Original Article

Optimization of a Validated UV-Spectrophotometric Methodology for Assessment of Apigenin in Bulk Powder

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ABSTRACT

Introduction: Apigenin (4,5,7 Trihydroxyflavone) is a natural compound of flavonoids class, which is found in various plant constituents. Since primitive years, Apigenin has been used as a traditional medicine, because it possesses biological functions. Common ayurvedic formulations comprising apigenin are in market. Hence quality control of preparation covering apigenin is desirable. **Objectives:** In present study, pointed to enhance and validate UV Spectrophotometric method to evaluation of apigenin in bulk powder. **Methods:** Using methanol as solvent, UV Spectrophotometric method was developed. Apigenin showed supreme absorbance wavelength at 267 nm. New technique enhanced and validated in terms parameters such Specificity, Discrimination, Linearity, Correctness, Ruggedness, Solution stability as per each ICH guidelines. **Results:** The detector response for apigenin was linear in the sure concentration range of 2μg/mL-10μg/mL with correlation coefficient of 0.9995. Newly developed method was found to be specific, Selective linear, Precise, Rugged, reproducible for estimation of apigenin with %RSD values less than 2%. **Conclusion:** In this study Development and Validation of UV-Spectrophotometric method can be employed for the apigenin assessment in bulk powder.

Key words: Apigenin, Absorbance, ICH guidelines, Ruggedness, Spectrophotometric, Ayurvedic Formulations.

INTRODUCTION

Apigenin is a very significant natural compound existing in various fruits and vegetables such as parsley, celeriac, celery, chamomile tea, orange, apple, tomatoes.[1] Apigenin in Figure 1 is a flavonoid that is used as a traditional medicine for decades, as it establish to show DPPH, antioxidant, *in vivo* animal anti-inflammatory, cytotoxic study, anti-bacterial, anti-viral and numerous biological activities. One of the very significant activities of apigenin is anticancer activity.[2-4] Numerous ayurvedic formulations comprising apigenin were marketed for health care delivery systems.[5] The quality control of formulations containing apigenin is important in the ayurvedic industries. Literature survey revealed that analytical methods such as spectrophotometric, HPLC and HPTLC methods[6-9] were reported for the estimation of apigenin in various plant extracts. The stated methods were having their own confines such as extra time consuming, use of costly and hazardous solvents. As per the literature review, there was no established UV spectrophotometric method for valuation of apigenin in the form of pure form. Henceforth, it is needed to develop and validate UV spectrophotometric method estimation of apigenin by using newer solvent system. In the present research an effort has been made to develop

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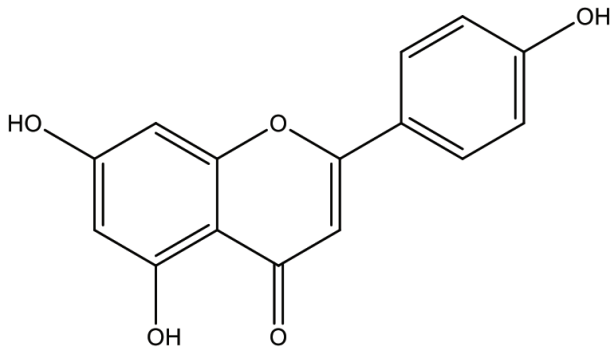

5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one

Figure 1: 2D structure of Apigenin.

and validate new UV-spectrophotometric method for estimation of apigenin in bulk powder.

MATERIALS AND METHODS

Chemicals

Apigenin was achieved as gift sample from Aktin chemicals, China. Beneficial chemicals and reagents used for the analysis pure, high analytical grade gotten from KAHER'S Dr. Prabhakar Kore Basic Science Research Center, Belagavi. UV-Spectrophotometer of Shimadzu make and model UV-1900 having UV probe software were used for analysis. Calibrated weighing balance was used for weighing drug sample in the study.

Method Optimization

Development also optimization of new UV Spectrophotometric method was accepted by selecting the proper solvent system the wavelength were recognized from review literature. Solubility of apigenin was screened by taking various solvents ethanol, methanol. In order to select the solvent system few trials were carried out, finally methanol was chosen as a solvent. Primary and secondary stock solution of apigenin was prepared by dissolving it in methanol. From these solutions working standard solution containing 10 µg/mL of apigenin was scanned between the wavelength regions of 400 - 200 nm against methanol as blank. The UV spectra were shown in and absorption curve showed characteristic absorption maxima at 267 nm and it was selected for analysis of apigenin.[10,11]

Method Validation

In order to validate newly developed method parameters as per the ICH guidelines (ICH guidance Q2A; Q2B) was followed.[12-15]

Specificity and selectivity

Employed standard solution containing 2 µg/mL Apigenin was observed between the range of 400-200nm. Spectrum of solvent as blank was obtained, analyzed for any interference of solvent at maximum wavelength of absorbance.

Linearity and range

While execution the study for linearity, 10mg of apigenin weighed and transferred into 10ml of volumetric flask and volume was attuned to the mark using methanol to obtained 1000µg/mL solution of apigenin. From this standard stock the working stock was prepared which consist of 1ml of standard stock was taken placed in another 10 mL volumetric flask covering methanol solvent and the volume was made up to the mark to 10mL, later further serial dilutions were made to prepare 2, 4, 6, 8 and 10 µg/mL solutions of apigenin. Solutions were ready in triplicates and absorbance's was measured at 267 nm.

Limit of Detection and Limit of Quantification

LOD and LOQ was calculated by using statistical calculations using formulas:

LOD = 3.3 × SD of y-intercept/ Slope of the calibration curve and LOQ= 10 x SD of y- intercept/ Slope of the calibration curve.

LOD = 3.3 × standard deviation of regression/ Slope

LOQ = 10 × standard deviation of regression/ Slope

Precision

Precision was measured in six replicates of solution containing apigenin that was prepared and the absorbance was recorded at 267nm on same day at different time intervals. On altered days the obtained system precision, intraday precision and interday precision data and absorbance were measured and % RSD was calculated.

Ruggedness and Reproducibility

In determine the ruggedness normally six replicates of solutions containing apigenin were prepared and absorbance of each replicate was measured by different analyst also by using different instruments and %RSD as calculated for absorbance.

Solution and standard stock solution stability

In order to check solution stability and obtained stability, fresh stock was prepared and dilutions were made using fresh solvent, absorbance's of each dilutions containing

apigenin was compared with that of old stock dilutions and % RSD for absorbance's was calculated.

RESULTS AND DISCUSSION

Optimization and validation

Solvent development step involves the use of methanol in which apigenin showed spectrum with maximum absorbance at 267 nm. Developed method parameters were presented below.

Specificity and selectivity

By obtaining solvent spectrum there was no interference of absorbance at 267nm hitch highlighted the specificity and method selectivity. The UV spectrum of solvent and apigenin is represented in Figure 2 and Figure 3 respectively.

Linearity and Range

Linearity and range was resolute by plotting standard calibration curve using concentration UV absorbance's obtained by linear dilution of apigenin. The absorbance range was between the concentration of 2, 4, 6, 8 and 10µg/mL. The regression equation of apigenin was 0.999. Linearity data as signified in Table 1. Overlay spectrum of linearity of apigenin is shown in Figure 4 with standard calibration curve was presented in Figure 5.

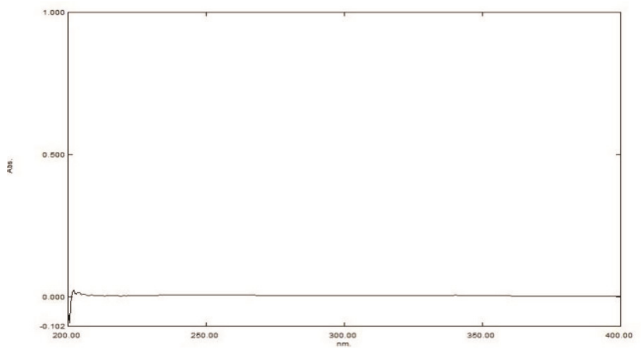

Figure 2: UV-Spectrum of Methanol.

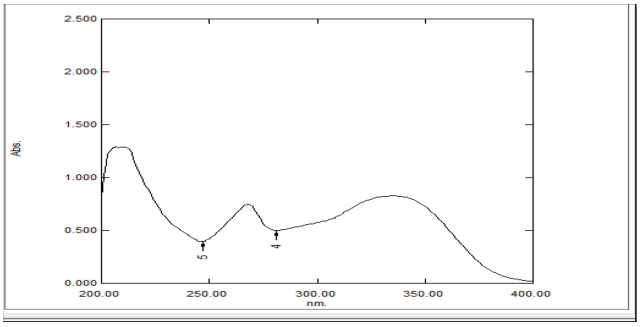

Figure 3: UV-Spectrum of Apigenin.

Table 1: Linearity and range data of apigenin.

Sr. No	Concentration	Absorbance at 267nm
1	2 µg/mL	0.179
2	4 µg/mL	0.288
3	6 µg/mL	0.401
4	8 µg/mL	0.519
5	10µg/mL	0.641
r^2		0.999
Slope		0.05775
LOD		0.27 µg/mL
LOQ		0.83 µg/Ml

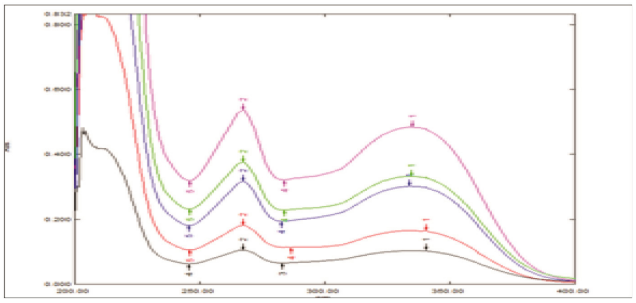

Figure 4: Overlay spectrum of Apigenin.

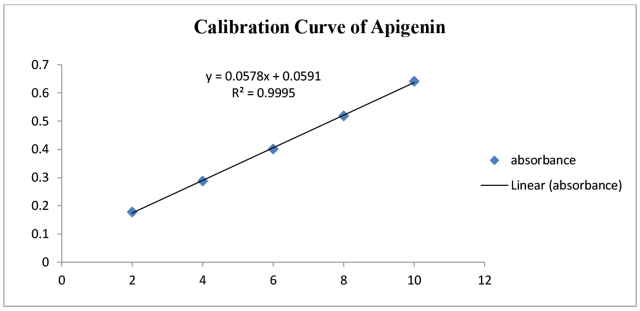

Figure 5: Standard calibration plot of Apigenin.

Limit of Detection and Limit of Quantification

By statistical calculation LOD and LOQ was found to be 0.27µg/mL and 0.83 µg/ml respectively.

Precision

Method was found to be precise as the %RSD calculated for six replicates solution of apigenin at each precision level was found to be less than 2% (Table 2).

Ruggedness and Reproducibility

The % RSD values calculated for apigenin was found to be less than 2% which indicates that the method was robust with slight change in the % composition of solvent system and reproducible as %RSD obtained for

Table 2: System, intraday and interday precision data of apigenin.						
Precision	**System precision**	**Intraday 1st hr**	**Intraday 5thhr**	**Interday -1 Precision**	**Interday -2 Precision**	**Interday -3 Precision**
2 µg/mL	0.164	0.171	0.168	0.171	0.155	0.161
2 µg/mL	0.167	0.174	0.172	0.174	0.159	0.165
2 µg/mL	0.168	0.172	0.172	0.172	0.154	0.169
2 µg/mL	0.166	0.167	0.174	0.167	0.157	0.167
2 µg/mL	0.167	0.164	0.176	0.164	0.159	0.164
2 µg/mL	0.166	0.166	0.177	0.166	0.161	0.166
%RSD	**0.82%**	**0.90%**	**1.87%**	**0.90%**	**1.69%**	**1.65%**
6 µg/mL	0.396	0.357	0.407	0.357	0.382	0.382
6 µg/mL	0.4	0.363	0.407	0.363	0.382	0.383
6 µg/mL	0.4	0.365	0.408	0.365	0.383	0.382
6 µg/mL	0.407	0.365	0.409	0.362	0.39	0.389
6 µg/mL	0.408	0.362	0.41	0.369	0.392	0.39
6 µg/mL	0.409	0.363	0.412	0.365	0.389	0.39
%RSD	**1.32%**	**0.81%**	**0.47%**	**0.81%**	**1.16%**	**1.04%**
10 µg/mL	0.633	0.591	0.639	0.633	0.631	0.619
10 µg/mL	0.634	0.597	0.641	0.634	0.636	0.622
10µg/mL	0.639	0.594	0.64	0.639	0.634	0.62
10 µg/mL	0.64	0.589	0.641	0.64	0.637	0.622
10 µg/mL	0.641	0.591	0.642	0.641	0.64	0.623
10 µg/mL	0.641	0.595	0.643	0.641	0.641	0.624
%RSD	**0.56%**	**0.50%**	**0.22%**	**0.56%**	**0.58%**	**0.29%**

Table 3: Ruggedness data of apigenin.					
Conc	**Absorbance**	**Conc**	**Absorbance**	**Conc**	**Absorbance**
2 µg/mL	0.168	6 µg/mL	0.368	10 µg/mL	0.633
2 µg/mL	0.172	6 µg/mL	0.369	10 µg/mL	0.639
2 µg/mL	0.172	6 µg/mL	0.369	10 µg/mL	0.64
2 µg/mL	0.174	6 µg/mL	0.362	10 µg/mL	0.641
2 µg/mL	0.177	6 µg/mL	0.363	10 µg/mL	0.641
2 µg/mL	0.176	6 µg/mL	0.365	10 µg/mL	0.642
%RSD	1.87%	%RSD	0.84%	%RSD	0.51%

absorbance's of each replicate of solutions was within the acceptance by change in the analyst and instrument (Table 3).

Solution stability

The solution stability for apigenin was determined by the %RSD or absorbance was calculated from prepared fresh solution and old solution containing apigenin. Results analyzed were found to be within the acceptance and data obtained revealed the standard stock solution.

Table 4: Solution stability data of apigenin.			
Solution stability		**Fresh stock dilutions**	**Old stock dilutions**
Replicates	**Conc.**	**Apigenin**	**Apigenin**
1	2 µg/mL	0.168	0.179
2	2 µg/mL	0.172	0.167
3	2 µg/mL	0.172	0.172
4	2 µg/mL	0.174	0.172
5	2 µg/mL	0.177	0.171
6	2 µg/mL	0.176	0.169
% RSD		1.87%	1.17%

Solution stability study was done for 4days and data have been displayed in (Table 4).

CONCLUSION

This paper presents in detail the development of a portable low-cost UV spectrophotometer which, by using an isolated compound, allows detection of developed, validated parameters, providing effective accurate results. Hence, the developed method is accurate, precise, reproducible and used as a quality control tool for analysis of apigenin in bulk. In future the methods

desirable to apply for estimation of apigenin in its various extract and other marketed dosage forms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

µg: Microgram; **mL:** Milliliter; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **UV:** Ultra violet spectrophotometer; **RSD:** Relative Standard Deviation; **ICH:** International Council of Harmonization.

Author Contribution

All the authors have equally contributed for the development and validation of apigenin. Ms. Priya Shetti contributed to develop and validate and method for apigenin in its pure form. Dr. Sunil S. Jalalpure guided for the present research work also in the reviving literature and writing the paper work.

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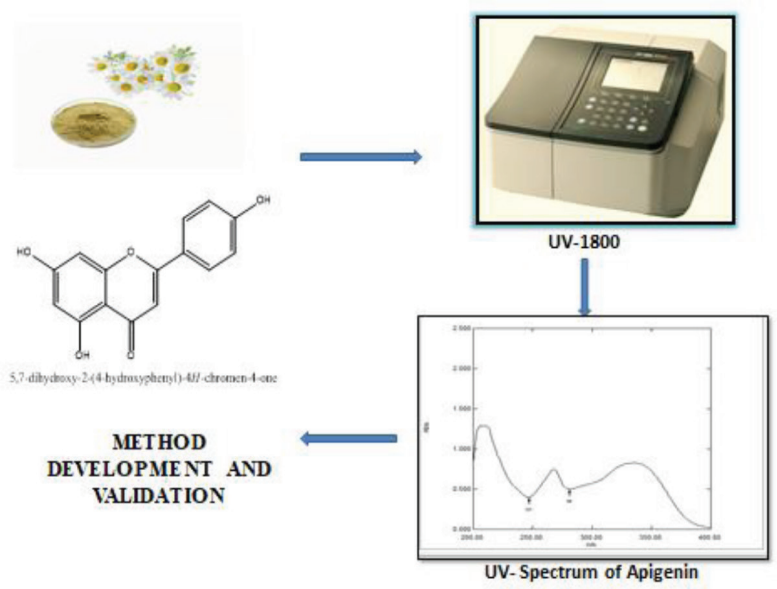
SUMMARY

The present study delivers a reference line data and analytical methods to contribution in the evaluation of apigenin, Plant flavonoid having capable chemo preventive activity counter to various cancers. Apigenin has low solubility which soluble in DMSO. Many studies on HPLC, HPTLC analysis for apigenin have been reported but till date UV Spectrophotometric method for apigenin isolated compound has not been reported. Here in this study the UV Spectra of apigenin was recorded by taking methanol as solvent, which sturdily absorbed gave two absorption spectra at a wavelength of 267 and 336nm herein we selected 267 nm as it produced a sharp peak compared to 336nm. Upright linearity was found with in concentration range of 2-10µg/mL with correlation coefficient (r^2) at 0.999. The data of precision study (interday and intraday) represent a good reproducibility with the RSD lower than 2.0% which shows that the method is precise. The use of costly analytical methods like HPLC, HPTLC limit the realizations of *in situ* studies because of their high cost, their limited portability, and even extend the time duration of the study. Henceforth the simple UV method was developed for the estimation of Apigenin in various extracts and in the marketed formulation and this developed method is validated according to the ICH guidelines.

PICTORIAL ABSTRACT



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