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Research Article

ESTIMATION OF QUERCETIN IN AYURVEDIC PROPRIETARY MEDICINE BY UV-SPECTROPHOTOMETRY

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ABSTRACT

A simple and reproducible U.V. Spectrophotometric method for the quantitative determination of Quercetin in *Madhujeevan churna (MJC)* was developed and validated. The hydroalcoholic extract of developed churna was obtained by continuous heat extraction method. The method was validated using parameters as linearity, precision, limit of detection, limit of quantification and recovery as per ICH guidelines. A new simple, rapid, sensitive, precise and economic spectrophotometric method in ultraviolet region has been developed for the determination of Quercetin in herbal formulation. The concentration of Quercetin present in raw material of madhujeevan churna was found to be 1.082 ± 0.011 w/w in Madhujeevan *churna (MJC)*. The samples were prepared in ethanol and methods obey Beers- Lamberts law in concentration ranges employed for evaluation. The result of analysis has been validated statistically and recovery studies confirmed the accuracy of the proposed method. Hence, the proposed method can be used for the reliable quantification of active marker compound in crude drug and its herbal formulations.

Keywords: *Madhujeevan churna*, Curcumin, Quercetin, U.V. Spectrophotometer.

INTRODUCTION

Madhujeevan churna (MJC) is well known ayurvedic Propritary formulation, traditionally used as anti-diabetics, anti-oxidant and anti-hyperlipidemic. *Madhujeevan churnam(MJC)* consist of 7 ingredients, *Curcuma longa, Aegle marmelos, Azardichata indica, Emblica officinalis Salacia reticulate Syzygium jambulanum,Stevia rebaudiana*. The world health organization (WHO) has emphasized the need to ensure the quality of medicinal plant products by using modern controlled technique and applying suitable standards[1]. For standardization of natural products, crude drugs, single chemical entities, "marker compounds", may be used as potency standards in U.V. analysis.[3]

In the past, the collection, identification, preparation of Ayurvedic medicines was done by the Acharys themselves. So drugs made by them were more efficacious, authentic and genuine. In the present age the suppliers make the collection. There are so many drugs, which lost their effectiveness with the passage of time. This causes the lowering the genuine character of the drug and make less efficacious. Until and unless, a method is not being developed to check the adulteration, it is too difficult to achieve the prestigious stage of Ayurveda. The checking of herbal drugs used in the preparation can be checked scientifically through certain well-established norms and standards through the research works.[2]

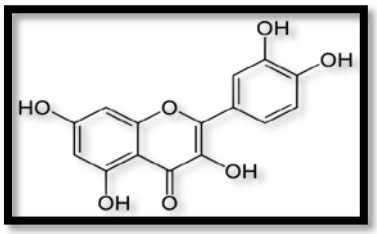

Fig. 1: Structure of Quercetin.

Quercetin is chemically, 2-(3,4-dihydroxy phenyl)-3,5,7-trihydroxy-4*H*-chromen-4-on Quercetin, a flavonol, is a plant-derived flavonoid found in fruits, vegetables, leaves and grains. It also may be used as an ingredient in supplements, beverages or foods. Quercetin is a flavonoid widely distributed in nature. Quercetin is frequently used therapeutically in allergic conditions, including asthma, hay-fever, eczema, and hives. Additional clinical uses include treatment of gout, pancreatitis, prostatitis, also in inflammatory conditions. Quercetin is used for treating conditions of the heart and blood vessels including "hardening of the arteries" (atheroscerosis), high cholesterol, heart disease, and circulation problems. It is also used for diabetes, cataract, hay fever, peptic ulcer, schizophrenia, inflammation, asthma, gout, chronic fatigue syndrome (CFS), preventing cancer and for treating chronic infections of the prostate. Quercetin is also used to increase endurance and improve athletic performance.

Literature survey reveals that several methods as U.V. [4,-6], HPLC [7,8], HPTLC [9,10], and Electrochemical determination of quercetin[11]; have been reported for estimation of Quercetin.

MATERIAL AND METHODS

Apparatus: Instrument used was an UV/Visible double beam spectrophotometer, SHIMADZU model 1800 (Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. An electronic analytical balance was used for weighing the sample.

Reagents and Materials

Ayurvedic Proprietary –MJC was procured as Gift sample from Dr. Wachasundar, Aniket Clinic, Magalwarpeth, karad. Dist- satara (Maharashtra).

All the chemicals and solvents were used of A. R. grade; standard. Quercetin was procured as gift sample from SDFCL, S.D. fine-chemicals limited, Mumbai.

Preparations of extract of madhujeevan churna (MJC)

The 300g of *Madhujeevan churna (MJC)* was extracted with a mixture of 95% ethanol & water (75:25) at 50-60^{0}C in a soxhlet apparatus separately. The extract was obtained concentrated to dryness in heating mental at a temperature of 35-40^0. The dried of the hydroalcoholic extracts weighed in a required dose and it was dissolved in known volume of distilled water, separately for further treatment.

Preparation of standard stock solution of Curcumin & Quercetin

The stock solution (100µg/ml)of Quercetin were prepared by dissolving accurately about 10mg of each drug in sufficient quantity of ethanol and then volume was adjusted to 100ml with ethanol. Further series of dilution were made with ethanol.

Calibration curve of Quercetin

A series of calibrated 10ml volumetric flask were taken and appropriate aliquots of the working standard solution of Quercetin were withdrawn and diluted up to 10ml with ethanol. The absorbance was measured at absorption maxima 256 nm against the reagent blank prepared in similar manner without the Quercetin.

Absorption maxima and Beer's law limit were recorded and data that prove the linearity and obeys Bee'r[12] law; limits were noted. The linear correlation between these concentrations (x-axis) and absorbance (y-axis) were graphically presented and slope (m), intercept (b) and correlation coefficient (R^2) were calculated for the linear equation (y=mx+b) by regression.

Estimation of Quercetin in MJC

The appropriate aliquots from extract of MJC churna were withdrawn in 10ml volumetric flask separately absorbance for aliquots of each was noted at 256nm for Quercetin. The corresponding concentration of Quercetin against respective absorbance value was determined by using the Quercetin calibration curve. The statistical analysis for checking uniformity in batches was also performed.

Table1: Content of Quercetin from MJC.

MJC	Quercetin content %w/w	1.082 ± 0.011

Validation of developed method

Linearity and range

The standard stock solution containing 100µg/ml each of Quercetin; was further diluted to get linearity concentration of 2-20µg/ml for Quercetin. Each concentration was analyzed in triplicates. Calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis. The relation between drug and its absorbance was expressed by equation y = mx+b, where m=slope, and b= intercept.

Limit of detection and limit of quantitation

LOD and LOQ of the drug were derived by calculating the signal -to-noise ratio (S/N, 3.3 for LOD and 10 for LOQ) using the following equation designated by ICH guidelines[13]. The residual standard deviation of regression line or standard deviation of y intercept of regression lines was used to calculate LOD and LOQ.

$$LOD = 3.3 \times \frac{D}{S}$$

$$LOQ = 10 \times \frac{D}{S}$$

Where, D=Standard deviation of y intercept of regression lines & S =Slope of calibration curve.

Recovery studies

It was carried out by standard addition method at three different levels. A known amount of drug was added to pre-analyzed sample and percentage recoveries were calculated.

Precision

The precision of the method was determined by carrying analysis of binary mixture of quercetin on same day (intra day precision) and on consecutive days (inter day).

RESULT AND DISCUSSION

The proposed method was validated as per ICH guideline. Method discussed in present work provides convenient and accurate way for simultaneous analysis of Quercetin.

Quercetin obeys Beer Lambert's law in concentration range 2-20µg/ml at the λ_{max} 256 nm. The correlation coefficiant (R^2) was calculated, where the (R^2) value 0.999 for Quercetin indicates the good linearity between the concentration and absorbance. The estimation of Quercetin in MJC was carried out. The concentration of Quercetin present in raw material was found to be 1.082 ± 0.011 w/w respectively in MJC.

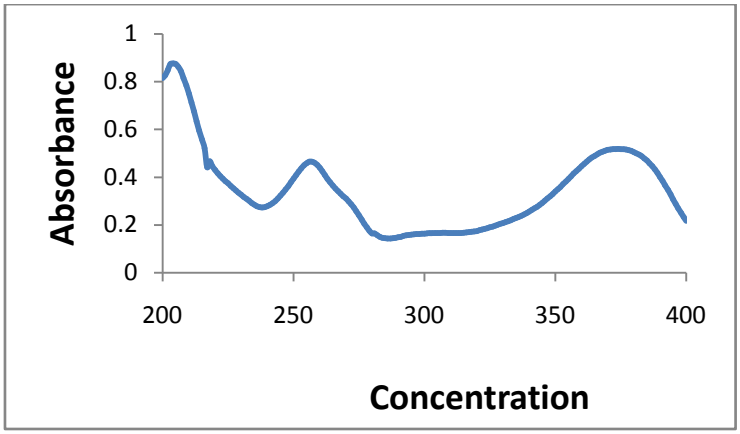

Fig. 2: Maxima absorption of Quercetin on U.V. spectrophotometer.

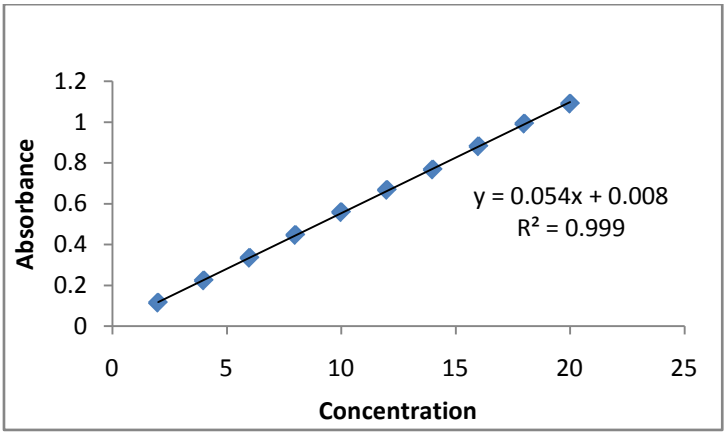

Fig. 3: Calibration curve of Quercetin on U.V. spectrophotometer.

In order to obtain precision and accuracy, the recovery study was performed at three levels by adding known amount of Quercetin with pre-analysed sample of extract of MJC. The experiment was repeated three times at three levels and result shows 99.39±0.17, 98.50±0.57 and 99.50±0.08 % recovery of Quercetin at all three levels with mean value 99.13±0.27 which prove reproductibility of the result. The % relative standard deviation (%RSD) value was found to be interday precision 0.51±0.0014 intraday precision 0.43±0.0058 for Quercetin .The low value of slandered deviation showed that, the method is precise. From the data; it is obvious that the present method of UV Spectrophotometric method determination of Quercetin is simple, precise, accurate and suitable for routine analysis of MJ churnna.

Recovery studies

Table 2: Recovery studies of Quercetin

Drug	Amount of drug taken(µg/ml)	Amount of drug added (%)	% Mean Recovery±S.D(n=3)
Quercetin	1	50	99.39±0.17
		100	98.50±0.57
		150	99.50±0.08

Validation parameter

Table 3: Validated parameters of Quercetin

Parameters		Method
Drugs		Quercetin
Wavelength range (nm)		256
Beer's law limit (µg/ml)		2-20
Regression equation y = mx+b, (m=slop, b= intercept)		y = 0.054x + 0.008
Slope (m)		0.054
Intercept (b)		0.008
Correlation Coefficient (r2)		0.999
Accuracy (Recovery) (n = 3)	I II III	99.39±0.17 98.50±0.57 99.50±0.08
Precision (% RSD, n=3)	Inter day Intra day	0.51±0.0014 0.43±0.0058
LOD (µg/ml)		0.09
LOQ (µg/ml)		0.27

CONCLUSION

Development and validation of spectrophotometric method for the estimation of Quercetin in MJC could be used as a valuable analytical tool in routine analysis, to check the batch to batch variations. After the drug is approved, pharmaceutical validation and development is necessary to ensure that the drug product will meet pharmaceutical standards for identity, strength, purity, stability, evaluation safety and efficacy. It provides strength and certain assurance of quality products. UV spectrophotometric estimation of active marker compound highlights assurance of batch uniformity and integrity of the product manufactured. Estimation of Quercetin by UV spectrophotometry can be used as one of the appropriate analytical method in MJC. UV analysis is most useful for quantitative estimation of target molecules in herbal products. UV detection of such compound is primary screening for further analysis of same by chromatoraphical technique.

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