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Simultaneous estimation using UV spectrophotometry of curcumin and silymarin in crude form and in prepared topical sunscreen cream

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Abstract

The current study's objective aimed at expanding and evaluate a spectrophotometric technique of simultaneous detection of curcumin and silymarin in natural and curcumin and silymarin containing cream dose forms. It is based on resolving a simultaneous equation. The absorbances of curcumin and silymarin were determined at their respective absorbance maxima (max) of 421 and 288 nm. In pure form and in curcumin and silymarin containing cream topical dosage form; the linearity range of curcumin and silymarin was 2-20 µg/ml at the corresponding chosen wavelengths. The coefficients of correlation for pure curcumin and its curcumin and silymarin containing cream topical dosage form at 421 nm are 0.9547 and 0.9764, respectively, while they are 0.9772 and 0.9673 for pure silymarin and in curcumin and silymarin containing cream topical dosage form at 288 nm. The methods proposed are simple, rapid, and proven, and they may be used successfully to quantify curcumin and silymarin in its natural and cream dosage forms on a regular basis.

1. Introduction

Curcumin is classified as a polyphenol flavonoid with betadiketone traits and the chemical name according to IUPAC is (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (Pratik *et al*., 2014; Nupur *et al*., 2021) (Figure 1) which is isolated from *Curcuma longa*. L. It has antioxidant properties. It shields human dermal fibroblasts (HDFs) from UVA rays that cause photoageing due to radiation exposure. Phototoxicity from UV-A light is frequently brought on by reactive oxygen species (ROS) (Tuba and Ilhami, 2008; Tamanna *et al*., 2020; Roopam and Jessy, 2021; Segu, 2022; Jyothilekshmi *et al*., 2022). Additionally, curcumin possesses hepatoprotective, anti-inflammatory, anticarcinogenic, antibacterial, cardiovascular effects, antiulcer, antioxidant, immunity-boosting properties, and is used to treat wounds (Patil *et al*., 2012; Abhijeet *et al*., 2015; Harithalakshmi *et al*., 2018; Komila *et al*., 2022).

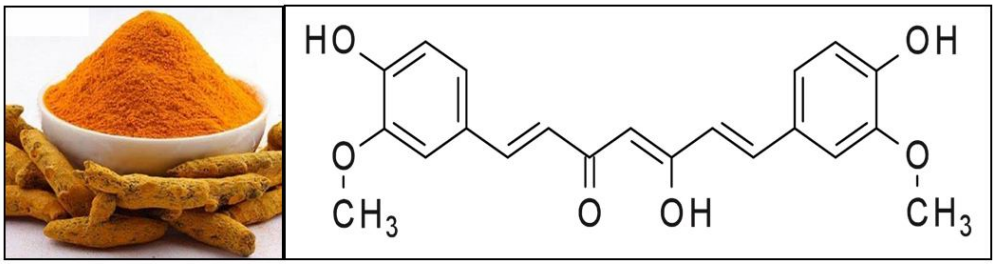

Figure 1: Structure of curcumin.

Silymarin is a flavanolignan compound with the chemical name as 3,5,7-trihydroxy-2-[3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydrochromen-4-one, (Figure 2), it is obtained from the milk thistle plant's seeds, *Silybum marianum* L., with antioxidant properties (Saber *et al*., 2020; Sailaja and Sasikala, 2022; Sapna *et al*., 2022). It causes induction of intracellular H_2O_2 by blocking UVB radiation. It prevents inducible nitric oxide synthase from being expressed when exposed to UVB, which lowers nitric oxide synthesis (Mudit *et al*., 2013; Santhosh *et al*., 2008). Additionally, silymarin has antiviral, hepatoprotectant and antidepressant effects (Shilpa *et al*., 2016). The simultaneous analytical examination gives the recognition of the chemical entities in pharmaceutical formulations for providing the specificity and assurance.

There are numerous techniques for measuring curcumin, including electrophoresis (Shanmugam *et al*., 2014), UV (Yuan *et al*., 2005), HPLC, GC (Ragsekaran *et al*., 1997), fluorescence spectroscopy, RP-UFLC (Krishnaveni *et al*., 2009), LC-MS (Rickling *et al*., 1995), and HPTLC (Sagar Kishore, 2017; Verma and Joshy, 2006). TLC, UV (Niraj *et al*., 2011) whereas, HPLC (Liu *et al*., 2006), capillary electrophoresis analysis, UPLC, RPHPLC (Mascher *et al*., 1993;

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Jahnavi and Ashok, 2022) and colorimetric methods (Pratik *et al*., 2014, Zarapkar *et al*., 2000) are reported for silymarin estimation. There are several UV spectrophotometric techniques for estimating curcumin and silymarin in combination with other phytochemicals, but no UV spectrophotometric techniques for curcumin and silymarin have yet been published. For the simultaneous assessment of curcumin along with silymarin as pure form and topical cream dosage form, we putforward simple and validated estimation of UV spectrophotometric technique in this work.

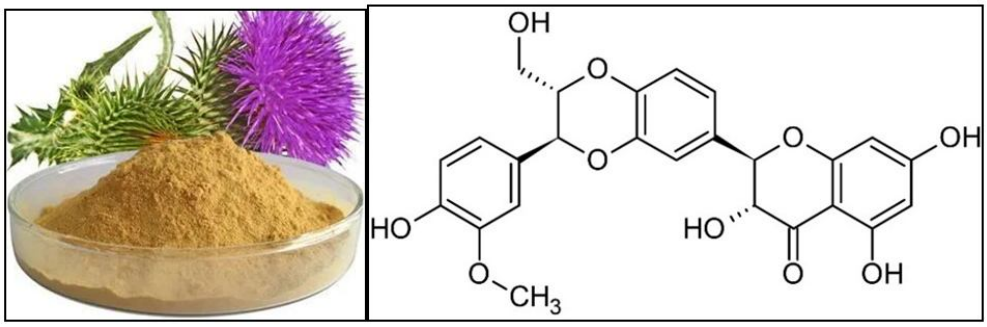


Figure 2: Structure of silymarin.

2. Materials and Methods

2.1 Instruments and apparatus

A double beam UV-visible spectrophotometer (UV-1700, Shimadzu, Japan), UV Probe 2.0, with a pair of identical quartz cells measuring a length of 1 cm, a 2 nm spectral width and 0.5 nm wavelength precision. The development along with validation of the suggested analytical method utilised borosilicate glass volumetric flasks and pipettes.

2.2 Reagents and chemicals

The reference standard silymarin was purchased from the bioven ingredients, while the curcumin was bought *via* Himedia, India. The reagents and chemicals utilised were all of the AR grade.

2.3 Development of calibration curve (Kollol Kumar *et al*., 2020)

The λ_{max} of curcumin was 421 nm and of silymarin was 288 nm obtained upon scanning between 200 nm-800 nm with 10 µg/ml of curcumin and silymarin separately. These λ_{max} wavelengths were selected for simultaneous estimation method.

2.3.1 Standard plot of curcumin

0.01g of curcumin was dissolved in 1ml of dichloromethane and pH 5.5 phosphate buffer was used to make up to 100 ml. This stock mixture was used for the preparation of solutions of various concentrations of curcumin in the range of 2-20 µg/ml. pH 5.5 phosphate buffer was used as a blank to determine the absorbance of the produced samples using a UV double beam spectrophotometer in triplicate. Concentration of solution and absorbance of solution were plotted on a standard curve using the X and Y axis, respectively.

2.3.2 Standard plot of silymarin

0.01 g of silymarin was dissolved in 1 ml of dichloromethane and pH 5.5 phosphate buffer was used to make up to 100 ml. This stock solution was used to prepare solutions with silymarin concentrations ranging from 2-20 µg/ml. In a UV double beam spectrophotometer, the spectra of this solution were measured between the 200 to 400 nm range. Silymarin was discovered to have a maximum absorption at 288 nm. The resulting samples' absorbance was tested in triplicate using a UV double beam spectrophotometer at a wave length of 288 nm with a blank of pH 5.5 phosphate buffer. The concentration of the solution on X-axis and absorbance of the solution on the Y-axis were plotted to get standard curve.

2.3.3 Simultaneous estimation of curcumin and silymarin through UV double beam spectrophotometer

(i) Sample preparation (Sukhjinde *et al*., 2017)

A mixture of curcumin and silymarin in pure form was prepared by mixing 1:1 ratio for comparison with CS cream during analysis. Curcumin-silymarin cream (CSC) was prepared with 1:1 ratio of curcumin and silymarin using olive oil, cetyl alcohol, stearic acid, cetosteryl alcohol, PEG200, beeswax, propyl paraben, triethanolamine, disodium EDTA, methyl paraben, rose oil as cream base ingredients. 1 g cream and 20 mg of pure mixture were weighed accurately and thereafter added separately to a 100 ml volumetric flask to dissolve in 1ml dichloromethane and make up to mark with phosphate buffer pH 5.5 to get a 100 µg/ml and prepared solution was filtered with Whatmann filter paper. Then, these were used to prepare dilutions for various concentrations with 2-20 µg/ml for simultaneous estimation of curcumin and silymarin at 421 nm and 288 nm with developed method.

(ii) Procedure

At selected wavelengths, a pair of simultaneous equations were generated by using the absorptivity values in the equation. Using the set of two simultaneous equations listed below, the concentrations of two phytochemicals in the solution were determined (Sagar Kishore *et al*., 2017).

$$C_a = \frac{Au_2 a_x y_1 - Au_1 a_x y_2}{Ax_2 a_x y_1 - a_x x_1 a_x y_2} \quad \text{... (Equation 1)}$$

$$C_b = \frac{Au_1 a_x x_2 - Au_2 a_x x_1}{Ax_2 a_x y_1 - a_x x_1 a_x y_2} \quad \text{... (Equation 2)}$$

where

C_a and C_b = concentration of curcumin and silymarin respectively, $a_x x_1$ and $a_x x_2$= curcumin absorptivity values at 421 nm and 288 nm,

respectively, $a_x y_1$ and $a_x y_2$ = silymarin's absorptivity measurements at 421 nm and 288 nm, Au_1 and Au_2 = absorbances of the sample solutions at 421 nm and 288 nm, respectively. Concentrations of C_a and C_b in cream was acquired by solving the above equations.

2.4 Validation of developed approach (Swetha *et al.*, 2022)

The devised simultaneous approach was validated by determining the limit of detection (LoD), limit of quantification (LoQ), and linearity using ICH recommendations.

2.4.1 Limit of detection and limit of quantification

The limit of detection (LoD) of an analyte in a test sample is its lowest concentration, where we can clearly differentiate from zero, and the limit of quantification (LoQ) is the minimal amount of an analyte in a testing sample that we can calculate with acceptable reproducibility as well as accuracy.

The drug's LoD and LoQ were established by computing peak height-to-noise in RMS ratio (H/N, LoQ is 10 and LoD is 3.3.) using the ICH-recommended equations. The standard residual deviation of the line of regression or the standard deviation of the Y intercept of the regression line were utilised in the LoD and LoQ calculations.

LoD = 3.3 × (SD/s) …… (equation 3)

LoQ = 10 × (SD/s) …… (equation 4)

where

SD= The y-intercept standard deviation on the correlation lines

and

s = calibration curve's slope.

2.4.2 Linearity

The method's linearity was tested using freshly produced samples across the analytical range. The curve for calibration was developed using a plot absorbance of solution (y) vs the drug concentration (x) in µg/ml. The developed approaches were used to evaluate standard stock solutions at the appropriate dilutions from 2-20 µg/ml for each drug.

3. Results

Calibration plots of curcumin and silymarin prepared individually were shown in Figure 3 (A and B). As per Figure 3 (A and B) the regression coefficient of curcumin is 0.9508 and silymarin is 0.9774.

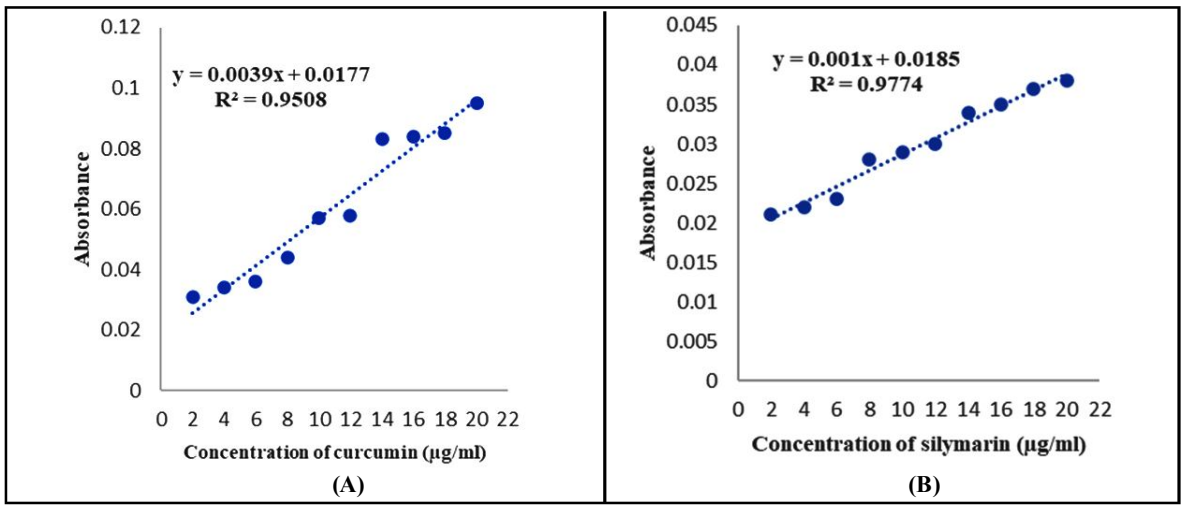
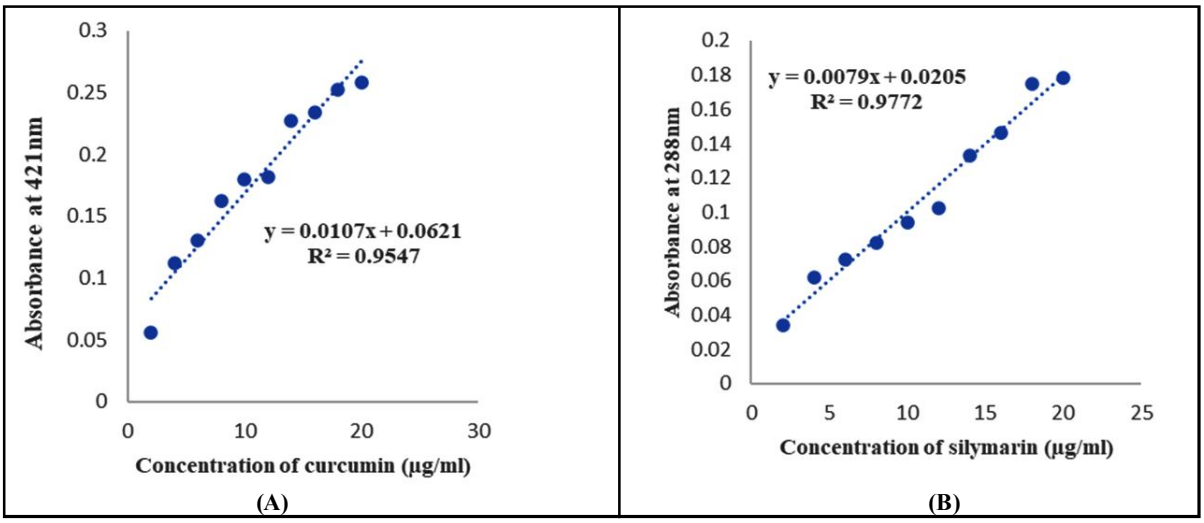


Figure 3: Calibration plot (A) Curcumin (B) Silymarin.

Then, the calibration plots on simultaneous estimation of the curcumin and silymarin in pure mixture form are shown in Figures 4 (A and B) and in cream form are as seen in Figures 4 (C and D).



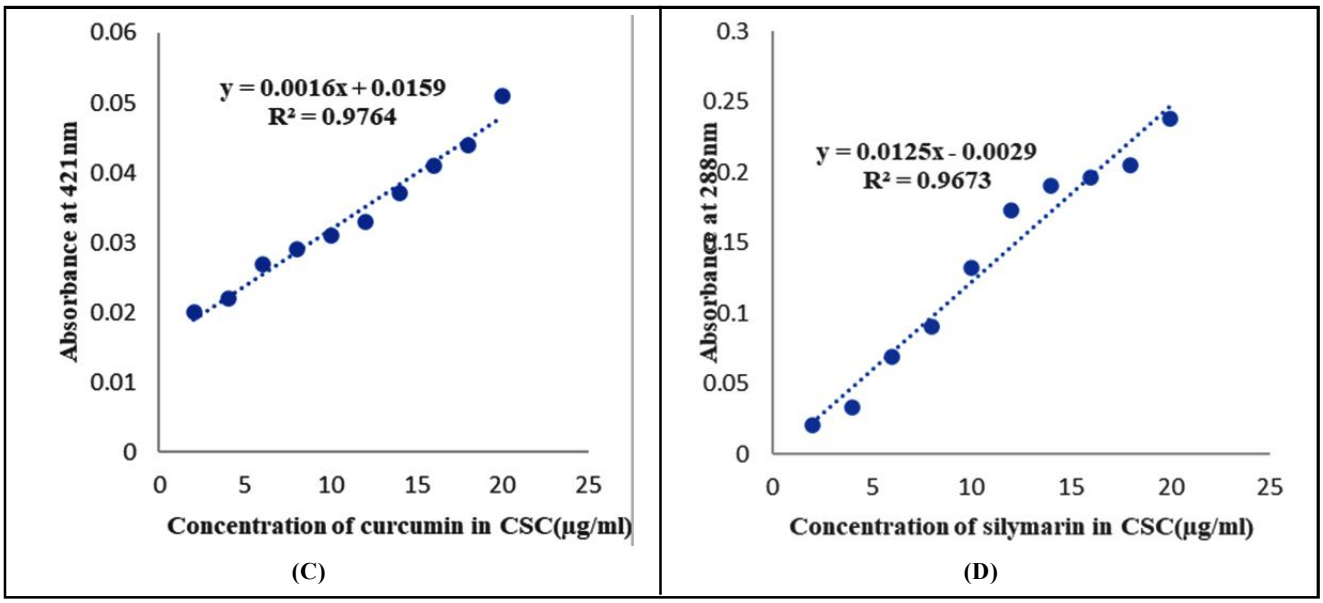


Figure 4: Calibration plots (A), Curcumin in pure form (B), Silymarin in pure form (C), Curcumin in CSC, (D) Silymarin in CSC.

The different validation parameters of developed methods for both curcumin and silymarin in pure form are given in Table 1 and in CS cream form were given in Table 2. Where in LoD and LoQ values of curcumin and silymarin in pure form and in CS cream are included.

Table 1: Validation parameters for curcumin and silymarin in pure form

S.No.	Parameter	Curcumin	Silymarin
1.	Wave length (λ_{max}) (nm)	421	288
2.	Range of Beer's law (µg/ml)	2-20	2-20
3.	Regression equation*(Y = mx+c)	Y = 0.0107x + 0.0621	Y = 0.0079x + 0.0205
4.	Slope (m)	0.0107	0.0079
5.	Intercept (c)	0.0621	0.0205
6.	Regression coefficient	0.9547	0.9772
7.	Limit of detection (LoD) µg/ml	0.781	9.143
8.	Limit of quantification (LoQ) µg/ml	2.366	27.707

Table 2: Validation parameters for curcumin and silymarin in CSC

S.No.	Parameter	Curcumin	Silymarin
1.	Wavelength (λ_{max}) (nm)	421	288
2.	Range of Beer's law (µg/ml)	2-20	2-20
3.	Regression equation* (Y = mx+c)	Y = 0.0016x + 0.0159	Y = 0.0125x – 0.0029
4.	Slope (m)	0.0016	0.0125
5.	Intercept (c)	0.0159	– 0.0029
6.	Regression coefficient	0.9764	0.9673
7.	Limit of detection (LoD) µg/ml	9.048	11.098
8.	Limit of quantification (LoQ) µg/ml	27.417	33.631

4. Discussion

The very two important potential phytochemicals curcumin and silymarin were used in combination in different formulations for various activities. For the estimation of these compounds in combined formulations, there is a need for development of a simultaneous estimation method. Therefore, the current work concentrated on developing and validating a UV spectrophotometric technique for simultaneous measurement of curcumin and silymarin in crude form and in topical cream dose form.

The linearity range of curcumin and silymarin in crude form and in CSC topical dosage form was 2-20 µg/ml, at respective wave lengths, *i.e*., 421 and 288 nm. Regression coefficient for curcumin in pure form and its CSC topical dosage form at 421 nm are 0.9547 and 0.9764, respectively (Figure 04) (Tables 1 and 2), while they are 0.9772 and 0.9673 for silymarin in pure form and in CSC topical dosage form at 288 nm. Both drugs curcumin and silymarin showed acceptable regression coefficients at their corresponding wavelengths and the outcomes of recovery trials showed that the suggested approach can reliably determine slight change in drug concentration in a solution or any dosage form.

UV simultaneous analysis identifies chemical entities in pharmaceutical formulations with high specificity and certainty. According to this investigation, one can determine two drugs easily in the formulations containing an equivalent amount of the both selected drugs.

5. Conclusion

Curcumin and silymarin in crude form and curcumin and silymarin containing cream (CSC) were used for simultaneous estimation by UV spectroscopy technique. The suggested spectrophotometric approach is simple, quick, and cost-effective, and it has been validated in terms of linearity in compliance with ICH criteria. There was no deviation from acceptable limits for any of the validation parameters. This approach may be used to estimate curcumin and silymarin together in topical preparations.

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Abbreviations

CSC : Curcumin and silymarin containing cream, EDTA : Ethylenediaminetetraacetic acid, GC: Gas chromatography, H_2O_2 : Hydrogen peroxide, HDF: Human dermal fibroblast, HPLC: High performance liquid chromatography, HPTLC: High performance thin layer chromatography, LC-MS: Liquid chromatography-mass spectrometry, LOD: limit of detection, LOQ : limit of quantification, nm : nanometer, PEG200 : polyethylene glycol 200, ROS: Reactive Oxygen Species, RP-UFLC: Reverse phase ultra-fast liquid chromatography, TLC: Thin layer chromatography, UPLC: Ultra performance liquid chromatography, UV: ultraviolet radiation, UVA : ultraviolet radiation A region, UVB: ultraviolet radiation B region, λ_{max}: maximum wavelength, µg/ml : microgram per milliliter.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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