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Development and Validation of UV-Spectrophotometric Method for Estimation of Vinpocetine in Marketed Formulation and Nanoformulation

Research Article

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Abstract

A quick, accurate, and cost-effective UV spectroscopy method was developed to estimate the Vinpocetine concentration in bulk, tablet dosage formulations and niosomes formulations, using a solvent ratio of (6:4) methanol: water. According to ICH guidelines, the proposed technique was validated and developed. In spite of linearity, precision, accuracy, specificity, Limit of Detection(LOD), and Limit of Quantification(LOQ), like parameters were validated by using UV/visible spectroscopy technique to analyze a spiked Vinpocetine solution. The wavelength at which the drug's maximum absorbance peak was obtained at 274 nm and the solvents used as methanol: water (6:4 w/v). The ethanol injection technique was used to prepare niosomes to analyze Vinpocetine in UV / visible spectrophotometric method. During the inter and intra-day studies, it was discovered that the developed UV technique was accurate, with % relative standard deviation ranging from 0.27 to 0.46 and 0.26 to 0.46, respectively. Vinpocetine overall recovery percentage was discovered to be between 98.42 to 99.82 %. LOQ and LOD were calculated to estimate the method's sensitivity, and they were observed to be 0.4565 µg/ml and 0.1506 µg/ml, respectively. The estimation of Vinpocetine content in bulk form, marketed formulations and niosomes was achieved using the developed methodology. **:** A quick, accurate, and economical UV spectrophotometric method has been developed that estimates the vinpocetine concentration in bulk, tablet dosage formulations, and niosomes formulations.

Keywords: UV- visible spectrophotometric method, Vinpocetine, Validation, Marketed formulations, Stability study, Periwinkle.

Introduction

Vinpocetine (ethyl-apovincamine, 14,-ethoxycarbonyl-(3alpha, 16alphaethyl)-14, 15-eburnamine) is perhaps the most well-known nootropic agent (1). Vinpocetine was first synthesized from the vincamine alkaloid about forty years ago in the late 1960s. It was extracted from the leaf of the periwinkle plant (*Vinca minor*) (Figure 1). Other compounds available in the vinca alkaloid family include vintoperol, vinburnine and brovincane (2,3). After its synthesis in Hungary, the nootropic and neuroprotective properties of this drug were discovered, and from thereafter it appeared under the name Cavinton in 1978 and has been used widely in over 40 countries including Germany, Hungary, Japan, Russia and Poland for prevention and treatment of stroke and other cerebrovascular-related disorders. Several clinical studies have confirmed the neuroprotective properties of this drug (4,5).

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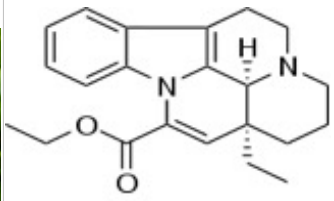
Vinpocetine (VPN) is a vincamine alkaloid derivative (Figure 2). It's mostly used to treat neurological illnesses including Alzheimer's and Parkinson's, as well as to increase brain blood flow (6,7). Vinpocetine works by inhibiting the enzyme phosphodiesterase type-1, which enhances cerebral blood flow specifically (8). Vinpocetine facilitates blood flow redistribution to ischemic regions and improves oxygen uptake and cerebral circulation (9). Because of its extensive first-pass metabolism, sluggish dissolution rate, poor water solubility, and limited absorption, Vinpocetine's bioavailability is extremely low. It also has a fast clearance rate and a short half-life, which leads to frequent drug administration (three times per day), which improves toxicity, and patient compliance (10,11).

Figure No 1: Vinpocetine (periwinkle) plant	Figure No 2: Chemical structure of Vinpocetine
	

Niosomes are self-assembled uni/multilamellar particles that are formulated by hydrating synthetic non-ionic surfactants with cholesterol or another lipid surfactant (12). Niosomes and liposomes are identical in terms of its structure and physical characteristics (13).

Despite Vinpocetine's potent therapeutic benefits and wide range of applications, no research on the development and validation of analytical techniques for its estimate in pharmaceutical dosage forms and niosome formulation has been found. Therefore, it was envisaged that developing a UV spectrophotometry method for estimation of vinpocetine in niosomes formulation and pharmaceutical dosage forms by using co-solvent system consisting of economic percentage of organic solvent will be valuable given the therapeutic importance of vinpocetine and the need for quick, rapid yet precise and robust analytical method for the same.

According to the literature review, several methods, including UV spectroscopy (14,15), high performance liquid chromatography (HPLC), gas, and mass spectroscopy (MS) methods, have been utilized to estimate vinpocetine in bulk, pharmaceutical preparations, and nanoformulation (16,17).

Furthermore, the aim of the current study is to develop an effective, rapid, precise and accurate, inexpensive and validated UV spectroscopic method following USP and ICH requirements for the determination and estimation of VNP in bulk, pharmaceutical tablet dosage forms and niosome formulations (18).

Materials and methods

Instrument

A UV-Visible double beam spectrophotometer (UV-1900, Shimadzu) was used having 2 matched 1cm matches quartz cells (19).

Chemicals and reagents

All chemicals used were of analytical grade. From Millipore's Milli-Q purification equipment, highly purified deionized water was collected. Vinpocetine was purchased from Micro Labs Ltd. India. Methanol was purchased from Hi-media.

Preparation of standard stock solutions

Utilizing methanol as the solvent, a standard stock solution of Vinpocetine (1000 µg/ml) was prepared. To reach a final concentration of 1000 µg/ml (stock-1), the standard Vinpocetine was precisely weighed and added into a (10 ml) volumetric flask, and mixed thoroughly and diluted up to the mark with a mixture of methanol: millipore water (6:4 v/v). To obtain a 100 µg/ml stock-2 solution, from stock-1 solution was further suitably diluted with mobile phase. To achieve 10 µg/ml reference solutions, 1 ml was diluted to 10 ml with the mobile phase. The reference solution was analysed between 200 and 400 nm in wavelength range (20,21).

Standard calibration curve

Vinpocetine was diluted appropriately from suitable aliquots of 100 µg/ml solution with mobile phase to achieve concentrations of 2, 4, 6, 8, 10, 12, 14, and 16µg/ml. An absorbance of every standard calibration was determined using the fixed wavelength measurement mode at a wavelength of 274 nm (22, 23).

Preparation of Vinpocetine loaded niosomes (VLN)

The ethanol injection technique was used to prepare Vinpocetine-loaded niosomes (VLN). Briefly, several ratios of cholesterol, span-60, and Vinpocetine (10mg) were precisely weighed and dissolved in 10ml of ethanol utilizing bath sonication at 60°C. The transparent organic solution was immediately transferred into the aqueous solution at 60°C while the mixture was vigorously stirred at 500 rpm on a magnetic stirrer (Remi) using a teflon coated bead (24, 25). The aqueous solution rapidly changed into milky solution suggested niosomes formation. To evaporate ethanol, the solution was allowed to evaporate under vacuum for 15min and stirring was remained for 1hr. Finally the volume of niosomal dispersion was maintained at 10ml by addition of water. The produced niosomal suspension was filtered via 2-20µm filter to ensure a homogeneous size distribution and to facilitate effective vesicle sealing (26, 27).

Characterization of Vinpocetine loaded niosomes

The characterization of VLN was carried out for the mean vesicle size, i.e., ploy dispersibility of index and zeta potential values in accordance with the standard protocols utilizing dynamic light scattering diffraction (DLS) techniques, respectively, utilizing Nano ZS ZS90 (Malvern Instruments, UK) (28).

Encapsulation efficiency

The VLN samples were centrifuged (High Speed Refrigerated Centrifuge, Floor Model, 7000 Kubota, Japan) at 19000 rpm for 60min. The supernatant was collected, diluted appropriately and determined for the VPN content by utilizing the UV/Visible spectroscopy analysis. The % EE was calculated by using following equation 1 (29).

$$\%EE = \frac{\text{Total entrapped drug} - \text{Amount of drug in supernatent}}{\text{Total entrapped drug}} \text{ X } 100 \ldots\ldots\ldots\ldots (1)$$

Method Validation

The method was developed by using UV technique for the detection of vinpocetine was validated in terms of parameters including linearity, range, accuracy, ruggedness, robustness, precision, limit of detection (LOD), and limit of quantification (LOQ), as depicted below (30, 31).

Linearity and range

Determining the various concentrations of the standard solution of Vinpocetine allowed for the evaluation of the linearity. For Vinpocetine, it was determined that the Beer-Lambert's concentration

range was 2 to 16 µg/ml. Figure 3 and Table 1 show the calibration curves for Vinpocetine, which were used to assess the linearity of the correlation between absorbance's and concentration.

Accuracy

The accuracy of the suggested ultravoilet-visible technique was validated utilizing recovery trials after standard addition of analyte. In triplicate, 3 separate Vinpocetine solutions were prepared at concentrations of 50, 100, and 150% of the standard Vinpocetine concentration of 20 µg/ml. The accuracy was calculated by using the following equation 2.

$$\%RC = (SPS-S/SP) \times 100 \ldots\ldots\ldots(2)$$

Where, SPS = Amount obtained in the spiked sample, % RC = Percent recovery, SP = Amount added to the sample, and S = Amount found in the sample,

Precision

Intraday and interday variation analysis was used to study the precision of proposed UV technique. By measuring the absorbance of triplicates of fixed concentration of the Vinpocetine (10µg/ml) at three distinct time periods on the same day and on 3 alternate days, the intraday precision and interday precision were calculated. The outcome of the precision studies was presented as % RSD (32).

Ruggedness and Robustness

Ruggedness of the sample was carried out by evaluating triplicate samples of Vinpocetine using two different instruments (UV-1900, Shimadzu and UV-1800, Shimadzu), two separate analysts in different laboratories by using UV spectroscopy. The robustness of the samples was estimated by observing the absorbance of a 10 µg/ml sample of vinpocetine at 270, 274 and 276 nm. The result was shown in terms of % RSD (33,34).

Limit of Quantification (LOQ) and Limit of Detection (LOD)

In the development of UV technique, the LOQ and LOD of Vinpocetine were calculated by using the following equations (3) and (4).

$$LOD = 3.3 \times SD/S \ldots\ldots\ldots\ldots\ldots (3)$$

$$LOQ = 10 \times SD/S \ldots\ldots\ldots\ldots\ldots (4)$$

Where, S= slope, SD= Standard deviation of Y-intercepts

Assay of marketed pharmaceutical formulation

Vinpocetine in marketed tablet formulation was estimated using the newly developed UV spectrophotometry. To get a maximum concentration of 0.1mg/ml, 10smg of vinpocetine tablet powder was dissolved in 100ml of methanol by stirring. After that, the mixture was filtered using Whatman filter paper of grade number 41. This filtrate was appropriately diluted with methanol to achieve the solution concentration of 20µg/ml. By measuring the solutions absorbance and using the calibration curve, the amount of Vinpocetine was determined (35,36).

Results and discussion

Characterization of Vinpocetine loaded niosomes (VLN)

Based upon the results obtained for VLN having the concentrations of span-60 (30mg) and cholesterol (20mg) was selected for further evaluation studies, as it is having maximum EE (81.5%) with less particle size (191 nm). The data are shown in table 1.

Table 1: Characterization of Vinpocetine loaded niosomes

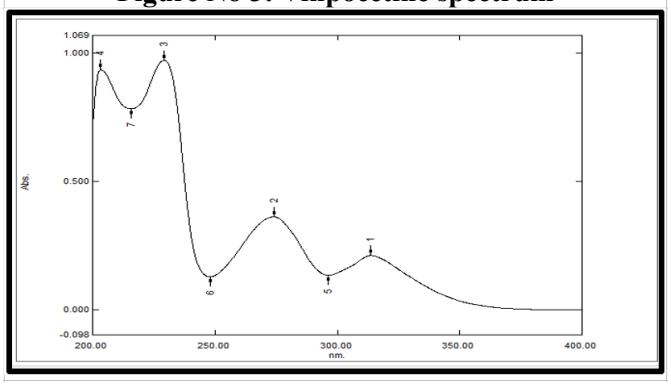
Formulation	Span 60 (X1)	Cholesterol (X2)	Particle size(nm)	Entrapment efficiency (%)	Zeta potential (mv)	PDI
VLN	30	20	191.4 ± 3.75	78.5 ± 1.21	-38.2 ± 0.26	0.592± 0.15

Method Validation

Determination of Maximum absorption of Vinpocetine

The absorption maximum of Vinpocetine was estimated by scanning the drug solution (10µg/ml) between 400-200nm regions on UV spectrophotometer. The obtained spectrum showed that the absorption maximum was 274nm for the Vinpocetine is represented in figure 3.

Figure No 3: Vinpocetine spectrum



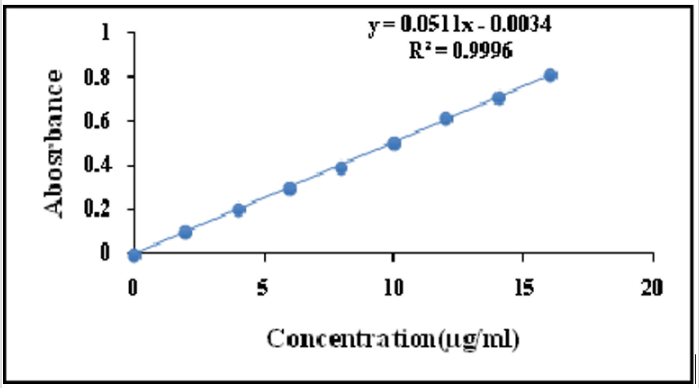
Determination of standard calibration curve of Vinpocetine

Standard calibration curve of drug was obtained using UV- absorption spectrophotometer (SHIMADZU-1900). Samples of various concentrations of drug was prepared and analyzed at 274 nm using methanol: millipore water as a solvent, the calibration curve obtained was linear giving R^2 value of 0.9996, slope of 0.0511 and intercept of 0.0034 at a concentration range of 2-16 µg/ml. Vinpocetine obeys the beer's law within the concentration range of (2-16 µg/ml). The standard calibration curve of Vinpocetine in ethanol is shown in figure 4.

Limit of quantification (LOQ) and Limit of detection

The lowest concentration that can be detected with appropriate precision and accuracy is denoted by

Figure 4.Standard Calibration Curve of Vinpocetine



the LOQ. Generally, limit of quantification is typically used as the initial calibration standard. According to table 2, the proposed UV method's shows LOQ and LOD were determined to be 0.495 and 0.1506µg/ml, respectively. The suggested technique would be acceptable for assessing samples containing even trace amounts of vinpocetine, according to the lower LOQ value. The LOD and LOQ data is depicted in table 2.

Accuracy

Accuracy of the sample was estimated by recovery experiments in which the mean recovery of the sample was within the limits of 98.47- 99.82 % which showed that the proposed method was an accurate method for estimation of Vinpocetine. The data are shown in table 3.

Table 2: Results of LOD and LOQ

Drug	LOD	LOQ
Vinpocetine	0.1506 µg/ml	0.4565 µg/ml

Table 3: Statistical analysis of Accuracy of the proposed method

Sample	Concentration (µg/ml)		% Recovery	Statistical analysis
	Standard	Formulation		
VS1 50%	10	20	98.65	Mean: 99.30 SD:0.598 %RSD: 0.60
VS1 50%	10	20	99.82	
VS1 50%	10	20	99.45	
VS2 100%	20	20	99.68	Mean: 99.24 SD:0.674 %RSD: 0.68
VS2 100%	20	20	98.47	
VS2 100%	20	20	99.59	
VS3 150%	25	20	99.56	Mean: 99.36 SD:0.224 %RSD: 0.23
VS3 150%	25	20	99.42	
VS3 150%	25	20	99.12	

Precision

The average % RSD values for the inter and intraday precision studies were determined to be 0.3686 % and 0.3356 %, respectively, indicating that the suggested method is precise. The results are shown in table 4 and 5.

Table 4: Statistical analysis of Intraday Assay of the proposed method

Sl.No.	Concentration (µg/ml)	Absorbance			Average %RSD
		Morning	Afternoon	Evening	
1	10	0.566	0.564	0.568	0.3356
2	10	0.565	0.567	0.566	
3	10	0.570	0.565	0.565	
%RSD		0.467	0.27	0.27	

Table 5: Statistical analysis of Interday Assay of the proposed method

Sl. No.	Concentration (µg/ml)	Absorbance			Average %RSD
		1st Day	2nd Day	3rd Day	
1	10	0.563	0.559	0.563	0.577
2	10	0.558	0.56	0.559	
3	10	0.561	0.565	0.567	
%RSD		0.448	0.572	0.710	

Ruggedness and Robustness

There were no significant differences in the results, as shown in tables 6a, 6b and 7, demonstrating the robustness and ruggedness of the suggested method.

Table 6a: Statistical analysis of Ruggedness of the proposed method

Sl.No.	Instrument UV-1900			Instrument UV-1800		
	Concentration (µg/ml)	Absorbance	Statistical analysis	Concentration (µg/ml)	Absorbance	Statistical analysis
1	10	0.566	Mean -0.565 S.D –0.0007 %RSD -0.13%	10	0.556	Mean -0.558 S.D –0.0028 %RSD -0.51%
2	10	0.565		10	0.560	

Table 6b: Statistical analysis for Ruggedness of the proposed method

Sl. No.	Analyst 1			Analyst 2		
	Concentration (µg/ml)	Absorbance	Statistical analysis	Concentration (µg/ml)	Absorbance	Statistical analysis
1	10	0.565	Mean -0.565 S.D –0.0007 %RSD -0.13	10	0.566	Mean -0.565 S.D –0.0014 %RSD -0.25
2	10	0.566		10	0.564	

Table 7: Statistical analysis for Robustness of the proposed method

Sl.No.	270 nm	274 nm	276 nm
1	0.547	0.566	0.556
2	0.545	0.565	0.555
3	0.550	0.570	0.560
Mean	0.547	0.567	0.557
SD	0.0025	0.0026	0.0026
%RSD	0.46%	0.47%	0.48%

Assay of marketed formulation

The assay data for the marketed formulations are represented in table 8. The proposed model has a significant correlation to the mean response.

Table 8: Results of tablet assay (n=3)

Drug	Label claim	Amount of drug estimated (mg/tablet)	Assay
Vinpocetine	5 mg	4.10 ± 0.1245	99.42± 0.2516

Stability Study

The sample was found to be stable in solution for 10 hours according to the short-term stability studies, which is within predetermined limits. The data are shown in table 9.

Table 9: Short term stability study

Concentration(µg/ml)	Concentration found (at 10 hours) Mean+ SD, (µg/ml)
2	0.108±0.0015
4	0.203±0.0012
6	0.275±0.0029

Conclusion

According to ICH guidelines, the method was developed and validated and determined to be quick, rapid, specific, accurate, and precise. It was discovered that the validation parameters' % RSD was found to be less than 2%. So, the suggested technology might be employed to routine estimation of these drugs in dosage formulations. Accuracy of the suggested technique was confirmed by conducting recovery trials that demonstrated that data within the limits. The suggested UV technique for accuracy was verified by running inter and intra-day precision tests. The results showed that the excellent scope of the UV-method for determining VPN in bulk and dosage formulations, niosomes, and they were well within acceptance criteria.

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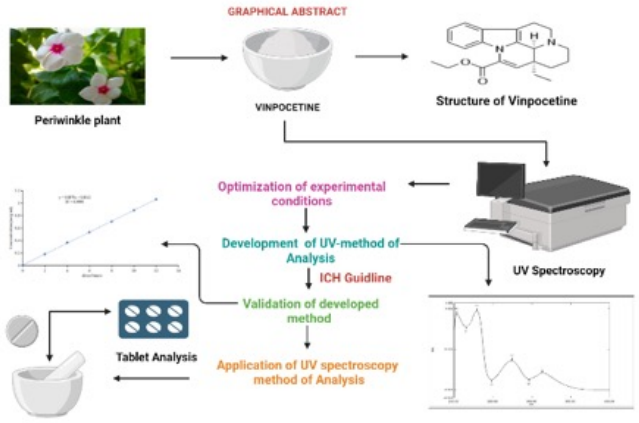
Conflict of Interest: The authors declare no conflict of interest.

Graphical Abstract



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