (ml/min)	9	4.602	0.89	121349	0.4847
		1	4.610	0.64	121431	0.3897
		1.1	4.614	0.94	121512	1.046
2	Mobile phase composition (%, v/v)	69.5:30.5	4.608	0.49	121314	0.6589
		70:30	4.612	0.34	121475	0.4028
		70.5:29.5	4.615	0.58	121475	1.1235

LOD and LOQ

LOD and LOQ of proposed HPLC method was found to be 0.1176and 0.8618 ng/ml. Lower LOQ value indicated that proposed method would be sensitive enough to quantify the Morin content of samples at its lower level.

Estimation of Morin content in pharmaceutical formulation

Proposed validated analytical method was successfully applied to the determination of morin in pharmaceutical formulation. By proposed HPLC method, Morin content in the proliposomes formulation was found to be 99.62 ± 0.015 %.Further, it was found that proposed HPLC method is specific for the Morin.

CONCLUSION

An accurate, precise, sensitive yet robust HPLC method was developed and validated for the determination of Morin in bulk and formulation. Proposed HPLC method was found to be specific for morin and was free from any interference of formulation excipients. Proposed HPLC method can be used for routine analysis of morin in bulk as well as formulation.

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References

[1] Marino J, Caballero J. Morin extended-release for the treatment of schizophrenia. Pharmacotherapy, 2008; 28(10): 1283-98.

[2] Lehman AF, Lieberman JA, Dixon LB, et al. Practice guideline for the treatment of patients with schizophrenia, second edition. American Journal of Psychiatry 2004; 161:1-56.

[3] Fowler JA, Bettinger TL, Argo TR. Morin extended release tablets for the acute and maintenance treatment of schizophrenia. Clin Ther 2008; 30:231-248.

[4] Sandra B, Krishna T, Luc J, Bart R, Marc D M, Stefaan R, Nancy van O, Marielle E, and Adriaan C, Journal of Clinical Pharmacology, 2009, 49 (11), 1318.

[5] Green and Ben, Morin: A clinical review, Current Drug Therapy. 2009, 4, 7.

[6] Rudragangaiah, et al., Stability indicating RP-HPLC method for the quantification of morin in bulk and solid dosage form to establish validation and stability indicating parameters, 2019; 53(4): 166.

[7] K Umamahesswar, G Ramu and C Rambabu, A reverse phase HPLC method development and validation for the determination of Morin in pure and dosage forms, 2013; 2(1): 41-46.

[8] Atul P. Sherje et al., Stability indicating HPLC method for determination of Morin in Bulk, IJPTR, 2015; 8(8): 157-163.

[9] Note for guidance on validation of analytical procedures: text and methodology. European Medicines Agency: 1995; 1-15.

[10] Validation of analytical procedures: text and methodology q2 (r1). ICH harmonised tripartite guideline, (1994).

[11] Atul P. Sherje et al., Stability indicating HPLC method for determination of Morin in Bulk, IJPTR, 2015; 8(8): 157-163