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Research Article

A validated spectrophotometric analysis for simultaneous estimation of vincristine sulfate and bovine serum albumin in pure preparations using Vierordt's method

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Abstract

The novel Vierordt's approach, or simultaneous equation method, was created and validated for the concurrent determination of vincristine sulfate (VCS) and bovine serum albumin (BSA) in pure solutions utilizing UV spectrophotometry. It is simple, precise, economical, rapid, reliable, and accurate. This method depends on measuring absorbance at two wavelengths, 296 nm and 278 nm, which correspond to the λ_{max} of VCS and BSA in deionized water, respectively. The calibration curves of VCS and BSA are linear at concentration ranges of 10–60 µg/mL and 200–1600 µg/mL, with correlation coefficient values (R^2) of 1 and 0.999, respectively. The limits of detection (LOD) and quantification (LOQ) were 0.465 µg/mL and 1.410 µg/mL for VCS and 41.096 µg/mL and 124.533 µg/mL for BSA. The precision investigation indicated that the relative standard deviation (RSD) value was within limitations (RSD < 2%). The percentage recovery varied between 99.40 and 103.20% for VCS and 97.90 and 102.54% for BSA at various concentration levels, demonstrating that the simultaneous equation technique is accurate. The suggested approach can be successfully applied to estimate VCS and BSA simultaneously in pure and pharmaceutical-marketed products comprising these two components.

Keywords

vincristine sulfate, bovine serum albumin, simultaneous equation method, UV spectroscopy

Introduction

VCS is a potent anticancer medication that belongs to a class of medicines known as vinca alkaloids. The VCS drug's anticancer mechanism is recognized to prevent cancer cell proliferation. As a result, it inhibits cancer growth and metastasis. VCS, marketed under the brand name Oncovin, is an antitumor medication commonly used in combination with other drugs to treat various types of cancer, including Hodgkin's disease, leukemia, lung cancer, and brain cancer (Al-Musawi et al. 2021). Fig. 1 depicts the chemical structure of VCS.

BSA is commonly used as a model protein instead of human serum albumin (HSA) due to its similar structure. BSA, like HSA, comprises a single polypeptide chain, which contains 583 amino acid residues. The structure

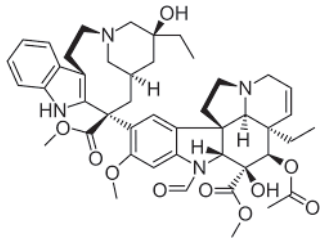

Figure 1. The chemical structure of VCS.

is kept stable by 17 disulfide linkages connecting cysteine (Cys) amino acid residues. It possesses a molecular weight of about 66.8 kDa and a heart-shaped structure with three homologous domains I–III. Each domain comprises two unique subdomains, A and B, with distinct binding characteristics. BSA contains two tryptophan (Trp) amino acid residues, Trp-134 and Trp-212, at subdomains IB and IIA, respectively (Jahanban-Esfahlan et al. 2019). Fig. 2 shows the structure of albumin (Jahanban-Esfahlan et al. 2020).

Various nanocarriers have been created to enhance the efficacy or lessen the adverse effects of active medicinal substances, resulting in more efficient medication delivery. Protein-based nanoparticles (NPs) are becoming increasingly popular as drug delivery systems in clinical and research settings due to their high transport efficiency, biodegradability, non-immunogenicity, and nontoxicity. Albumin is an excellent choice for developing medication delivery systems, such as nanocarriers. In 2005, the US FDA authorized Abraxane, the albumin-bound form of paclitaxel. This product is the first example of a drug-loaded protein nanoparticle product to be successfully sold on the market (Luo et al. 2021).

Albumin can interfere with UV spectroscopy analysis of drugs as it absorbs light in the UV range (peptide bond at around 220 nm). This can lead to overlapping signals and make it difficult to measure the drug concentration accurately (Rasoulzadeh et al. 2010; Xu et al. 2013; Korkmaz et al. 2015).

A detailed literature search was conducted, and the results revealed that only a few analytical procedures, such as HPLC-UV (Rodrigues et al. 2009), UV (Bakmaz et al. 2021), RP-HPLC (Umrethia et al. 2010), UPLC-MSMS (Yang et al. 2015), and HPLC (Stewart et al. 2021), have been reported for estimating VCS and BSA alone and in combination with other drugs. Numerous techniques have been previously reported for the analysis of vincristine in biological samples following the delivery of vincristine injection, ranging from HPLC-UV (Junping et al. 2003), HPLC combined with electrochemical detection technique (Gidding et al. 1999), to LC–MS (Schmidt et al. 2006), then to LC–MS/MS (Ling et al. 2010).

However, these test procedures are pretty complex; often, the mobile phases contain multiple components, and pH values must be adjusted. The last two approaches require MS equipment, which increases the test cost. Furthermore, the sample processing technique is more severe than the classic HPLC-UV technique to remove endogenous chemicals that may have an additive effect and hurt quantitative MS detection (Chen et al. 2011).

Embree et al. reported the analysis of VCS from human plasma following the administration of VCS liposome containing injection; the mobile phase was pretty complex, made up of diethylamine aqueous solution, acetonitrile, and methanol, and its pH value was adjusted to 7.0. In the present study, multiple mobile phases were employed to assess the concentration of VCS in the plasma specimens after the administration of its nanoparticle suspension. (Embree et al. 1997).

A recent literature review has found no UV spectrophotometric investigations of VCS and BSA in combined dosage form in pharmaceutical products. The current work aims to create a new spectrophotometric method that can be used for routine analysis in pharmaceutical companies, hospitals, and research laboratories. This method will estimate VCS and BSA in combined dosage form with reasonable accuracy, simplicity, precision, and economy over other chromatographic methods.

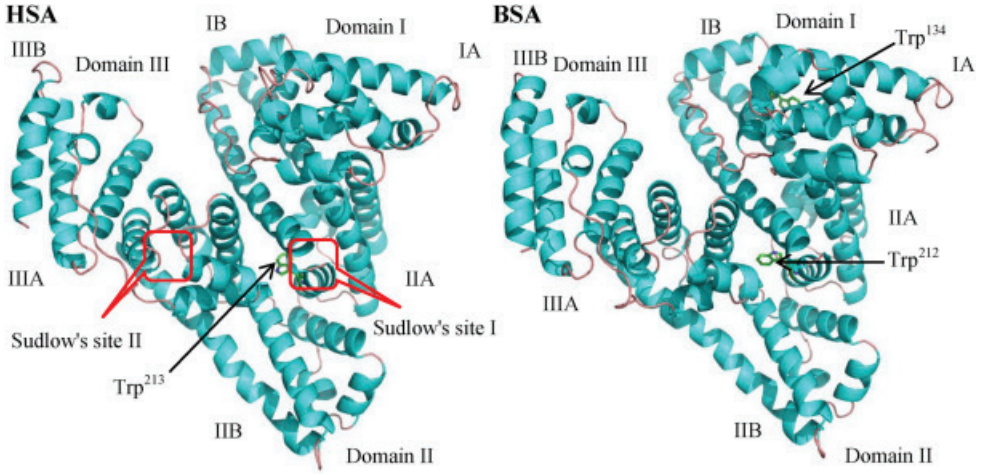

Figure 2. The HSA structure consists of three primary domains, I, II, and III; each one contains a pair of subdomains, known as A and B. Sites I and II, which are located in subdomains IIA and IIIA, respectively, are BSA's two critical binding sites.

Experimental

1. Materials and reagents

Vincristine sulfate and bovine serum albumin were purchased from Hangzhou Hyper Chemicals Limited, China. Deionized water was provided by Rafidain Environment Company, Iraq.

2. Instruments

Sensitive balance (Denver Instrument Germany), ultrasonic cleaner (Fuyang Technology China), hotplate magnetic stirrer (Joanlab/China), and two UV-visible spectrophotometers (double-beam Shimadzu-1900i/Japan and single-beam Shimadzu UVmini-1240/Japan) with UV probe software were utilized. Absorbance was measured with a pair of 1-cm-matched quartz cells.

3. Selection of a common solvent

Deionized water was chosen as a standard solvent for examining the spectrum properties of the selected compounds.

4. Preparation of typical stock solutions

A typical stock solution of VCS (100 µg/mL) was created by dissolving 10 mg using deionized water in a 100-mL volumetric flask. Sonication of the resulting solution was done for 5 minutes, followed by the volume adjustment to 100 mL with deionized water. From this typical stock solution, 6 mL was taken and diluted to 10 mL with deionized water to prepare a working standard solution of 60 µg/mL. Similarly, a typical stock solution of BSA (2000 µg/mL) was created by dissolving 200 mg using deionized water in a 100-mL volumetric flask. Sonication of the resulting solution was done for 10 minutes, followed by the volume adjustment to 100 mL with deionized water. From this typical stock solution, 8 mL was taken and diluted to 10 mL with deionized water to prepare a working standard solution of 1600 µg/mL (Giriraj and Sivakkumar 2014).

5. Determination of λ_{max}

These working standard solutions (60 µg/mL for VCS and 1600 µg/mL for BSA) were scanned in the whole UV range (200–400 nm) to figure out the λ_{max}. Absorption maxima of VCS and BSA were identified, and overlain spectra were recorded (Makvana and Sahoo 2019).

6. Preparation of calibration curves

Appropriate aliquots of each component were pipetted from the standard stock solutions into a series of 10-mL volumetric flasks, and the volume was filled up to the line with deionized water to get concentrations of 10–60 µg/mL of VCS and 200–1600 µg/mL of BSA. For each material, solutions of the series of concentrations were evaluated at their corresponding wavelengths, and absorbance was measured (Bhaskar et al. 2020; Mohammed and Abed 2023).

7. Simultaneous equation method development

After plotting calibration curves to confirm Beer's law, the absorptivity values were calculated at the corresponding wavelength for each material, and these values were used to build the simultaneous equations, as illustrated below:

$$\text{At } \lambda_1: A_1 = ax_1bC_X + ay_1bC_Y \quad (1)$$

$$\text{At } \lambda_2: A_2 = ax_2bC_X + ay_2bC_Y \quad (2)$$

For measurements using cells of 1 cm, b = 1
Rearrangement of equation (2)

$$C_Y = A_2 - ax_2C_X / ay_2$$

Substitution of C_Y in equation (1) and rearrangement

$$C_X = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2 \quad (3)$$

$$C_Y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2 \quad (4)$$

Where:
λ_1 = 296 nm and λ_2 = 278 nm
A1 and A2 are the absorbance at 296 nm and 278 nm respectively.
C_X and C_Y are the concentration of VCS and BSA, respectively.
ax_1 and ax_2 are the absorptivity of VCS at 296 nm and 278 nm, respectively.
ay_1 and ay_2 are the absorptivity of BSA at 296 nm and 278 nm, respectively.

The concentrations of both VCS and BSA in the sample solutions were calculated by utilizing two simultaneous equations (3) and (4) (Panchale et al. 2020).

8. Calculation of the absorptivity value

When the solutions of each material were scanned in triplicate versus the solvent blank at the designated wavelengths, the absorptivity of each solution was calculated using the following equation:

$$\text{Absorptivity, A (1\%, 1 cm)} = \text{Absorbance at specified wavelengths / concentration in g/100 mL} \quad (5)$$

(Begum et al. 2013).

9. Validation of the developed method

In method validation, International Conference of Harmonization (ICH) guidelines (Shabir 2005) recommendations for specificity, linearity, accuracy, precision, ruggedness, robustness, stability, limits of detection, and quantification were carried out (Yaseen et al. 2023).

Specificity

Specificity is the ability to evaluate the analyte definitively in the presence of additional substances that are expected to be present (Ravisankar et al. 2015).

Specificity is investigated by the absorbance measurements of VCS and BSA at 296 nm and 278 nm, respectively, against the blank and the comparison of the absorbance of drug solutions with the blank (Venkatesan and Kannappan 2014).

Linearity

The linearity of VCS and BSA calibration graphs at their respective absorbance maxima was observed in the 10–60 µg/mL concentration ranges for VCS and 200–1600 µg/mL for BSA (Sakhare et al. 2016).

Accuracy

By definition, it is how close a measurement is to the accepted value. A recovery study using the standard addition method at three distinct levels (80%, 100%, and 120%) equal to 8/160, 10/200, and 12/240 mg of VCS/BSA, respectively, was performed to evaluate the accuracy of the proposed approaches. The percentage recovery by the intended method was calculated using the following formula:

$$\text{Recovery} = (A - B) / C \times 100 \quad (6)$$

Where:
A is the overall quantity of drug calculated (mg).
B is the quantity of drug present at the pre-analyzed level (mg).
C is the quantity of bulk drug added (mg) (Shetty and Patil 2014; Al-Momani and Al Souqi 2023).

Precision

The method's precision had been evaluated by checking its repeatability with intermediate precision. Repeatability is assessed with three replicates of the concentration of the analyte during one day at various intervals under identical experimental circumstances (intraday). The intermediate precision was examined by running the study on three distinct days with three replicates investigated each day (interday). The percentage relative standard deviation (%RSD) must be smaller than 2 (Abed and Hussein 2019; Al-Uzri et al. 2023).

After calculating the mean and standard deviation (SD), the relative standard deviation (RSD) is provided by the following equation:

$$\text{RSD (\%)} = \text{SD} \times 100 / \text{Mean} \quad (7)$$

(Solano-Cueva et al. 2023).

Ruggedness

Ruggedness is an investigation that was undertaken to study the impact of variation in analysts, labs, and instruments in triplicate measurements according to the experimental procedure (Venkatesan and Kannappan 2014).

Robustness

The robustness of an analytical technique is the capacity of an optimized approach to remain unaltered despite slight parameter variations. The robustness of the established UV technique was determined by altering the wavelength by (±1 nm) and measuring the absorbance (Qureshi et al. 2021).

Stability

The stability study was tested by storing the drug solutions at room temperature for nine days, reading the absorbance of the sample solution each day, and checking for drug content (Giriraj and Sivakkumar 2017; Salih et al. 2018).

Sensitivity

LOD represents the minor concentration of a substance in the sample that can be reliably identified with the mentioned possibility, although it may not be quantified as a precise value. LOD is the same as "sensitivity," "analytical sensitivity," and "detection limit." LOQ is the minor concentration of analyte in the sample, which can be quantitatively determined under specified experimental conditions with sufficient precision and accuracy (Forootan et al. 2017).

Both the LOD and LOQ were calculated using the following equations:

$$\text{LOD} = 3.3 \times \sigma / S \quad (8)$$

$$\text{LOQ} = 10 \times \sigma / S \quad (9)$$

Where:
σ is the standard deviation of the intercept.
S is the slope (Mali et al. 2015; Habib et al. 2023).

Statistical analysis

The experiment data were presented as the mean of triplicate samples ± standard deviation (Hussein and Rajab 2018).

Results and discussion

1. Determination of λ_{max}

The UV scanning showed a spectrum exhibiting λ_{max} of 296 nm and 278 nm for VCS and BSA, respectively, as shown in Fig. 3 (Jeewantha 2017; Malarkani et al. 2018).

2. Preparation of calibration curves

Calibration curves for VCS and BSA were constructed and shown in Figs 4, 5.

3. Simultaneous equation method development

Figs 6–8 illustrate the overlain spectra of both materials.

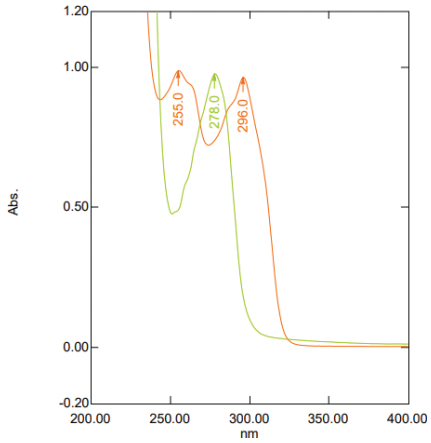

Figure 3. λ_{max} of BSA and VCS.

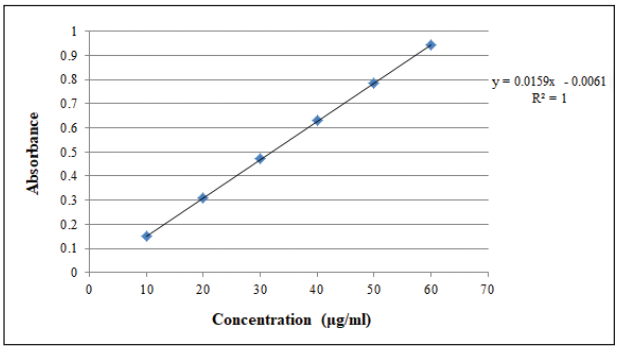

Figure 4. Calibration curve of VCS in deionized water (10–60 µg/mL).

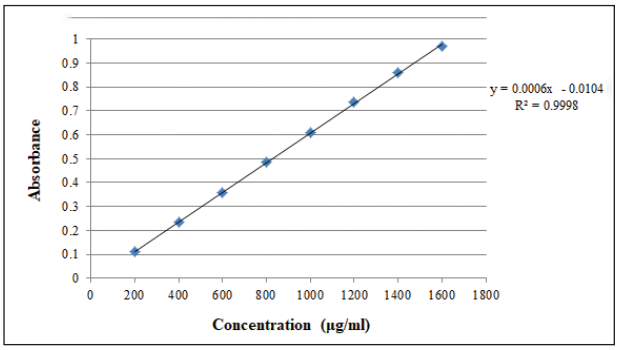

Figure 5. Calibration curve of BSA in deionized water (200–1600 µg/mL).

4. Determination of absorptivity value

Tables 1, 2 show the absorptivity values calculated for each concentration.

Table 1. Absorptivity values for VCS.

Concentration (µg/mL)	Absorbance λ_1–296	Absorptivity λ_1–296	Absorbance λ_2–278	Absorptivity λ_2–278
10	0.152	152.00	0.112	112.00
20	0.309	154.50	0.230	115.00
30	0.471	157.00	0.355	118.33
40	0.631	157.75	0.476	119.00
50	0.787	157.40	0.596	119.20
60	0.943	157.16	0.715	119.16
	Absorptivity for λ_1	155.986	Absorptivity for λ_2	117.115

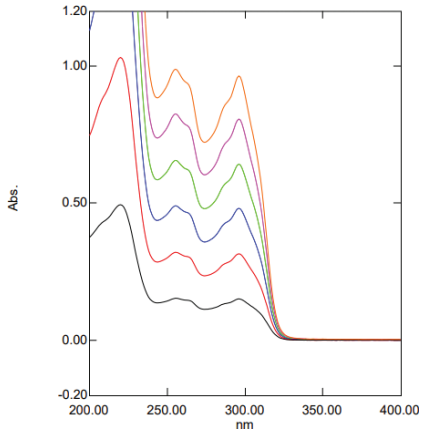

Figure 6. Overlain spectra of VCS.

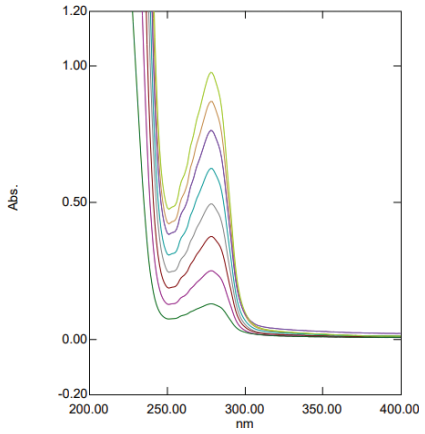

Figure 7. Overlain spectra of BSA.

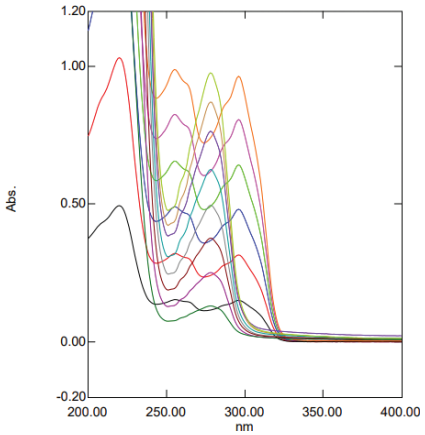

Figure 8. Overlain spectra of VCS and BSA.

Table 2. Absorptivity values for BSA.

Concentration (µg/mL)	Absorbance λ_1–296	Absorptivity λ_1–296	Absorbance λ_2–278	Absorptivity λ_2–278
200	0.023	1.15	0.113	5.65
400	0.043	1.07	0.235	5.87
600	0.067	1.11	0.358	5.96
800	0.089	1.11	0.486	6.07
1000	0.111	1.11	0.608	6.08
1200	0.138	1.15	0.736	6.13
1400	0.161	1.15	0.859	6.13
1600	0.180	1.12	0.971	6.06
	Absorptivity for λ_1	1.121	Absorptivity for λ_2	5.993

Validation of the developed method

Specificity and linearity

The specificity of the method was determined by calculating the absorbance of VCS and BSA separately at 296 nm and 278 nm against deionized water as a blank; their absorbance was compared with the blank. There was no interference at 296 nm or 278 nm, showing that the approach is specific. The calibration curves were linear in the concentration ranges from 10, 20, 30, 40, 50 to 60 µg/mL and 200, 400, 600, 800, 1000, 1200, 1400 to 1600 µg/mL for VCS and BSA, with correlation coefficient values (R^2) of 1 for VCS and 0.999 for BSA. Results revealed that a good correlation happens between the concentrations of the sample and their absorbance (Giriraj and Sivakkumar 2014).

Accuracy

Table 3 shows the results of calculating the quantity of VCS and BSA recovered at each level. The recovery tests were performed to ensure the technique's reproducibility and reliability. A known amount of the standard drugs was added to the pre-analyzed samples at three distinct levels (80%, 100%, and 120%). These results indicate high accuracy, as the percent recoveries of the three concentrations were close to 100% (Sachin et al. 2014).

Table 3. Recovery results for VCS and BSA.

Concentration (%)	Added amount (mg)		Amount recovered (mg)		Amount recovered (%)	
VCS/BSA	VCS	BSA	VCS	BSA	VCS	BSA
80	8.00	160.00	17.952	356.640	99.40	97.90
100	10.00	200.00	20.320	405.096	103.20	102.54
120	12.00	240.00	22.014	435.720	100.86	98.21

Precision

Table 4 shows the results of the percentage relative standard deviation (%RSD). The %RSD for intraday and interday precision for VCS and BSA was less than 2%, indicating a good level of precision (Chitlange et al. 2008).

Ruggedness

Table 5 shows the results of calculating the %RSD for each condition. The current procedure demonstrated good ruggedness when used by various analysts and instruments of the same make. The results are within acceptable limits, suggesting no substantial analyst-to-analyst or instrument-to-instrument variation and, therefore, the method's ruggedness (Donepudi and Achanta 2019).

Robustness

The results are presented in Table 6. A small change in the wavelength within (±1 nm) relative to the λ_{max} of VCS and BSA exhibited no influence, further indicating the validity of the created approach, with %RSD less than 2% falling within the permissible level (Ali and Elsaman 2021).

Stability

The results are presented in Table 7. The sample solution of VCS and BSA in deionized water is stable at room temperature without degradation for up to nine days (Giriraj and Sivakkumar 2017).

Table 4. Intraday and interday precision results.

Parameters	Sampling time	VCS			BSA		
		Amount present (mg)	Amount present (%)	RSD (%)	Amount present (mg)	Amount present (%)	RSD (%)
Intraday precision	0 h	10.204	102.04 ± 0.09	0.90	206.432	103.21 ± 2.13	1.03
	1st h	10.138	101.38 ± 0.18	1.82	207.338	103.67 ± 3.80	1.83
	2nd h	10.036	100.36 ± 0.13	0.13	197.442	98.72 ± 1.38	0.70
Interday precision	1st day	10.224	102.24 ± 0.13	1.33	200.025	100.01 ± 3.78	1.89
	2nd day	9.956	99.56 ± 0.07	0.73	202.554	101.27 ± 2.28	1.28
	3rd day	10.158	101.58 ± 0.07	0.75	207.726	103.86 ± 2.30	1.10

Table 5. Ruggedness results for VCS and BSA.

Parameters		VCS			BSA		
		Amount present (mg)	Amount present (%)	RSD (%)	Amount present (mg)	Amount present (%)	RSD (%)
Instrument	I						
Analyst	I	10.216	102.16 ± 0.19	1.06	209.277	104.63 ± 3.02	1.44
Analyst	II	10.266	102.66 ± 0.08	0.81	205.846	102.92 ± 3.98	1.93
Instrument	II						
Analyst	I	10.436	104.36 ± 0.14	1.41	210.177	105.08 ± 2.33	1.11
Analyst	II	10.254	102.54 ± 0.15	1.52	206.300	103.15 ± 2.45	1.19

Table 6. Results observed by altering the wavelength ± nm.

VCS				BSA			
Wavelength (nm)	Amount present (mg)	Amount present (%)	RSD (%)	Wavelength (nm)	Amount present (mg)	Amount present (%)	RSD (%)
295	10.240	102.40 ± 0.17	1.72	277	215.268	107.63 ± 2.91	1.35
297	10.305	103.00 ± 0.90	0.92	279	208.886	104.44 ± 1.34	0.64

Table 7. Stability data for VCS and BSA.

Day	VCS		BSA	
	Amount present (mg)	Amount present (%)	Amount present (mg)	Amount present (%)
1	10.26	102.60	206.62	103.31
2	10.15	101.50	207.40	103.70
3	10.06	100.60	208.36	104.18
4	10.08	100.80	206.82	103.41
5	10.10	101.00	197.88	98.94
6	10.01	100.10	205.46	102.73
7	10.16	101.60	201.73	100.86
8	10.22	102.20	203.90	101.95
9	9.81	98.10	200.62	100.31

Sensitivity

The LOD and LOQ were computed theoretically for VCS and BSA and found to be 0.465 µg/mL, 1.410 µg/mL, 41.096 µg/mL, and 124.533 µg/mL, respectively.

Our study developed a simple, effective UV method to determine the concentration of VCS and BSA, with good accuracy and precision compared to the previously mentioned literature, which has used complex and time-consuming analytical procedures.

Conclusion

The created and validated UV estimating method described here is speedy, simple, accurate, sensitive, and specific, with a lack of time in comparison with the HPLC analysis method, which needs preparation of the samples for analysis like extraction of solvent, degassing, and heating technique. This approach was also effectively applied to the quantitative calculation of VCS and BSA in combination dosage form. Thus, the disclosed method is critical and has wide application in the industry for quality control and assessment of VCS and BSA in mixed-dose forms.

Contribution of authors

The authors did this work mentioned in this article, and the authors will bear all liabilities pertaining to claims relating to the article's content. Hamid Jabbar Hasan achieved the experimental work in the study. Mowafaq Mohammed Ghareeb reviewed the article, provided comments on the study design, drafted the manuscript, and supervised this study.

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