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Validated UV Spectrophotometric Method for Simultaneous Estimation of Curcumin and Rutin

Running title: Simultaneous estimation of curcumin and rutin by UV

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ABSTRACT
Anabsorption correction method of estimation for the simultaneous estimation of curcumin and rutin in their bulk powder was developed and validated as per ICH guidelines. The method involved measurement of absorbance of the curcumin and rutin at the λmax of the drugs i.e., 424nm and 257 nm in methanol. Calibration curves of curcumin and rutin were found to be linear in the range 1-7 µg/mL and 10-70 µg/mL respectively. The correlation coefficient values R^2 was found to be 0.9987 and 0.9993. LOD and LOQ were 0.0012 µg/mL and 0.7097 µg/mL for curcumin, 0.037.6 µg/mL and 2.15 µg/mL for rutin.The percentage recovery was 99.2 ± 0.278 for curcumin and 98.66 ± 0.260 for rutin was estimated, thereby confirming accuracy of the method developed. In the precision study, the %RSD was found to be less than 2%, indicating the method is precise. Hence it can be concluded that the method developed is simple, accurate, precise, and economical.

Keywords: Curcumin, Rutin, Simultaneous estimation and Validation.

I. INTRODUCTION

Curcuma longa (Zingiberaceae) contains an active principle compound curcumin,[1] chemically known as 1, 7 bis (4- hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5 dione. It has been used for treatment of several diseases since centuries due to its therapeutic benefits on autoimmune, cancer, cardiovascular, neurodegenerative and pulmonary diseases, where inflammation is involved as major mechanistic pathway.[2]Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonoid compound.Rutin displayseveral pharmacological properties, including antioxidant, anti-cancer, cytoprotective, anti-platelet, anti-thrombotic, vasoprotective, cardioprotective, and neuroprotective activities.[3] Even though both drugs possess a wide range of physiological and pharmacological properties, several studies revealed it's limitation of therapeutic applicability in terms of low bioavailability in the intestine. [4,5]Literature survey reveals that there was no method of estimation available for the selected drug combination by UV. Hence this works aims at developing a simple, rapid and economic method for simultaneous estimation ofcurcumin and rutin by UV by applying absorption correction method.

II. MATERIALS & METHODS

Instrument details: -A double beam UV-visible spectrometer (UV-1900I, Shimadzu) was used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Curcumin wasobtained from Konark Herbals & Health Care, Mumbai. Rutin was purchased by Kshipra biotech PVT. LTD. Methanol was purchased from SD Fine-Chem Ltd. The other chemicals were of analytical grade.

Selection of solvents for the simultaneous estimation

Thorough selection of solvent system was done based on the stability of the drugs individually and in combination as depicted in the table 1. The stability was decided based on %RSD of interday and intraday studies.

Simultaneous estimation

If the identity, concentration and absorptivity of the absorbing interferents are known, it is possible to calculate their contribution to the total absorbance of a mixture. The concentration of absorbance component of interest is then calculated from the corrected absorbance (total absorbance minus the absorbance of the interfering substances) in the usual way.

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Preparation of standard solutions

Accurately weighed quantities (10 mg) of Curcumin and Rutin were taken in 10mL volumetric flasks separately, dissolved by adding 10 mL of methanol (1000 µg/ml). This solution was used as primary working standards solution.

From the primary standard stock solution series dilutions were made to get curcumin 1-7 µg/mL and rutin 10 - 70 µg/mL concentration

The solutions were scanned from 500 to 200 nm and the absorbance was noted at both the wavelengths 257 nm and 424 nm, standard graph was plotted for each drug at both the wavelengths.

By using absorbance correction method[6]

In this method, solution of Curcumin and rutin (10 µg/mL, each), was prepared by - appropriate dilution of standard stock solution with Methanol and scanned in the spectrum mode from 500 to 200 nm. The λmax of curcumin in methanol was 424 nm(λ2), where Rutin has no significant absorbance and λmax of Rutin in methanol was 257 nm(λ1), where curcumin also shows absorbance at 257 nm. To get concentration of rutin at 257 nm the absorbance was corrected.

Steps for calculation.

- Since rutin doesn't absorb at 424 nm, The concentration of curcumin was determined by the absorbance at 424 nm
- The absorbance of curcumin at 257 nm was determined
- The absorbance got at 257 nm was subtracted with determined absorbance of curcumin at 257 nm to get corrected absorbance of rutin
- The concentration of rutin from corrected absorbance at 257 nm was calculated.

The concentrations of drugs in sample solution were determined by using the following formula:

A = abc.....(1)

Where,

A – Absorbance
a - Absorptivity
b - Path length
c – concentration

III. VALIDATION OF ANALYTICAL METHOD[7]

Method Validation: The method was validated according to ICH Q2 (R1) guidelines for the following parameters.

3.1 Linearity and range: Linearity of the developed method was established from the calibration curves prepared using linear regression analysis. Based on the measurement of the absorbance for the calibration standards, graph was plotted against the respective concentration to obtain the standard calibration graph. The procedure was repeated three times and the average values of absorbance were calculated. The data obtained was statistically evaluated to obtain the standard deviation of the said values and regression coefficient.The calibration curve range was decided depending upon the Beer-lamberts law adherence.

3.2 Accuracy: Accuracy was assured by standard addition technique, performed by addition of known amounts of pure CUR and RUT to known concentrations of sample solution. The resulting mixtures were assayed in triplicate and results obtained were compared with expected results (Table3).

3.4 Precision

3.4.1 Repeatability: Repeatability was determined by analyzing different levels of drug concentrations in mixed standards from independent stock solutions with minimum three replication within in the same day i.e., 0, 8th and 24th hour (Table4).

3.4.2 Intermediate precision: Inter-day variations in estimation were determined to assess intermediate precision of the proposed method. The absorbance of seven determinations of working solution was taken on different days. Concentration ofcurcumin and rutin in each replicate was calculated. The standard deviation was calculated from the concentrations of curcumin and rutin in seven determinations of working solution (Table 4).

3.5 Limit of Detection and Limit of Quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the drugs by the proposed method were determined using calibration standards. (Table1). The LOD and LOQ were calculated as per equations 1 and 2 respectively,

LOD = 3.3 (SD Intercept / Slope) ....(2)

LOQ = 10 (SD Intercept / Slope) ....(3)

Where "SD intercept" is the standard deviation of the intercept of regression line and "Slope" is the slope of the calibration curve.

IV. RESULTS AND DISCUSSIONS

Curcumin and rutin act as anti-inflammatory drugs which have been used in the treatment of several disease from ancient times, hence development of analytical method which is simple and economic is the need of the hour. There are various techniques for estimation of the drugs,

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of all the simplest and most economic method is UV-spectrophotometric method, because the method is cost effective and less laborious in comparison to the HPLC method of analysis.

4.1 Selection of solvents for the simultaneous estimation

Table 1 Screening of solvents for stability

S No.	Solvents used	Inference in curcumin	Inference in rutin
1	Phosphate buffer pH 7.4	Not stable	Stable
2	PB pH 7.4 +2% ascorbic acid	Stable	Not stable
3	BHT + methanol in PB pH 7.4	Turbidity	Turbidity
4	BHT + ethanol in PB pH 7.4	Turbidity	Turbidity
5	BHT + chloroform in PB pH 7.4	Turbidity	Turbidity
6	BHT + DMSO in PB pH 7.4	Turbidity	Turbidity
7	BHT + ethanol in PB pH 7.4 (1:1) ratio	No turbidity	No turbidity
8	Ethanol	Stable	Stable
9	Ethanol: PB pH 7.4 (1:9)	Not stable	Stable
10	Ethanol: PB pH 7.4 (2:8)	Not stable	Stable
11	Ethanol: PB pH 7.4 (3:7)	Not stable	Stable
12	Ethanol: PB pH 7.4 (4:6)	Not stable	Stable
13	Ethanol: PB pH 7.4 (1:1)	Stable	Stable
14	Methanol	Stable	Stable

Thorough selection of solvent system was done based on the stability of the drugs individually and in combination as depicted in the table 1. The stability was decided based on %RSD of interday and intraday studies.

Initially the trial was done in phosphate buffer pH 7.4, wherein rutin was stable but curcumin exhibited stability less than 4 hours. Hence, 2% ascorbic acid was used to stabilize curcumin which resulted in Curcumin's stability for 2 days but rutin was not found to be stable.

By literature survey butylated hydroxytoluene (BHT) which was synthetic antioxidant was used, BHT was insoluble in water or other buffers as it was lipophilic organic compound. Hence, BHT was solubilized using organic solvents like methanol, chloroform, DMSO individually and in combinations. All the organic solvents tested resulted in visual turbidity, whereas the ethanol combination didn't show any turbidity. However, it was observed that BHT was exhibiting absorbance at 271 nm which is similar to the rutin λmax, hence was not used further. In Ethanol both the drugs were stable, in order to reduce the use of organic solvents, mixture of ethanol and PBS pH 7.4 was used. Different ratios were screened, where only in the ethanol: PB pH 7.4 (1:1) ratio both the drugs were stable.

In ethanol + PBS pH 7.4 (1:1) ratio the method reproducibility and accuracy were less, whereas, in methanol the method was reproducible and accurate for analysis of curcumin and rutin with %RSD less than 2. Therefore, methanol was selected as stable solvent in the simultaneous estimation of curcumin and rutin.

The simultaneous estimation was done by using absorbance correction method for estimation of rutin as curcumin was showing absorbance at the λmax of rutin. However, Curcumin was estimated by single component method as rutin did not exhibit interference at λmax of curcumin. (Figure 1)

4.2 Absorbance correction method

The utility of dual wavelength data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. For elimination of the effects of an interfering component, two specific wavelengths are chosen.

1. First wavelength λ_1 was the wavelengths at which the maximum absorbance of RUT was observed, and also CUR gives some absorbance at this wavelength (257.0 nm).
2. Second wavelength λ_2 at which maximum absorbance of CUR was observed and there was no interference of RUT at this wavelength (424.0 nm).

In this proposed method the absorbance of RUT alone in a mixture of CUR and RUT was determined using absorbance correction method. To remove the interference of CUR to the absorbance at 257.0 nm (λ_1), the wavelength of maximum absorbance for CUR, another wavelength 424 nm (λ_2) was found out at which the

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absorbance of RUT was sufficiently small hence, can be ignored. (This condition is satisfied if the absorbance of the interfering substance is less than 1%) This was confirmed by measuring the absorbance of various dilution of RUT in methanol at 257.0 nm and 424 nm. These two selected wavelengths were employed to determine the concentration of RUT from the mixture of CUR and RUT (Figure1). The difference in absorbance at these two wavelengths ($A_{257} - A_{424}$) cancels out the contribution of absorbance of CUR in mixture.

4.3 Linearity: The non overlapping spectra (Fig 1) of CUR and RUT exhibit lambda max of 424nm and 257nmrespectively**.** The calibration curve was plotted by taking absorbance versus concentration in the range of 1-7µg/mL and 10-70µg/mL forCUR and RUT respectively (Fig 2 & 3). The curves were linear and the correlation coefficient value(R^2**)** was 0.9984 and 0.9993 for CUR and RUT respectively.

4.4 Accuracy: The accuracy of the method is required to be obtained within the linearity range of the method developed. Accuracy of the developed method was dogged from recovery studies.The recovery of CUR and RUT from the standard mixture solution was found to be in the range of 99-101% (table 2) and the %RSD was found to be less than 2%, indicating the method is accurate and reproducible.

4.5 Precision: Precision of the method was verified by repeatability and intermediate precision studies. Intermediate precision of the method was checked by assay the sample solution on three different days (table 3). This study indicates that the solutions can be analyzed up to 120 hours without affecting the stability of the drug in the solvent. The % RSD value was found to be less than 2%, indicating the method is precise.

4.6 Limit of Detection and Limit of Quantification: LOD and LOQ was found to be 12.4ng/mL and 37.6ng/mL for curcumin, 709.7ng/mL and 2150.6ng/mL for rutin respectively. Hence it can be concluded that the method can be utilized to detect the curcumin at nanogram and rutin at micrograms level. (table 2)

V. CONCLUSION

The method developed was found to be simple, precise and accurate. Further the method was selective for the simultaneous estimation of curcumin and rutin in pure form. Hence can be used for the routine analysis in the quality control laboratories.

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Table 2: Regression analysis data and summary of validation parameters for the proposed absorbance correction method

Parameters	**Absorbance Correction Method**	
	CURCUMIN	**RUTIN**
λmax	424 nm	257 nm
Concentration range (µg/ml)	1-7	10-70
Slope	0.1183	0.014
Intercept	0.0081	0.0079
Correlation Coefficient (r^2)	0.9984	0.9993
LOD (µg/ml)	0.0124	0.0376
LOQ (µg/ml)	0.7097	2.150
Accuracy (% recovery, n = 3)	99.2 ±0.278	98.66 ± 0.260
Repeatability (%RSD, n = 3)	0.280	0.264
Precision (% RSD)		
Interday (n = 7)	0.187 – 0.280	0.40 – 0.264
Intraday (n = 7)	0.147 – 0.318	0.264 – 0.422

Table 2: Recovery results of CUR and RUT

Drug in standard mixture (µg/ml)		**Amount of drug added (µg/ml)**		**Mean % Recovery**	**%RSD**	**Mean % Recovery**	**%RSD**
CUR	**RUT**	**CUR**	**RUT**	**CUR**		**RUT**	
1	1	10	10	99.2 ± 0.278	0.280	98.66±0.260	0.264
1	1	10	10				
1	1	10	10				

Table 3: Precisions results of CUR and RUT

Parameters	**Sampling time**	**Curcumin**		**Rutin**	
		% Assay	**% RSD**	**% Assay**	**% RSD**
Repeatability (n=3)	0 hour	99.2± 0.278	0.280	98.66 ± 0.260	0.264
	8^{th} hour	99.42 ± 0.186	0.187	98.5 ± 0.4	0.40
	24^{th} hour	99.73 ± 0.246	0.246	98.63 ± 0.5	0.51
Intermediate Precision (n=3)	Day 1	99.2 ± 0.278	0.280	98.66 ± 0.260	0.264
	Day 2	99.83 ± 0.147	0.147	98.43 ± 0.416	0.422
	Day 3	100.23 ± 0.319	0.318	98.96 ± 0.324	0.324

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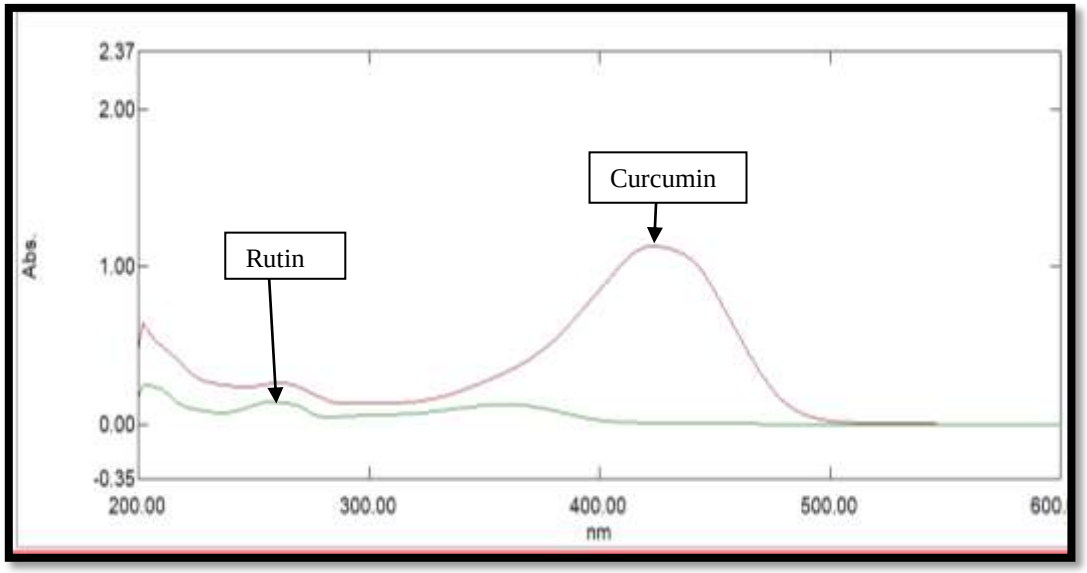

Figure: 1 Spectrum of curcumin and rutin (1:1) ratio exhibiting λmax at 424 and 257 nm respectively

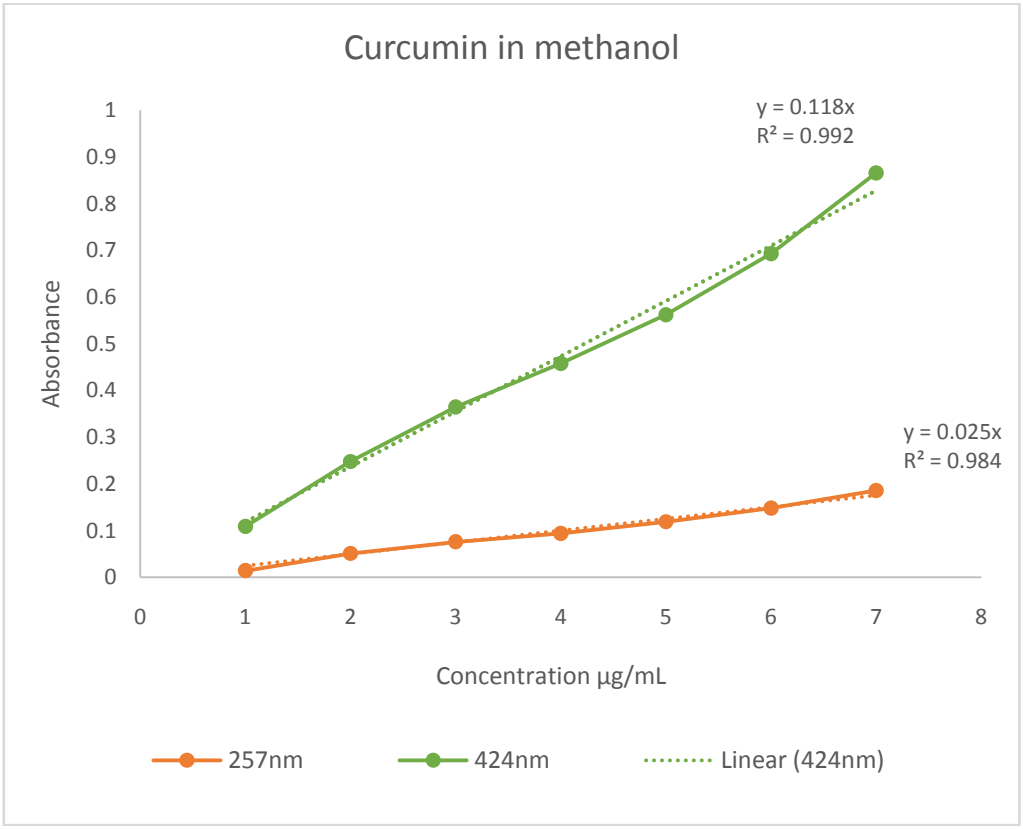

Figure 2 Standard graph of curcumin at 257 nm and 424 nm in methanol

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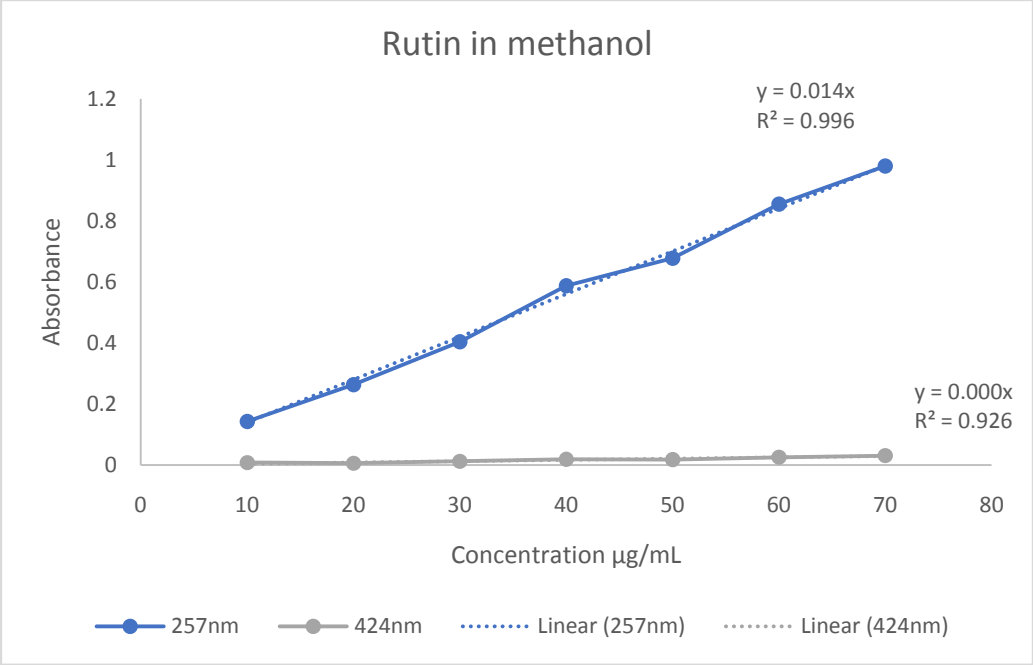

Figure 3 Standard graph of rutin at 257 nm and 424 nm in methanol