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UV Spectrophotometric Quantification of Vincristine and Vinblastine in *Catharanthus roseus* Extracts

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Abstract: Background, *Catharanthus roseus* is widely used in traditional medicine due to its richness in secondary metabolites, particularly alkaloids and flavonoids. In modern pharmacology, *Vinca* alkaloids such as vincristine and vinblastine are of major interest for their antineoplastic properties. However, accessible and cost-effective methods for their quantification in plant matrices remain limited. Methodology, a qualitative phytochemical screening of ethanolic extracts from whole *C. roseus* plants was performed to identify major classes of secondary metabolites. Alkaloid fractions were extracted and weighed to estimate relative content. UV spectrophotometry was employed for compound identification and quantification, with calibration curves established for vincristine ($\lambda_{\max} = 221$ nm) and vinblastine ($\lambda_{\max} = 208$ nm). Differential subtraction and spectral comparison with standards were used to confirm compound identity. Results and discussion, the phytochemical screening revealed the presence of 8 out of 10 tested families of secondary metabolites, including alkaloids, anthocyanins, anthraquinones, catechic tannins, flavonoids, phenols, polyphenols and triterpenes, while saponins and steroids were absent. Alkaloid content ranged between 0.30 g/100 g and 0.55 g/100 g of dry matter, consistent with literature values. UV spectra confirmed similarities between isolated fractions and standards, though artifacts were observed between 220–250 nm, likely due to incomplete subtraction of carbonyl signals. Quantification yielded vincristine levels between 93.78 ± 0.56 mg and 177.65 ± 26.78 mg per plant, and vinblastine levels between 31.75 ± 0.12 mg and 67.01 ± 5.40 mg per plant. Relative percentages of vincristine and vinblastine accounted for up to 99% of total alkaloids in certain batches. These findings highlight both the potential and limitations of UV spectrophotometry, with matrix effects and spectral artifacts requiring methodological refinements. Conclusion, this study demonstrates that differential UV spectrophotometry provides a simple, rapid, relatively precise, and low-cost method for routine quantification of vincristine and vinblastine in *C. roseus*. While limitations such as spectral artifacts and matrix effects persist, validation of this approach could offer an accessible alternative for laboratories lacking advanced chromatographic techniques, thereby expanding routine monitoring of *Vinca* alkaloids in plant matrices.



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1. Introduction

Reliable analytical data are fundamental to pharmaceutical and biomedical research and rely on analytical methods that are fit for purpose and appropriately validated [1]. Beyond analytical performance, method development should consider technical feasibility, operational costs, equipment availability, and implementation in the intended laboratory setting [1]. Such considerations are particularly important in low- and middle-income countries, where limited infrastructure and resources often restrict the routine use of advanced analytical technologies [2]. This challenge is especially relevant in sub-Saharan Africa, where the rising cancer burden underscores the need for robust, affordable analytical methods to support pharmaceutical research and improve access to quality anticancer therapies [3]. In Cameroon, where cancer incidence continues to increase amid resource constraints, accessible analytical approaches are essential for strengthening local research capacity and pharmaceutical quality control [4].

Vincristine (VCR) and vinblastine (VLB) are two clinically important anticancer alkaloids widely used in the treatment of haematological malignancies and several solid tumours, including lymphomas, leukaemia, breast cancer, and lung cancer [5,6]. These compounds belong to the class of vinca alkaloids isolated from *Catharanthus roseus* and exert their anticancer activity through disruption of microtubule assembly, leading to inhibition of cell division [6]. The only structural difference between the compounds is the functional group attached to the nitrogen atom of the vindoline portion of the molecule: a methyl group in VLB and a formyl group in VCR (Figure 1). VCR and VLB naturally occur only in trace amounts in plant tissues, with yields estimated at approximately 0.0005% of the dry weight of aerial plant parts [6,7]. Such low concentrations make extraction difficult and economically challenging while also creating analytical challenges for their reliable detection and quantification in plant matrices. The importance of these compounds has stimulated the development of numerous analytical methods for their determination in biological, clinical, and pharmaceutical samples [5,8]. UV spectrophotometry was among the earliest techniques used for their detection and was progressively complemented by more advanced approaches such as liquid chromatography, electrochemical methods, capillary electrophoresis, and improved spectrophotometric techniques [5,8]. Among these methods, high-performance liquid chromatography (HPLC) has become one of the most commonly employed techniques for the quantification of VCR and VLB because of its robustness, sensitivity, and compatibility with various detection systems [5,9]. However, HPLC generally requires extensive sample preparation procedures, expensive instrumentation, and highly trained personnel, which may limit its application in laboratories with restricted resources [5,9].

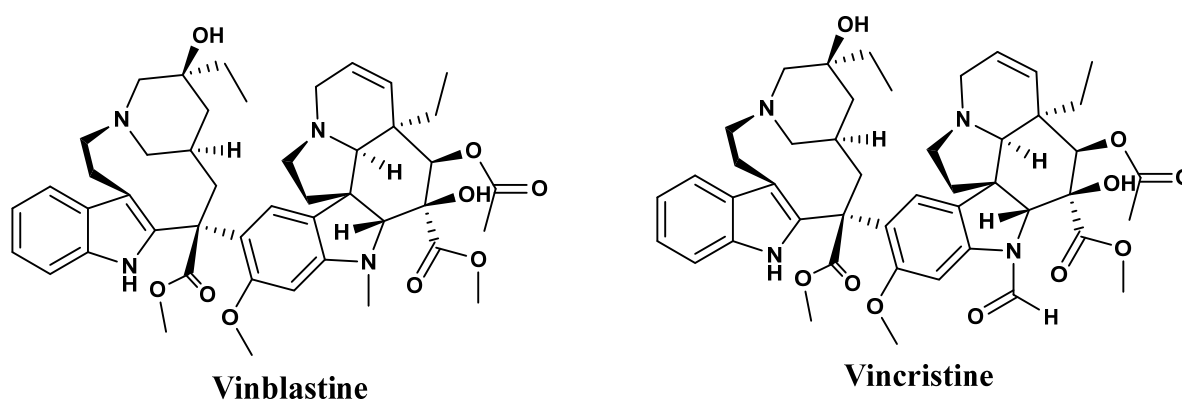


Figure 1. Structures of vinblastine and vincristine.

Several analytical methods exploit the spectral characteristics of VCR and VLB for their determination in pharmaceutical preparations, dosage forms, and biological matrices [8,10,11]. Despite their analytical performance, many of these approaches require considerable technical expertise and financial resources that may not be readily available in developing countries such as Cameroon, where scientific research infrastructure remains limited despite the increasing burden of cancer [12]. Although UV spectrophotometry exhibits lower sensitivity and selectivity than chromatographic techniques, it remains attractive because of its simplicity, rapidity, affordability, and suitability for routine analyses [13]. It has been proven useful for the rapid determination of vincristine and vinblastine from various

matrices including human serum, pharmaceutical dosage forms, and clinical preparations [5,6,8]. Therefore, the present study aimed to develop and preliminary evaluate a simple UV spectrophotometric method for the quantification of VCR and VLB in *Catharanthus roseus* extracts. To the best of our knowledge, this is the first study reporting the application of differential UV spectrophotometry for the simultaneous quantification of these alkaloids directly in a *Catharanthus roseus* plant matrix.

2. Methodology

2.1. Reagents and Equipment

Vincristine sulphate (Vinlon[®], Celon Laboratory Pvt. Ltd., Hyderabad, India) and vinblastine sulphate (Myoblastin[®], Eli Lilly and Company, West Ryde, Australia) were obtained from Pharmacie La Référence SARL. Absorbance measurements were performed using a BIOBASE BK-UV1800PC UV—visible spectrophotometer operating within a wavelength range of 190–1100 nm. The instrument specifications included a wavelength accuracy of ± 0.5 nm, photometric accuracy of ± 0.002 absorbance units (AU) within the range of 0–0.5 AU and ± 0.004 AU within the range of 0.5–1.0 AU, and transmittance accuracy of $\pm 0.5\%$ within the range of 0–100% transmittance. The instrument also had a noise level of ± 0.0005 AU. The light sources consisted of a deuterium lamp for the ultraviolet region and a tungsten lamp for the visible region, while detection was achieved using a silicon photodiode detector.

2.2. Plant Material

Catharanthus roseus (L.) G. Don plants were obtained from experimental plants cultivated at the Regional Laboratory for Biological Control and Applied Microbiology, Institute of Agricultural Research for Development (IRAD), Yaoundé, Cameroon. Seeds were germinated under different conditions (See Table S1, Supporting Material) at a depth of 1.5 cm in a sterilized soil-sand substrate composed of one-quarter sand and three-quarters soil, without any pre-germination treatment. Transplantation into nursery beds was carried out 30 days after germination, and plant cultivation was performed according to standard forest nursery management practices [14]. The plant material was identified at the Cameroon National Herbarium by comparison with the reference specimen voucher number N° 18590.

2.3. Collection and Processing of Plant Material

Six batches, each consisting of 10 plants, were collected after 30 days of cultivation in humus-rich soil. The plant materials were air-dried in the absence of direct sunlight for 10–15 days, cut into smaller pieces using pruning shears, and pulverized using an electric grinder. The resulting powders (10 g) were extracted by four repeated macerations in 96% ethanol (100 mL) during twelve hours' period each, until complete exhaustion of the plant material. The obtained extracted was concentrated to dryness and kept at 4 °C until further usage.

2.4. Phytochemical Characterization

2.4.1. Qualitative Screening

Qualitative phytochemical characterization of the ethanolic extract was performed using modified methods described by Harborne (1998) and Evans (2009) [15,16]. The metabolite classes investigated included alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, polyphenols, saponins, steroids, tannins (gallic and catechic) and triterpenes.

2.4.2. Alkaloids Quantification

Extraction of alkaloids was performed using a modified method described by Gupta et al. [17]. The ethanolic extract obtained above was acidified using 3% hydrochloric acid (HCl) and partitioned with hexane to remove fats and neutral compounds. The resulting aqueous acidic phase was adjusted to pH 8–9 using ammonia solution, followed by liquid-liquid extraction with dichloromethane (CH₂Cl₂). This procedure yielded a CH₂Cl₂ fraction enriched in primary, secondary, and tertiary alkaloids, including vincristine and vinblastine, while the remaining basic aqueous phase contained N-oxide alkaloids and quaternary amines [18]. The presence of alkaloids in the recovered extract was confirmed using Mayer's reagent test [15]. The CH₂Cl₂ fraction obtained after extraction was weighed to estimate the content of total extractable alkaloids. Since vincristine and vinblastine are expected to be present in this fraction, only the CH₂Cl₂ extract was retained for subsequent analyses.

The percentage of alkaloids extracted was calculated using the following formula: Alkaloid content (%) = (Mass of alkaloid fraction/Mass of initial dry plant material) × 100.

2.5. Development of the Quantification Method

The quantification method was developed in three stages: (i) preparation of calibration solutions, (ii) UV scanning and spectral isolation of VCR and VLB signals from the plant extract matrix, and (iii) quantification of VCR and VLB using calibration curves generated by linear regression analysis. The method was adapted from Jeewantha et al. [8].

2.5.1. Preparation of Calibration Solutions

Stock solutions of vincristine sulphate and vinblastine sulphate at a concentration of 100 µg/mL were prepared from Vinlon® and Myoblastin® formulations by dilution with 96% ethanol. Working standard solutions were prepared by transferring 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the stock solutions into separate 10 mL volumetric flasks, followed by dilution to volume with 96% ethanol, to obtain final concentrations of 5, 10, 15, 20, and 25 µg/mL, respectively.

2.5.2. UV Scanning of Standards and Signal Isolation

UV Scanning of Standard Solutions

UV spectra of VCR and VLB standard solutions at different concentrations were recorded over a wavelength range of 190–400 nm using 96% ethanol as the blank solution. The absorption maxima of the standards were identified and selected for subsequent analyses.

Spectral Isolation of Vincristine and Vinblastine from Plant Extracts

Dried alkaloid extracts were dissolved in 96% ethanol to obtain extract solutions with concentrations ranging from 50 to 315 µg/mL, allowing suitable absorbance measurements within the detectable range of the spectrophotometer. The UV absorption spectra of the extracts were first recorded using 96% ethanol as the blank. Subsequently, additional scans were performed using 5 µg/mL vincristine sulphate solution and 5 µg/mL vinblastine sulphate solution as reference blanks, respectively. This procedure enabled the reduction of spectral interference from co-extracted matrix constituents and facilitated identification of absorbance regions corresponding to vincristine and vinblastine.

2.5.3. Quantification of Vincristine and Vinblastine

The isolated spectra obtained from plant extracts were compared with those of the standard compounds to verify the presence of VCR and VLB through similarities in their spectral profiles and absorbance maxima. Calibration curves were constructed by plotting absorbance against concentration for each standard solution. Linear regression equations derived from these calibration curves were subsequently used to determine the concentrations of vincristine and vinblastine in the *Catharanthus roseus* extracts.

2.6. Statistical Analysis

All experiments were performed in independent triplicates, and the data are expressed as mean ± standard deviation. The calculation of means and errors, regression data analysis, inverse prediction equation modeling, and data interpolation were conducted using GraphPad Prism software 9.3 (GraphPad Software Inc., San Diego, CA, USA). In the case of missing values, the dataset was not discarded, provided that it did not compromise the accuracy of the performed analysis.

3. Results

3.1. Preliminary Phytochemical Analysis

3.1.1. Qualitative Screening

The results of the qualitative phytochemical screening of the ethanolic extracts are presented in Table 1. Phytochemical analysis revealed the absence of gallic tannins, saponins and steroids in all batches examined. In contrast, alkaloids, anthocyanins, anthraquinones, catechic tannins, flavonoids, phenols, polyphenols and triterpenes were detected consistently across all batches. Since alkaloids represent the metabolite family of interest

in the present study, their presence was further confirmed in the alkaloid-enriched matrices obtained from each of the six batches.

Table 1. Qualitative phytochemical screening of ethanolic extracts from different batches.

Batch	Alc.	Phe.	Po. Phe.	Tannins		Sap.	Flav.	Trit.	Ster.	Antho.	Anthr.
				Gal.	Cat.						
Batch 1	+	+	+	—	+	—	+	+	—	+	+
Batch 2	+	+	+	—	+	—	+	+	—	+	+
Batch 3	+	+	+	—	+	—	+	+	—	+	+
Batch 4	+	+	+	—	+	—	+	+	—	+	+
Batch 5	+	+	+	—	+	—	+	+	—	+	+
Batch 6	+	+	+	—	+	—	+	+	—	+	+

(—) = absent, (+) = present, Alkaloids (Alc.), Phenols (Phe.), Polyphenols (Po. Phe.), Gallic (Gal.), Cathechic (Cat.), Saponins (Sap.), Flavonoids (Flav.), Triterpenes (Trit.), Steroids (Ster.), Anthocyanins (Antho.), Anthraquinones (Anthr.).

3.1.2. Estimation of Primary, Secondary, and Tertiary Alkaloid Content

Table 2 presents the estimated alkaloid content of the *Catharanthus roseus* extracts, determined by gravimetric analysis of the fractions containing primary, secondary, and tertiary alkaloids, including vincristine and vinblastine, obtained from the different plant matrices. Variations in alkaloid yield were observed among the matrices, with total alkaloid content ranging from 0.20 ± 0.00 g to 0.54 ± 0.05 g per 100 g of dried plant material, indicating differences in alkaloid accumulation between the analysed batches.

3.2. Identification of Compounds of Interest

Figure 2 presents the superimposed UV spectra of the plant matrices, the isolated spectra of VCR and VLB, and their corresponding standard compounds. Analysis of the vincristine standard spectrum revealed characteristic absorption peaks at 204, 221, 256 and 297 nm, whereas the vinblastine standard spectrum exhibited absorption maxima at 203, 208 and 258 nm. The spectra isolated from the plant matrices displayed absorption peaks at 205 nm, 224 nm and 288 nm for vincristine, and 207 nm and 236 nm for vinblastine. Despite slight shifts in wavelength positions, the isolated spectra exhibited overall spectral profiles comparable to those of the corresponding standards. These similarities suggest the presence of vincristine- and vinblastine-related signals within the analyzed *Catharanthus roseus* extracts. The absorption maxima, wavelengths of 221 nm for vincristine and 208 nm for vinblastine were selected as the optimal analytical wavelengths for subsequent quantification.

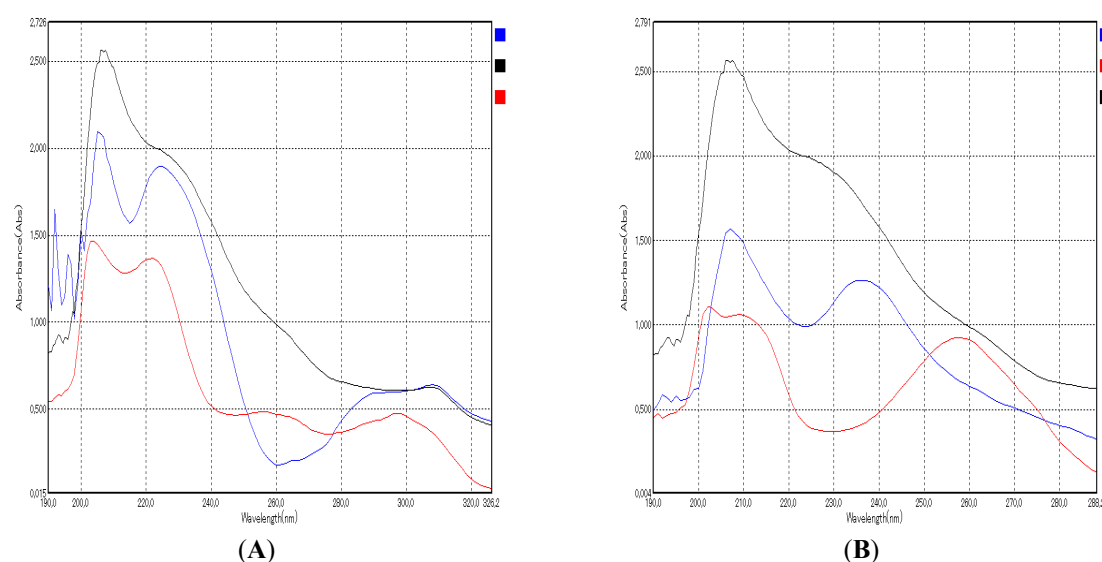


Figure 2. Superimposed UV spectra of 25 µg/mL vincristine sulphate (A) and 25 µg/mL vinblastine sulphate (B) (red line), the plant matrix (black line), and the corresponding isolated VCR or VLB spectra obtained from the matrix (blue line).

Table 2. Relative masses of alkaloids obtained after extraction.

Batch	Alc. Mass (g/100 g Dry Matter)
Batch 1	0.54 ± 0.05
Batch 2	0.20 ± 0.00
Batch 3	0.36 ± 0.04
Batch 4	0.50 ± 0.04
Batch 5	0.30 ± 0.00
Batch 6	0.33 ± 0.00

3.3. Quantification of Vincristine and Vinblastine

For the quantification of VCR and VLB, optical density measurements of the standard solutions were used to construct calibration curves. Linear regression analysis yielded the following equations: $\text{Abs} = 0.05186 [\text{VCR}] - 0.3965$ ($R^2 = 0.9990$) and $\text{Abs} = 0.1389 [\text{VLB}] - 0.8575$ ($R^2 = 0.9607$) for vincristine and vinblastine, respectively (See supporting material Table S2; Figures S1 and S2).

The concentrations of vincristine and vinblastine in the different *Catharanthus roseus* batches were subsequently determined using the corresponding calibration equations, and the results are presented in Table 3. Variations in the content of both alkaloids were observed among the analyzed batches. Vincristine content ranged from 93.78 ± 0.56 mg to 177.65 ± 26.78 mg, whereas vinblastine content ranged from 31.75 ± 0.12 mg to 67.01 ± 5.40 mg per 100 g of dried plant material.

When these values were compared with the total alkaloid content determined for each batch, vincristine and vinblastine together represented a substantial proportion of the extracted alkaloid fractions. Detailed results are presented in Table 4.

Table 3. Vincristine and Vinblastine content per *Catharanthus roseus* plant.

Batch	VCR (mg)	VLB (mg)
Batch 1	154.96 ± 22.20	50.95 ± 02.13
Batch 2	139.90 ± 00.04	58.31 ± 00.07
Batch 3	109.34 ± 15.83	50.00 ± 07.03
Batch 4	177.65 ± 26.78	67.01 ± 05.40
Batch 5	139.28 ± 00.04	49.23 ± 00.15
Batch 6	93.78 ± 00.56	31.75 ± 00.12

Table 4. Relative content of Vincristine and Vinblastine compared to total alkaloids.

Batch	VCR (%)	VLB (%)	Total
Batch 1	28.25 ± 1.75	9.34 ± 0.39	37.59
Batch 2	69.95 ± 0.02	29.15 ± 0.04	99.10
Batch 3	30.60 ± 5.51	14.00 ± 2.53	44.60
Batch 4	35.54 ± 4.73	13.42 ± 0.82	48.96
Batch 5	46.43 ± 0.02	16.41 ± 0.05	62.84
Batch 6	28.16 ± 0.02	9.53 ± 0.04	37.69

4. Discussion

Qualitative phytochemical screening of the ethanolic extract of *C. roseus* (whole plant) revealed a diversity of secondary metabolites including alkaloids, anthocyanins, anthraquinones, catechic tannins, flavonoids, phenols, polyphenols and triterpenes. The presence of alkaloids and flavonoids may partly explain the widespread use of *C. roseus* in traditional medicine and its broad range of pharmacological activities. Previous studies have demonstrated antioxidant, antiviral, antidiabetic, antimicrobial, antidiarrheal, antineoplastic, and anticancer properties associated with *C. roseus* alkaloids and other secondary metabolites [6,19,20]. The absence of saponins and steroids in *C. roseus* extracts observed in the present study is consistent with previous reports [21].

Estimation of primary, secondary, and tertiary alkaloid content after fractionation of the ethanolic extract yielded values ranging from 0.30 to 0.55 g/100 g. These values fall within the range generally reported in the literature (0.3–1.2% dry weight) [22,23]. The extraction yields obtained here were lower than those reported by Mall et al. who observed higher alkaloid yields in the aerial parts of *C. roseus* [24]. Such differences may be attributed to variations in extraction conditions, plant material composition, and the possible retention of more polar alkaloids, such as N-oxide alkaloids and quaternary amines, in the aqueous phase during fractionation [18].

Analysis of the UV spectra obtained from the plant extracts revealed differences between the spectra expected after differential subtraction and the isolated spectra obtained experimentally. Although fractionation reduces spectral interference from co-extracted constituents, differential spectrophotometric approaches remain

susceptible to artifacts resulting from incomplete subtraction of overlapping signals. This phenomenon may explain the additional spectral features observed for vinblastine between 220 and 250 nm, instead of the single expected absorption peak around 258 nm. Similar analytical challenges have previously been reported in UV-based analyses. It has been demonstrated that derivative spectrophotometry can improve spectral resolution and reduce the occurrence of false peaks generated by overlapping signals [25]. Likewise, Caudron et al. employed UV spectral discrimination during HPLC analysis of several *Vinca* alkaloids and demonstrated the usefulness of spectral characteristics in differentiating structurally related compounds exhibiting overlapping absorbance regions [11]. Additional spectral variations may also arise from matrix effects associated with residual co-extracted compounds present after fractionation. These interactions can alter the electronic environment of the compounds of interest and induce wavelength shifts or distortions in absorption profiles [26–28]. Such effects have been reported for aromatic compounds and may contribute to the differences observed between standard and isolated spectra [26].

The selection of analytical wavelengths for quantification was based on similarities observed between isolated spectra and reference standards, leading to the use of 221 nm for vincristine and 208 nm for vinblastine. These values differ slightly from previously reported absorption maxima for these compounds [11]. Nevertheless, slight variations during development of a UV spectrophotometric method for vincristine and vinblastine analysis is common [8]. The calibration curves obtained in the present study showed good linearity, with R^2 values of 0.9990 for vincristine and 0.9607 for vinblastine, indicating acceptable relationships between absorbance and concentration within the tested range of 5–25 $\mu\text{g/mL}$. Comparable studies involving plant-derived compounds such as quinine and curcumin have also reported R^2 values ranging from 0.90 to 0.99 [29,30].

UV spectrophotometric methods have been developed and demonstrated satisfactory analytical performance for the quantification of a wide range of pharmaceutical compounds, including gatifloxacin, dexamethasone, carbohydrates, and vinca alkaloids, with reported coefficients of determination (R^2) ranging from 0.93 to 0.99 [8,31,32]. Although high-performance liquid chromatography (HPLC) and liquid chromatography—mass spectrometry (LC—MS/MS) remain the reference techniques for the analysis of vinca alkaloids because of their superior sensitivity and selectivity [5,9], their routine application is often limited by high instrumentation costs, complex sample preparation, and the need for specialized expertise, particularly in resource-constrained laboratories [5]. Consequently, UV spectrophotometry remains an attractive alternative for routine analysis where rapid, inexpensive, and easily implemented analytical methods are required. The present study addresses the limited availability of accessible methods for the determination of vincristine and vinblastine in *Catharanthus roseus* plant matrices. The findings suggest that differential UV spectrophotometry provides a practical and cost-effective strategy for quantifying these alkaloids, particularly in laboratories where access to advanced chromatographic instrumentation is constrained. One limitation of the present study is that the experiments were conducted using *C. roseus* plants subjected to experimental treatments that may have influenced alkaloid production and consequently affected the measured concentrations of vincristine and vinblastine. This variability was particularly evident in Batch 2, in which vincristine and vinblastine represented approximately 99% of the quantified alkaloid content detected, suggesting that the experimental treatment may have influenced alkaloid accumulation. This factor limits broader interpretation of the quantified levels obtained in the present work. Nevertheless, comprehensive validation, including evaluation of precision, accuracy, specificity, limits of detection and quantification, and robustness, is required before routine analytical application.

5. Conclusion

The present study demonstrates that differential UV spectrophotometry has the potential to provide an accessible analytical platform for the routine quantification of vincristine and vinblastine in *C. roseus*, particularly in laboratories where advanced chromatographic instrumentation is unavailable. Although the proposed method showed promising performance, certain limitations remain, particularly those related to spectral artifacts and matrix effects that may influence signal resolution and quantification accuracy. Additional validation studies are therefore required to establish the reliability and reproducibility of the method.

Supplementary Materials

The additional data and information can be downloaded at: <https://media.scilit.com/articles/others/2607291701105604/NPA-26060184-SI.pdf>. *Calibrations curves* are provided in the supplementary materials. Figure S1: Regression line of vincristine (VCR) from standard solutions. Figure S2: Regression line of vinblastine (VLB) from standard solutions. Table S1: Application modes of treatments administered to *C. roseus*. Table S2: Concentrations of standard solutions and their absorbance.

Author Contributions

J.P.N.O.: Investigation, data analysis, data curation, validation, and writing—original draft preparation, review and final editing; R.N.N.N.: Data curation, writing—original draft preparation, review and final editing; S.T.G.: Data analysis, data curation, validation, writing—original draft preparation, review and final editing; E.N.M.A.: Investigation, review and final editing; J.M.M.M.: Conceptualization, Supervision, writing—original draft preparation, review and final editing; N.N.: Conceptualization, Supervision, writing—original draft preparation, review and final editing; All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare no conflict of interest. Given the role as Editorial board member, Stephanie Tamdem Guetchueng had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

During the preparation of this manuscript, the authors used Copilot and ChatGPT to improve grammar, spelling, overall language clarity, and to assist with graphical abstract generation. The authors carefully reviewed and edited all outputs and take full responsibility for the content of the publication.

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