



## SIMULTANEOUS ESTIMATION OF CURCUMIN AND QUERCETIN IN AYURVEDIC PROPRIETARY MEDICINE BY U.V. SPECTROPHOTOMETRY

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### ABSTRACT

A simple and reproducible U.V. spectrophotometric method for the quantitative determination of Curcumin and 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 such 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 and Curcumin in herbal formulation. The concentration of Quercetin and Curcumin present in raw material of madhujeevan churna was found to be  $1.082 \pm 0.011$  w/w and  $2.163 \pm 0.550$  w/w respectively. 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 Proprietary formulation, traditionally used as anti-diabetics, antioxidant and anti-hyperlipidemic. Madhujeevan churna (MJC) consist of seven ingredients, *Curcuma longa*, *Aegle marmelos*, *Azardichata indica*, *Emblica officinalis*, *Salacia reticulata*, *Syzygium jambolanum*, *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<sup>1</sup>. For standardization of natural products, crude drugs, single chemical entities, "marker compounds" may be used as a potential standards in U.V. analysis.<sup>2</sup>

In the past; the collection, identification, preparation of Ayurvedic medicines were done by the Acharyas 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 them 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 a certain well-established norms and standards through the research works.<sup>3</sup>

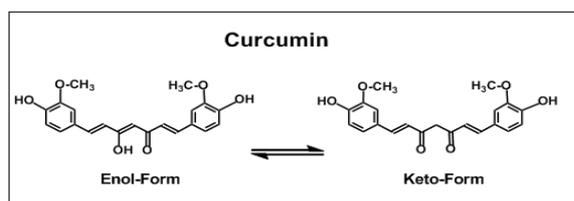


Figure 1: Structure of Curcumin

Curcumin is chemically, (1E, 6E)-1, 7-bis (4-hydroxy-3-methoxy phenyl) -1, 6-heptadiene-3,5-dione. It is the principle curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (*Zingiberaceae*). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are natural phenols and which are responsible for the yellow colour of turmeric. Curcumin has a long history of use for maintaining a healthy inflammatory response, via its effects on cyclooxygenase, prostaglandin and leukotriene metabolism. Curcumin appears to maintain healthy cell cycle function and provide important anti-oxidant defense. Furthermore, it supports the body's natural detoxification system and helps to maintain healthy hepatic function.

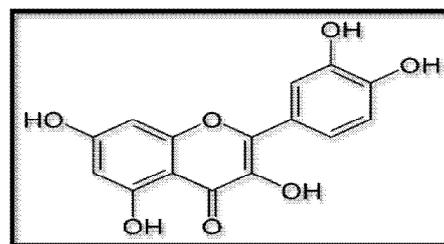


Figure 2: Structure of Quercetin

Quercetin is chemically, 2-(3,4-dihydroxy phenyl)-3,5,7-trihydroxy-4H-chromen-4-one. Quercetin, a flavonol, is a plant-derived flavonoid found in fruits, vegetables, leaves and grains. It also may be used as an ingredient of 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 and prostatitis; also used in inflammatory conditions. Quercetin is used for treating conditions of the heart and blood vessels including "hardening of the arteries"

(atherosclerosis), high cholesterol, and heart disease and circulation problems. It is also used for diabetes, cataract, hay fever, peptic ulcer, schizophrenia, inflammation, asthma, gout, chronic fatigue syndrome (CFS), and 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 such as U.V.<sup>4-6</sup>, HPLC<sup>7,8</sup>, HPTLC<sup>9,10</sup>, and Electrochemical determination of quercetin<sup>11</sup>, have been reported for estimation of Quercetin. Also Spectrofluometric estimation<sup>12</sup> like U.V.<sup>13</sup> HPLC<sup>14-16</sup>, and HPTLC<sup>17-19</sup>, have been reported for estimation of Curcumin. Not a single U.V., HPLC or HPTLC method is reported so far for simultaneous analysis of curcumin and quercetin in herbal dosage form.

**MATERIAL AND METHODS**

**Apparatus:** Instrument used was an UV/Visible double beam spectrophotometer, SHIMADZU model no.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 all the samples.

**Reagents and Materials**

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

All the other chemicals and solvents were used, are of A.R. grade; standard curcumin was procured as gift sample from Synthite Industries Ltd, kolenchery kerla, India. Quercetin was procured as gift sample from SDFCL, S.D. fine-chemicals limited, Mumbai.

**Preparations of extract of madhujeevan churna (MJC)**

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

**Preparation of standard stock solution of Quercetin and Curcumin**

The stock solution (100µg/mL) of Quercetin and Curcumin 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 dilutions were made with ethanol.

**Calibration curve of Quercetin and Curcumin**

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 Quercetin. Same procedure was applied for Curcumin and absorbance was measured at 263 nm, against reagent blank prepared in similar manner without Curcumin. Absorption maxima and Beer’s law limit were recorded and data that prove the linearity and obeys Bee’r law; limit were noted. The linear correlation between these concentrations (x-axis) and absorbance (y-

axis) were graphically presented. Slope (m), intercept (b) and correlation coefficient (R<sup>2</sup>) were calculated from the linear equation (Y=mx+b) by regression.

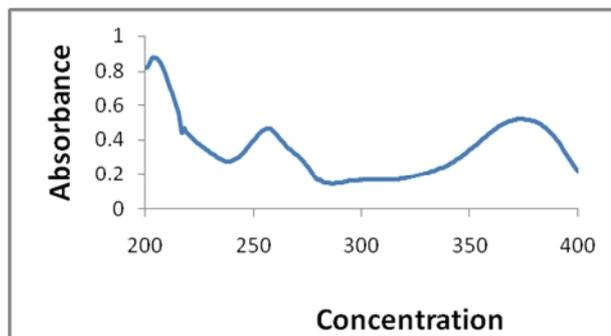


Figure 3: Maxima absorption of Quercetin on U.V. spectrophotometer (256 nm)

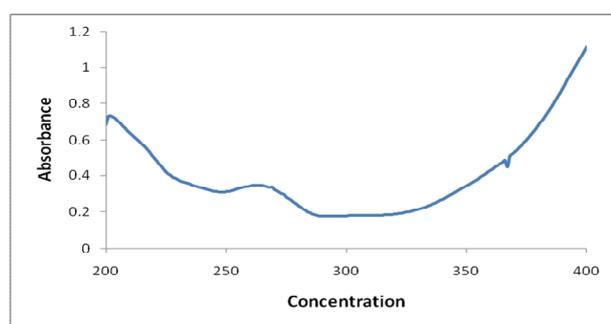


Figure 4: Maxima absorption of curcumin on U.V. spectrophotometer (263 nm)

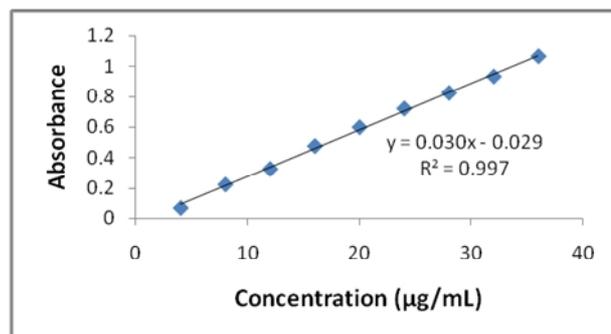


Figure 5: Graph showing calibration curve of curcumin

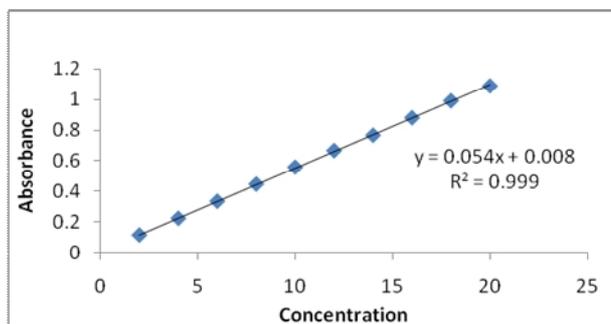


Figure 6: Calibration curve of Quercetin on U.V. spectrophotometer

**Estimation of Quercetin and Curcumin in MJC**

The appropriate aliquots; from the extract of MJC churna were withdrawn in 10mL volumetric flask separately, absorbance for aliquots of each was noted at 256 nm and 263 nm for Quercetin and Curcumin respectively. The corresponding concentration of Quercetin and Curcumin against respective absorbance value was determines using

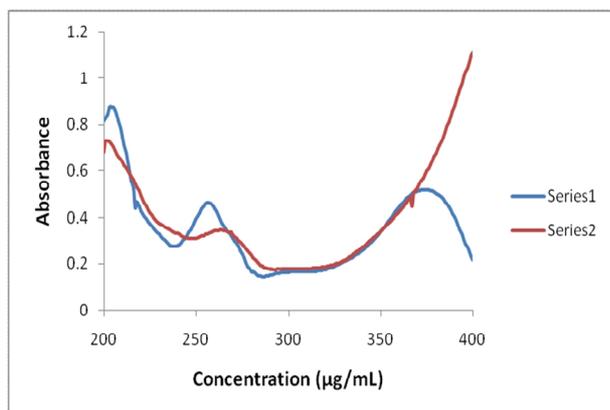
the Quercetin and Curcumin calibration curve. The statistical analysis for checking the uniformity in all batches was performed.

**Table 1: Estimated results of Quercetin and Curcumin with S.D. in MJC**

MJC	Quercetin content %w/w	1.082 ± 0.011
	Curcumin content % w/w	2.163 ± 0.550

**Simultaneous equation method**

From the overlain spectra (show in figure 7) of Quercetin (10µg/mL) and Curcumin (10µg/mL), two wavelengths i.e. 256nm as λ<sub>max</sub> of Quercetin and 263nm as λ<sub>max</sub> of Curcumin were selected as the working wavelength, at which both drugs showed absorbance for each other. The absorptivity of these two drugs was determined at 256nm and 263nm. A set of two simultaneous equations were formed using absorptivity values as given below, at selected wavelength. The concentrations of two drugs in mixture were calculated using set of two simultaneous equations.<sup>20</sup>



**Figure 7: Overlay of Maxima absorption of Quercetin and Curcumin on U.V. spectrophotometer**

$$C_x = \frac{A_2 ay_1 - A_1 ay_2}{Ax_2 ay_1 - ax_1 ay_2} \text{-----(1)}$$

$$C_y = \frac{A_1 ax_2 - A_2 ax_1}{Ax_2 ay_1 - ax_1 ay_2} \text{-----(2)}$$

Where, C<sub>x</sub> and C<sub>y</sub> are concentrations of Quercetin and Curcumin in µg/mL respectively in known sample solution. A<sub>1</sub> and A<sub>2</sub> absorbances of sample solutions at 256nm and 263nm respectively.

ax<sub>1</sub> and ax<sub>2</sub> are absorptivity of Quercetin at 256 nm and 263 nm, ay<sub>1</sub> and ay<sub>2</sub> are absorptivity of Curcumin at 256 nm and 263 nm.

The concentration of C<sub>x</sub> and C<sub>y</sub> in herbal formulation can be obtained by solving equation (1) and (2). Validity of above framed equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components.

**Validation of developed method**

**Linearity and range**

The standard stock solution containing 100µg/mL each of Quercetin and Curcumin was further diluted to get linearity concentration of 2-20µg/mL for Quercetin and 4-36µg/mL for Curcumin. 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 is expressed by equation y = mx+b, where m=slope, and b= intercept.

**Limit of detection and limit of quantization**

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<sup>21</sup>. 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=Slop 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 intraday precisions were determined by estimating the corresponding response 3 times on the same day for Quercetin and Curcumin; whereas the interday precision were determined by estimating the corresponding response on 3 different days over a period of 1 week. The results were reported in terms of relative standard deviation (RSD).

**RESULT AND DISCUSSION**

The proposed method was validated as per ICH guideline<sup>21</sup>. Method discussed in present work provides convenient and accurate way for simultaneous analysis of Quercetin and Curcumin.

Quercetin and Curcumin obeys Beer Lambert’law in concentration range 2-20µg/mL at the λ<sub>max</sub> 256 nm, 4-36µg/mL at the λ<sub>max</sub> 263 nm respectively. The correlation coefficient (R<sup>2</sup>) was calculated, where the (R<sup>2</sup>) value 0.999 for Quercetin and 0.997 for Curcumin indicates the good linearity between the concentration and absorbance. The estimation of Quercetin and Curcumin in MJC was carried out. The concentration of Quercetin and curcumin present in raw material was found to be 1.082 ± 0.011 w/w and 2.163 ± 0.550 w/w respectively in MJC.

In order to obtain precision and accuracy, the recovery study was performed at three levels by adding known amount of Quercetin and Curcumin with pre-analysed sample of extract of MJC.

The experiment was repeated three times at three levels and result shows  $99.39 \pm 0.17$ ,  $98.50 \pm 0.57$ ,  $99.50 \pm 0.08$  % recovery of Quercetin at all three levels with mean value  $99.13 \pm 0.27$  and  $99.07 \pm 0.88$ ,  $99.37 \pm 0.24$ ,  $99.01 \pm 0.62$  % recovery of Curcumin at all three level with mean value of  $99.15 \pm 0.20$  which prove reproductibility of the result. The % relative standard deviation (%RSD) value was found to be interday precision  $0.51 \pm 0.0014$  and

$0.76 \pm 0.00215$ , intraday precision  $0.43 \pm 0.0058$  and  $0.56 \pm 0.0041$  for Quercetin and Curcumin respectively. The low value of standard deviation showed that, method was precise. From the data, it indicate that the present method of UV Spectrophotometric method determination of Quercetin and curcumin is simple, precise, accurate and suitable for routine analysis of MJ churnna.

### Recovery studies

**Table 2: Recovery study of Curcumin and Quercetin**

Drug	Amount of drug taken( $\mu\text{g/mL}$ )	Amount of drug added (%)	% Mean Recovery $\pm$ S.D (n=3)
Quercetin	1	50	$99.39 \pm 0.17$
		100	$98.50 \pm 0.57$
		150	$99.50 \pm 0.08$
Curcumin	2	50	$99.07 \pm 0.88$
		100	$99.37 \pm 0.24$
		150	$99.01 \pm 0.62$

### Validation parameter

**Table 3: Validated parameters for Curcumin and Quercetin**

Parameters		Method	
Drugs		Quercetin	Curcumin
Wavelength range (nm)		256	263
Beer's law limit ( $\mu\text{g/mL}$ )		2-20	4-36
Regression equation $y = mx + b$ , (m=slop, b= intercept)		$y = 0.054x + 0.008$	$y = 0.030x - 0.029$
Slope (m)		0.054	0.030
Intercept (b)		0.008	-0.029
Correlation Coefficient (r2)		0.999	0.997
Accuracy (Recovery) (n = 3)	I	$99.39 \pm 0.17$	$99.07 \pm 0.88$
	II	$98.50 \pm 0.57$	$99.37 \pm 0.24$
	III	$99.50 \pm 0.08$	$99.01 \pm 0.62$
precision (% RSD, n=3)	Inter day	$0.51 \pm 0.0014$	$0.76 \pm 0.00215$
	Intra day	$0.43 \pm 0.0058$	$0.56 \pm 0.0041$
LOD ( $\mu\text{g/mL}$ )		0.09	0.105
LOQ ( $\mu\text{g/mL}$ )		0.27	0.318

### CONCLUSION

Development and validation of Spectrophotometric method for the estimation of Quercetin and Curcumin in MJC could be used as a valuable analytical tool in routine analysis. 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 and Curcumin by UV spectrophotometry can be used as one of the appropriate analytical methods 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 chromatographical technique.

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