

SIMULTANEOUS ESTIMATION OF PIPERINE, QUERCETIN, AND CURCUMIN IN A MIXTURE USING U.V-VISIBLE SPECTROPHOTOMETER AND METHOD VALIDATION.

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ABSTRACT

U.V Spectrophotometric method have been widely employed in determination of individual components in a mixture or fixed dose combination. For the ternary mixture containing Quercetin, Curcumin and Piperine (Anti-inflammatory action) no spectrophotometric method for simultaneous evaluation has been reported so far. Thus our aim is to develop simultaneous equation method for estimation of the ternary mixture using U.V spectrophotometry. Methanol was used as solvent for dissolving each component and preparation of all the dilutions. The method involves formation and solving of simultaneous equations. Mixture of Quercetin, Curcumin and Piperine, having concentration 5.0 ppm, 5.0 ppm and 2.5 ppm, was subjected to overlay scan in U.V spectrophotometer from which λ_{max} of same were found to be 371.31 nm, 424.68 nm, 343.76 nm respectively. Calibration curve depicted the concentration range following Beer Lambert's law to be 5-30 μ g/ml, 1-5 μ g/ml and 1-10 μ g/ml respectively. Final concentration of Quercetin, Curcumin and Piperine in the mixture was found to be 5.3 ppm, 5.06 ppm, 2.25 ppm respectively. Accuracy and precision were also assessed in order to validate the method.

KEYWORDS: Quercetin, piperine, curcumin, simultaneous estimation, spectrophotometric method.

INTRODUCTION:

Quercetin, a flavanol, is a plant-derived flavonoid (3,5,7,3',4'-pentahydroxyflavone) found in fruits, vegetables, leaves and grains [1]. Quercetin has neither been confirmed scientifically as a specific therapeutic for any condition nor been approved by any regulatory agency. The U.S. Food and Drug Administration have not approved any health claims for quercetin. However preliminary research says that it has a role in cancer, inflammation,

fibromyalgia, MAO inhibitor; it has also been claimed that it has antiviral activity [1,2].

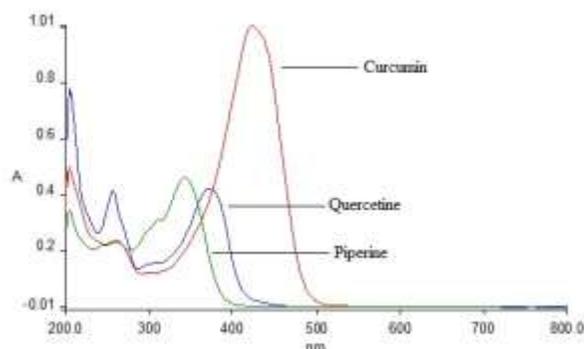


Figure 1. Overlay spectra for Quercetin, Curcumin and piperine in stock solution.

Curcumin is the principal curcuminoid of turmeric, which is a member of the ginger family (Zingiberaceae). The curcuminoids are natural phenols, (1E,6E)-1,7-bis(4hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione). It has been found to be a potent anti-inflammatory agent, used in post operative inflammation and inflammatory bowel disease. Other use includes anti tumor activity, ulcerative colitis [3,4].

Piperine is the alkaloid responsible for the pungency of black pepper and long pepper, 5-(3,4-methylenedioxyphenyl)-2,4-pentadienoyl-2-piperidinepiperoylpiperidine [4]. Piperine is known to possess anti-inflammatory, analgesic, anti neoplastic, and anxiolytic activities, also acts as digestive enzyme stimulator [5].

The ternary mixture can be used for its anti-inflammatory action. Earlier studies have been reported that spice active principle Quercetin, Curcumin and Piperine has antiinflammatory properties [6,7,8]. Literature survey has revealed that

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simultaneous estimation of Quercetin, Curcumin and Piperine in a mixture, has not been reported yet. Thus our aim is to develop simultaneous equation

TABLE 1: DATA FOR ACCURACY STUDIES.

DRUG	TRUE VALUE (ppm*)	AMOUNT FOUND (Mean of six estimation)	TRUE VALUE% ± S.D**
Quercetin	5	5.13	102 ± 0.2049
Curcumin	5	5.08	101.6 ± 0.1824
Piperine	2.5	2.43	97.2 ± 0.1262

*ppm – Parts per million

**S.D – Standard deviation

(Vierodt's) method for estimating all the three compounds simultaneously in a mixture. In this proposed method, no separation is required unlike HPLC; hence method is fast and economical.

MATERIALS AND METHOD

Instruments, reagents and chemicals: A dual beam PERKIN ELMER, U.V-Visible Spectrophotometer was used (with a pair of 1cm quartz cells). Analytical grade methanol was used as solvent for preparation of stock solution. Samples of Quercetin, Piperine and Curcumin were procured from. Distilled water was used for further dilutions.

Preparation of standard and sample solution: A standard stock solution of 1000ppm of Quercetin, Piperine and Curcumin was prepared by taking 100mg in 100ml of methanol. From standard stock solution, aliquots portion were suitably taken and diluted to different concentration using distilled water to get final concentration of 5ppm, 2.5ppm, 5ppm respectively and were scanned in the wavelength range of 200- 400 nm to determine λ_{max}. (Solutions were filtered using waltmann filter paper)

Simultaneous equation method: This method of analysis was based upon the absorption of drugs at wavelength maximum of each other. Three wavelengths selected for development of simultaneous equations were 371.31 nm, 424.68 nm, 343.76 nm. The absorptivity values E (1%, 1 cm) were determined for three drugs at all wavelengths

^[9]. The concentration of three drugs in a mixture was determined using following equation:

$$C_Q = \frac{A_1 (a_y \lambda_3 \cdot a_z \lambda_2 - a_y \lambda_2 \cdot a_z \lambda_3) + A_2 (a_y \lambda_1 \cdot a_z \lambda_3 - a_z \lambda_1 \cdot a_y \lambda_3) + A_3 (a_z \lambda_1 \cdot a_y \lambda_2 - a_z \lambda_2 \cdot a_y \lambda_1)}{a_y \lambda_1 (a_x \lambda_2 \cdot a_z \lambda_3 - a_x \lambda_3 \cdot a_z \lambda_2) + a_y \lambda_2 (a_x \lambda_3 \cdot a_z \lambda_1 - a_x \lambda_1 \cdot a_z \lambda_3) + a_y \lambda_3 (a_x \lambda_1 \cdot a_z \lambda_2 - a_x \lambda_2 \cdot a_z \lambda_1)}$$

$$C_C = \frac{A_1 (a_x \lambda_3 \cdot a_z \lambda_2 - a_x \lambda_2 \cdot a_z \lambda_3) + A_2 (a_x \lambda_1 \cdot a_z \lambda_3 - a_x \lambda_3 \cdot a_z \lambda_1) + A_3 (a_x \lambda_2 \cdot a_z \lambda_1 - a_x \lambda_1 \cdot a_z \lambda_2)}{a_y \lambda_1 (a_x \lambda_3 \cdot a_z \lambda_2 - a_x \lambda_1 \cdot a_z \lambda_3) + a_y \lambda_2 (a_x \lambda_1 \cdot a_z \lambda_3 - a_x \lambda_3 \cdot a_z \lambda_1) + a_y \lambda_3 (a_x \lambda_2 \cdot a_z \lambda_1 - a_x \lambda_1 \cdot a_z \lambda_2)}$$

$$C_P = \frac{A_1 (a_x \lambda_3 \cdot a_y \lambda_2 - a_x \lambda_2 \cdot a_y \lambda_3) + A_2 (a_x \lambda_1 \cdot a_y \lambda_3 - a_x \lambda_3 \cdot a_y \lambda_1) + A_3 (a_x \lambda_2 \cdot a_y \lambda_1 - a_x \lambda_1 \cdot a_y \lambda_3)}{a_y \lambda_1 (a_z \lambda_3 \cdot a_x \lambda_2 - a_z \lambda_2 \cdot a_x \lambda_3) + a_y \lambda_2 (a_x \lambda_3 \cdot a_z \lambda_1 - a_x \lambda_1 \cdot a_z \lambda_3) + a_y \lambda_3 (a_x \lambda_1 \cdot a_z \lambda_2 - a_x \lambda_2 \cdot a_z \lambda_1)}$$

Where, C_Q, C_C, C_P are the concentration of quercetin, curcumin, piperine in a mixture and A₁, A₂, A₃ are the absorbance of the mixture at 371.31 nm, 424.68 nm, 343.76 nm respectively.

a_xλ₁, a_xλ₂, a_xλ₃ are absorptivity values for quercetin at 371.31 nm, 424.68 nm, 343.76 nm respectively.

a_yλ₁, a_yλ₂, a_yλ₃ are absorptivity values for curcumin at 371.31 nm, 424.68 nm, 343.76 nm respectively.

a_zλ₁, a_zλ₂, a_zλ₃ are absorptivity values for piperine at 371.31 nm, 424.68 nm, 343.76 nm respectively.

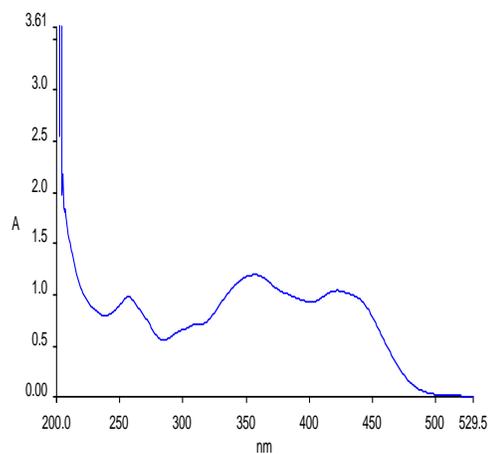


Figure 2. Spectra for mixture containing

OBSERVATION

Validation of proposed method:

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between

the value which is accepted either as a conventional true value or an accepted reference value and the value found ^[10]. Accuracy studies for the proposed method were carried out, and respective data obtained is mentioned in table 1.

simultaneous estimation of three drugs, and hence, such method can also be developed for the marketed formulations containing three components. It can be said that Quercetin, piperine, curcumin combination can serve as a novel formulation to target inflammatory disorders, where

TABLE 2: DATA FOR PRECISION STUDIES.

DRUG	INTRA-DAY PRECISION \pm S.D*	INTER-DAY PRECISION \pm S.D*		
		1 st DAY	2 nd DAY	3 rd DAY
Quercetin	\pm 0.0195	\pm 0.0348	\pm 0.0329	\pm 0.0601
Curcumin	\pm 0.0287	\pm 0.0478	\pm 0.0651	\pm 0.0803
Piperine	\pm 0.0157	\pm 0.0471	\pm 0.0302	\pm 0.0563

Precision:

- A. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability was assessed using 6 determinations at 100% of the test concentration ^[10].
- B. Inter-day and intra-day precision: It was calculated by assay of sample solution (n = 6) on the same day and on different days at different time intervals ^[10].

Precision studies for the proposed method were carried out, and respective data obtained is mentioned in table 2.

piperine enhances bioavailability of anti inflammatory agents quercetin and curcumin. This combination allows each component to selectively interfere with successive steps resulting in efficient anti inflammatory activity ^[11, 12]. Hence, simpler dosage schedule, increase compliance and therefore improves treatment outcomes^[13]. The mean percentage drug estimated in mixture form was 102 ± 0.2049 , 101.6 ± 0.1824 , and 97.2 ± 0.1262 for quercetin, curcumin and piperine respectively. Results for validation parameters are mentioned in table 3. Results obtained are within the acceptable limits; hence the above method for simultaneous evaluation for FDC having three drugs is accurate and precise for routine drug analysis.

Table 3: Validation Parameters

PARAMETERS	RESULTS		
	Quercetin	Curcumin	Piperine
Slope (m)	0.015	0.203	0.073
Intercept (c)	0.358	0.006	0.247
Correlation coefficient	0.984	0.994	0.992
Beer's Law limit (μ g/ml)	5-30	1-5	1-10
LOD (μ g/ml)	4.291	0.492	0.710
LOQ (μ g/ml)	13.005	1.493	2.153

RESULTS AND DISCUSSION

The proposed method was validated as per ICH guidelines. The proposed method was found to be accurate and economical as well for routine

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