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Spectral estimation of vincristine Sulphate in pharmaceutical Formulations

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Abstract

A novel spectrophotometric technique has been introduced for quantifying minuscule quantities of the anticancer drug vincristine sulfate (VCS). This approach is based on the combination of vincristine sulfate with 4-chloroaniline. The resulting dye displays a molar absorptivity of 1.47×104 l.mol-1. cm-1 at its peak absorption wavelength of 455 nm. The method adheres to Beer's law over a range of 10-300 µg of vincristine in 10 ml (equivalent to 1.0 – 30 ppm) and exhibits low detection limits (LOD) of 0.12 µg/ml and quantification limits (LOQ) of 0.40 µg/ml. The color reaction remains highly stable and maintains its absorbance without significant deviation (within acceptable analytical error) for up to 24 hours, demonstrating a relative error between +0.37 to +1.02% and a relative standard deviation ranging from ±0.42 to ±1.05%, depending on the concentration level. This method has been effectively utilized for quantifying vincristine sulfate in pharmaceutical formulations.

***Keywords*:** vincristine sulphate; 4-chloro aniline; spectrophotometry

Introduction

Vincristine belongs to a class of bisindole alkaloids characterized by a dihydro indole nucleus in the lower portion of their molecular structure. This compound, referred to as VCS, plays a crucial role in the treatment of various conditions such as Hodgkin's disease, lymphocytic lymphoma, histiocytic lymphoma, advanced testicular cancer, advanced breast cancer, Kaposi's sarcoma, and Letterer-Siwe disease. Given the ongoing challenge posed by neoplastic disorders in medicine, it is imperative to develop highly sensitive and accurate methods for detecting and quantifying these antineoplastic drugs, including VCS, in diverse clinical and biological applications.

Several methodologies have been devised for analyzing these substances, encompassing high-performance liquid chromatography, polarography, radioimmunoassay, electroanalytical techniques, and thin-layer chromatography. Additionally, VCS has received official recognition in both the British Pharmacopoeia and the United States Pharmacopoeia. In this study, we explore the utility of 4-chloroaniline as a coupling agent for determining vincristine in both its pure form and pharmaceutical preparations.

Instrumentation

Apparatus

A double-beam UV-Vis spectrophotometer, specifically the Spectroscan Model 50 from Japan, was equipped with 1.0 cm matched cells for all spectral readings.

Materials and Chemicals

All chemicals and solvents were of analytical reagent quality, and the water used was subjected to double distillation.

Vincristine sulfate (VCS), in a solution of 1000 µg/ml concentration, was prepared by dissolving 0.1g of vincristine in distilled water with 2 ml of 5M HCl. The solution was then brought up to a final volume of 100 ml in a volumetric flask using distilled water. Working solutions were subsequently prepared through further dilutions of the stock solution.

A solution of 4-Chloro aniline at a concentration of 0.1% was prepared by dissolving 0.1 g of 4-Chloro aniline in a 100 ml volumetric flask filled with distilled water and containing 2 ml of 5M HCl.

A solution of Sodium nitrite at a concentration of 0.5% was prepared by dissolving 0.5 g of Sodium nitrite in 100 ml of distilled water.

A solution of Sulphamic acid at a concentration of 5% (w/v) was prepared by dissolving 5.0 g of sulphamic acid in 100 ml of distilled water.

Additionally, 5M HCl solutions were prepared as well.

Procedure and Calibration Curve

In 10 ml calibrated flasks, 3.0 ml of the 0.1% 4-chloro aniline solution were transferred. These flasks were then cooled in an ice bath, and 0.5 ml of a 0.5% aqueous sodium nitrite solution was added, followed by 0.5 ml of 5M HCl. After cooling for 5 minutes, 0.5 ml of a 5% aqueous sulphamic acid solution was introduced, and the reaction mixture was allowed to cool for an additional 5 minutes with occasional shaking. Standard solutions of VCS (ranging from 10 to 300 mg) were added to each flask and heated in a boiling water bath for 10 minutes. After cooling, the flasks were diluted with distilled water to the mark.

Subsequently, absorbance measurements were carried out at 455 nm against a blank reagent. A linear calibration curve was established within the concentration range of (10-300) µg of VCS per 10 ml. It is important to note that higher concentrations exhibit a negative deviation from Beer's law (as depicted in Figure 1). The apparent molar absorptivity was determined to be 1.47×104 l.mol-1. cm-1.

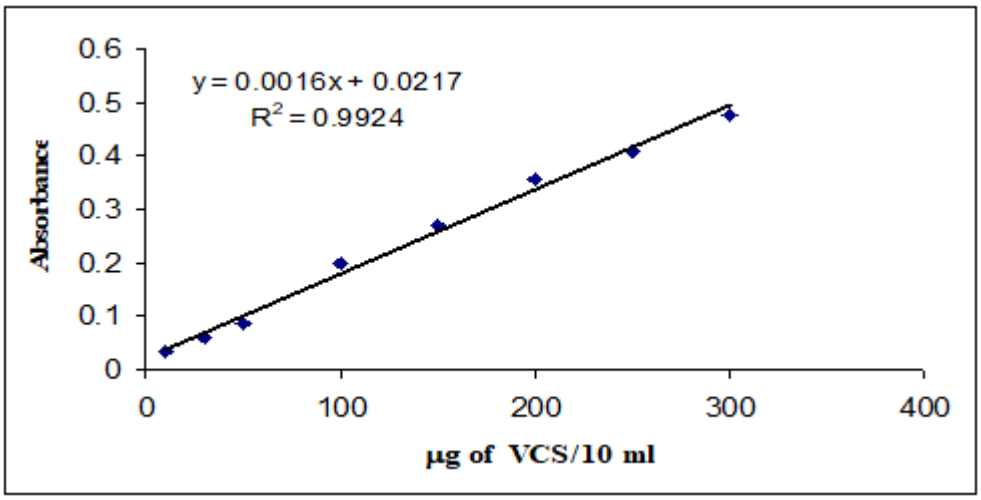


Fig. 1: Calibration graph for Vincristine determination using the proposed method

Results and Discussion

Influence of Acid:

Various acids, including HCl, CH3COOH, HNO3, and H2SO4, were examined to determine their impact on the diazotization of 4-chloroaniline to produce a vibrant colored dye, maintaining a strong color constant and minimizing the baseline absorbance. The experimental findings revealed that a 0.5 ml volume of 5M HCl solution was chosen for the reaction due to its ability to yield a highly intense dye with correspondingly low reagent blank absorbance.

Influence of Sodium Nitrite Quantity and Reaction Time:

The influence of different amounts (ranging from 0.25 ml to 1.0 ml) of 0.5% sodium nitrite, coupled with varying reaction durations, was examined on the resulting azo dye's absorbance. The outcomes indicated that a 0.5 ml quantity of 0.5% sodium nitrite solution, in conjunction with a 5-minute reaction time, was deemed optimal and thus recommended for subsequent experiments.

Influence of Sulphamic Acid Quantity and Reaction Time:

Based on the experimental results, it was determined that employing 0.5 ml of 5% sulphamic acid with a standing time of 5 minutes was most effective for enhancing the formation of the resultant-colored azo dye.

Influence of Reagent (4-chloroaniline) Quantity:

The impact of varying amounts of 4-chloroaniline on the absorbance of the azo dye was thoroughly investigated. The results indicated that utilizing 3 ml of 0.1% 4-chloroaniline was the most suitable option for achieving the highest intensity value for the azo dye, accompanied by a favorable determination coefficient (r2 = 0.9924).

Absorption Spectra

The absorption spectrum of the resultant-colored dye, formed through the coupling of VCS with 4-chloroaniline in an acidic environment, exhibits a peak absorption at 455 nm, which stands in stark contrast to the absorbance of the reagent blank (refer to Figure 2).

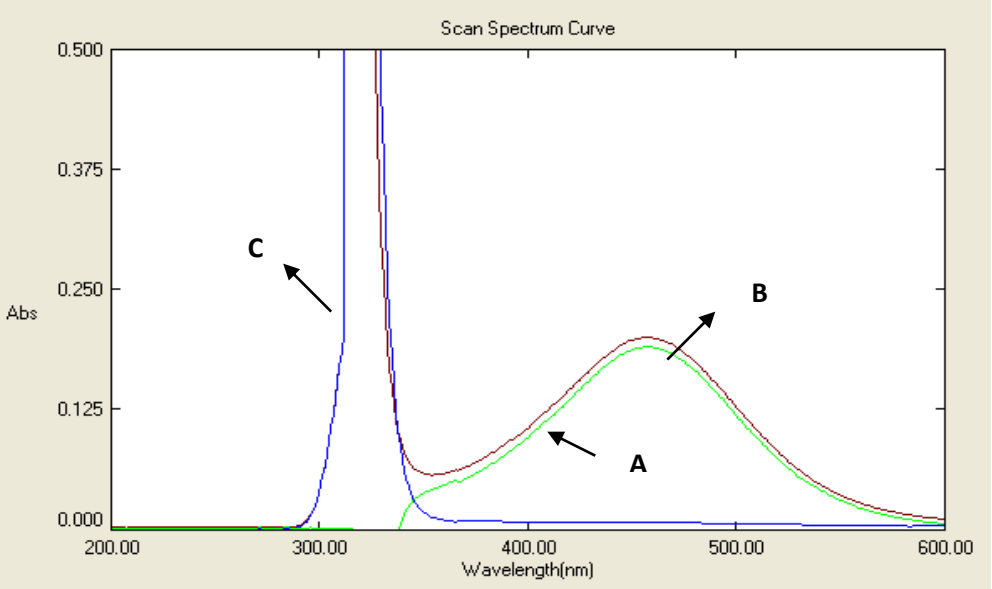


Fig. 2: Absorption spectra of 100 µg VCS treated according to the recommended procedure and measured against (A) blank, (B) distilled water, and (C) blank measured against distilled water

Interferences

To assess the effectiveness and specificity of the suggested analytical approach, a comprehensive investigation was conducted on common excipients typically found in pharmaceutical dosage forms, such as glucose, lactose, gum Arabic, and starch. The experimental findings indicated no interference observed from these additives or excipients at concentrations of up to 1000 µg in the current method, as demonstrated in Table 1.

Table 1. Effect of foreign compounds for assay of Vincristine

Foreign compound	**Recovery (%) of 100 µg VCS per µg foreign compound added**		
	500	**1000**	**1500**
Glucose	99.3	100.0	98.9
Gum Arabic	100.0	99.7	99.3
Lactose	100.2	100.6	100.4
Starch	100.2	99.5	100.6

Accuracy and precision

To assess the accuracy and consistency of the calibration curve, vincristine was quantified at three distinct concentrations. The outcomes presented in Table 2 confirm the reliability of the method.

Table 2: Accuracy and precision of the calibration curve

Amount of Vincristine taken, µg	Relative error, %*	Relative standard deviation, %*
50	1.02	±3.16
100	0.37	±0.43
200	0.91	±2.18

*Average of five determinations

Stoichiometry of reaction

The product resulting from the reaction between diazotized vincristine and 4-chloroaniline was analyzed for its stoichiometry using the continuous variation method, commonly known as Job's (as depicted in Figure 3).

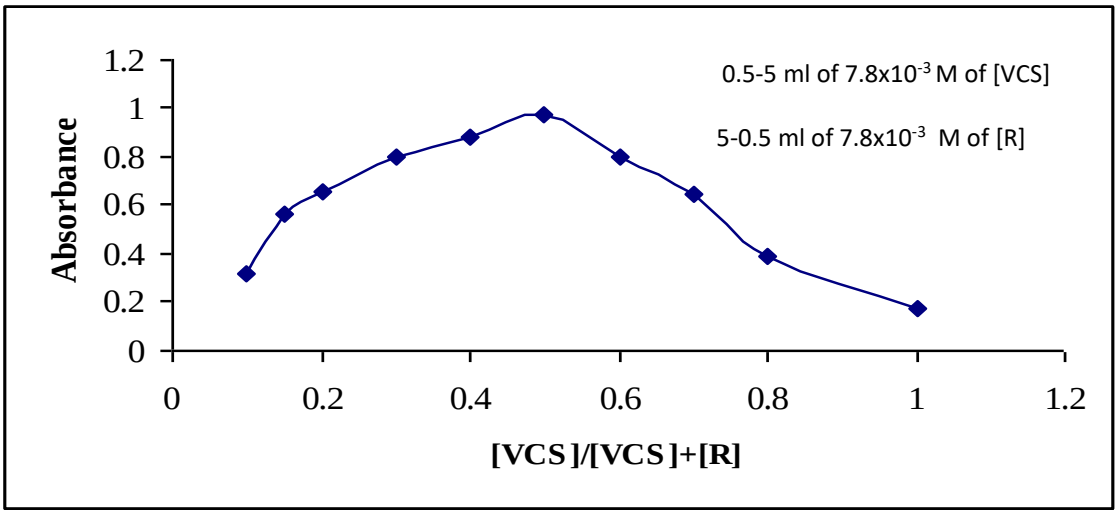
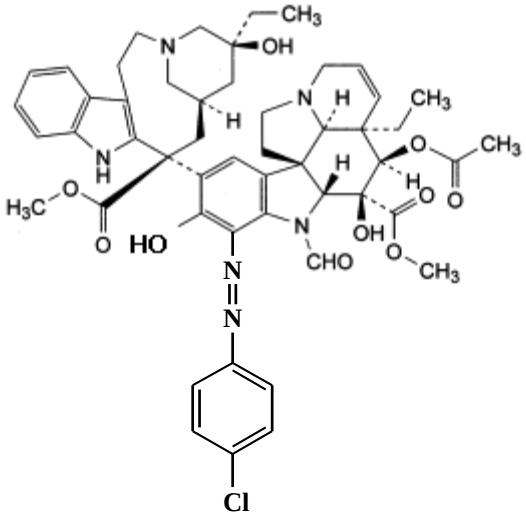


Fig.3: Job's plot for vincristine and 4-chloro aniline

The results indicate that azo dye was formed in a ratio of 1:1 , indicating a monoazo dye with probably the following structure.



Yellow-orange azo dye

Application of the Method

The suggested approach was effectively employed to quantify vincristine in pharmaceutical formulations. The findings, as presented in Table 3, demonstrate a favorable recovery rate.

Table 3: The recovery of Vincristine in pharmaceutical preparations

Drug	**□g Vincristine present / 10 ml**	**□g Vincristine measured / 10 ml**	***Recovery, %**
Ninacristine	50	46.9	93.8
1mg/ml	100	96.9	96.3
NDI(Iraq)	200	196.4	98.2
Vincristine	50	47.8	95.6
1mg/ml	100	97.3	97.3
Hospira(Turkey)	200	198.8	99.4

***Average of five determinations

Comparison of the methods and t-test

The efficacy of the proposed method was evaluated by computing the t-test compared to a standard method (as described in reference [22]) at a 95% confidence level with eight degrees of freedom. The results revealed that the t-value was lower than the critical value, signifying no substantial discrepancies between the proposed method and the standard method for estimating VCS (as demonstrated in Table 4).

Table 4. Analytical applications of the proposed method and t-values experimental.

Drug	Recovery, %*		t.exp
	British Pharmacopeia method	Present method	
Ninacristine 1mg/ml NDI(Iraq)	93.8	96.3	±1.575
Vincristine 1mg/ml Hospira (Turkey)	95.6	97.3	±0.837

*Average of five determinations of 100 µg VCS

Table 5 compares certain analytical parameters between the current method and another spectrophotometric method described in the literature (reference [23]). The proposed method offers an advantage over the previously published method in that it operates at ambient temperature and is not limited to a specific set of conditions.

Table 5. Comparison of the methods

Analytical parameters	**Present method**	**Literature method**
Temperature ^{0}C	At room temperature	At room temperature
λ_{max} (nm.)	455	430
Medium of reaction	Aqueous	Aqueous
Reagent	4-Chloro aniline	4-Nitro aniline
Beer's law range (ppm)	1-30	0.5-10
Molar absorptivity (l.mole^{-1}.cm^{-1})	1.478×10^4	3.434×10^4
Sandell's sensitivity, mg/cm–2	0.062	0.026
Colour of the dye	Yellow-orange	Yellow
Application of the method	Pharmaceutical preparations	Pharmaceutical preparations

Conclusion

In the literature review of spectrophotometric techniques for analyzing these anticancer drugs, the proposed method stands out as a pioneering approach. Compared to previously documented instrumental methods, this method is characterized by its simplicity, swiftness, high sensitivity, and cost-effectiveness. As a result, the suggested approach is well-suited for determining vincristine in both its pure form and pharmaceutical formulations.

References

1. Neuss, N., Gorman, M., Hargrove, W., Cone, N.J., Biemann, K., Buchi, G., Manning, R.E. Vinca alkaloids : XXI. The structures of the oncolytic alkaloids vinblastine and vincristine. *J. Am. Chem. Soc.,* **86**: (1964) 1440-1442.

2. Hesse, M. Alkaloid Chemistry, Wiley, New York. (1981).

3. Goodman, Sanford, L., Gilman, A., Gilman, A.G. The Pharmacolo- gical basis of Therapeutics, 8th ed., Pergamon press, New York. (1990).

4. Van Tellingen, O., Beijnen, J.H., Baurain, R., Tenbokkel Huinink, W.W., Vanderwonde, H.R., Nooyen, W.J. High performance liquid chromatographic determination of vinblastine, 4-0-deacetyl vinblastine and the potential metabolite 4-0-deacetylvinblastine-3-oic acid in biological fluids. *J. chromatogr.,* **553(1)** (1991) 47-53.

5. Volkov, S.K. Determination of vinblastine and vincristine by HPLC. *Khim. Farm. Zh.,* **30(3)** (1996) 58-62.

6. Volkov, S.K., Grodmitskaya, E.I. Application of HPLC to the determination of vinblastine in cantharus roseus. *J. chromatogr. B. Biomed. Appl.,* **660(2)** (1994) 405-408.

7. Chu, I.H., Bodnar, J.A., Bowman, R.N., White, E.L. Determination of vincristine and vinblastine in catharanthus roseus plants by HPLC – electrospray ionization mass spectrometry. *J. Liq. Chromatogr. Related Technol.,* **20(8)** (1997) 1159-1174.

8. Embree, L., Gelmon, K.A., Tolcher, A.W., Hudon, N.J., Heggie, J.R., Dedhar, C., Webb, M.S., Bally, M.B., Mayer, L.D. Validation of a high-performance liquid chromatographic assay method for the quantification of total vincristine sulphate in human plasma following administration of vincristine sulphate liposome injection. *J. Pharm Biomed. Anal.,* **16(4)** (1997) 675-687.

9. Kovbuz, M.O., Felitsin, N.M., Khimyak, Y., Ziminkovskii, B.S. Polarographic determination of some anticancer drugs. *Farm. Zh(Kiev).,* **2**(1995) 60-63.

10. Langone, J.J., D'Onofrio, M.R., Van-Vunakis, H. Radio-immunoassays for the vinca alkaloids vinblastine and vincristine. *Anal. Biochem.,* **95(1)** (1979) 214-221.

11. Sethi, V.S., Burton, S.S., Jackson, D.V. Sensitive radio-immunoassay for vincristine and vinblastine. *Cancer chemother. Pharmacol.,* **4(3)** (1980) 183-187

12. Huhtikangas, A., Lehtola, T., Lapinjoki, S., Lounasmaa, M. Specific radio-immunoassay for vincristine. *Planta Med.,* **53(1)** (1987) 85-87.

13. Chu, I., Bodnar, J.A., White, E.L., Bowman, R.N. Quantification of vincristine and vinblastine in catharanthus roseus plants by capillary zone electrophoresis. *J. Chromatogr. A.,* **755(2)** (1996) 281-288.

14. Kamau, G.N., Rusling, J.F. Resolution of ascorbic acid or catechol amine and indole alkaloid mixtures by pulse voltammetry at highly polished glassy carbon. *Electronal.,* **6(6)** (1994) 445-450.

15. Rusling, J.F., Scheer, B.J., Haque, I.U. Voltammetric oxidation of vinblastine and related compounds. *Anal. Chim Acta,* **158(1)** (1984) 23-32.

16. Temizer, A. Electroanalytical determination of vinca alkaloids used in cancer chemotherapy. *Talanta,* **33(10)** (1986) 791-794.

17. Horvath, P., Ivanyi, G. Quantitative analysis of natural drugs. III. Densitometric determination of vinblastine and other alkaloids of catharanthus roseus. *Acta Pharm.Hung.,* **52(4)** (1982) 150-157.

18. Kaleagasioglu, F. Identification of antineoplastic agents by Thin-layer chromatography. *Acta pharm. Turc.,* **34(4)** (1992) 115-119.

19. British Pharmacopoeia. The Stationary Office. London. (1998) Vol. 2, p.1147-1148.

20. United States Pharmacopoeia XXIV. USP convention Inc. Rockville. MD 20852, (2000). p. 1744-1746.

21. Delevie, R. Principles of quantitative chemical analysis, McGraw-Hill, international Edn, Singapore. (1997).

22. British Pharmacopoeia. The Stationery office. London. (1980).

23. Nagaraja, P.,Vasantha, R. Métodos sensitivos para la determinación espectrofotométrica de los compuestos antineoplásicos . *Ars Pharmaceutica,* **43:3-4**(2002). 121-133.

التقدير الطيفي لكبريتات الفنكريستين في بعض المستحضرات الصيدلانية

الملخص

تم اقتراح طريقة طيفيه لتقدير كميات متناهية في الصغر من الدواء المضاد للسرطان الفنكريستين. تعتمد الطريقة على الاقتران مع 4- كلورو انيلين. بلغت قيمة معامل الامتصاص المولاري للصبغة المتكونة 1.47 × 10^4 لتر.مول$^{-1}$.سم$^{-1}$ عند الطول الموجي الاعظم 455 نانوميتر. وتتبع الطريقه قانون بير في مدى التراكيز من 10 - 300 مايكروغرام/ 10 مل (10-30 جزء/ مليون) وبحدود كشف 0.12 مايكروغرام/ مل وتقدير كمي 0.40 مايكروغرام/ مل. يمتاز التفاعل اللوني باستقرارية عالية ولا يوجد تغيير ملحوظ في الامتصاصية (ضمن الخطأ المسموح) لمدة لاتقل عن 24 ساعة وبخطأ نسبي يتراوح بين 0.37+ و % 1.02+ وانحراف قياسي نسبي بين 0.43± و 3.16± % اعتمادا على مستوى التركيز. تم تطبيق الطريقة بنجاح في تقدير الفنكريستين في المستحضرات الصيدلانية.