

Simultaneous Determination of Strychnine and Brucine in Herbal Formulation by UV Derivative Spectrophotometry

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Abstract: A simple, precise and accurate UV first order derivative spectrophotometric method has been developed and validated for the quantitative determination of strychnine and brucine in herbal formulation. The response of strychnine and brucine was linear over the range of 10-50 µg/mL respectively. The strychnine and brucine in herbal formulation were quantified using the first order derivative spectrum in which strychnine responded at 265.4 nm and brucine responded at 256.4 nm. The developed UV method was validated in terms of precision, accuracy, stability, LOD and LOQ.

Key words: Strychnine, brucine, first order derivative spectrophotometry.

Introduction

Strychnos nuxvomica Linn. is (Fam. Loganiaceae) commonly known as Kuchila, poison nut, and semen strychnos. In herbal medicine, it is traditionally recommended for upset stomach, vomiting, and bitter stomachic. It stimulates the muscular coat of the intestine, increases peristalsis, and hence is given for constipation in an atonic condition of the intestine, problems related to menopause, and migraine headaches¹⁻³. The main constituents of the seeds of *Strychnos nuxvomica* are known to be strychnine and brucine. The structures of strychnine and brucine are shown in figure 1.

The multi-component dosage forms prove to be effective due to the combined mode of action on the body. The complexity of dosage forms including multiple drug entities possesses considerable challenge to the analytical chemist during the development of assay procedures for herbal or ayurvedic formulations. For the estimation of drugs present in multi-component formulations, derivative spectroscopy can be employed as it eliminates the interferences occurred during the estimation of the desired analyte. There are few works reported for the determination of strychnine and brucine by HPLC⁴, TLC⁵, but no UV derivative spectrophotometric method for has been reported in herbal formulation. The present study deals with the

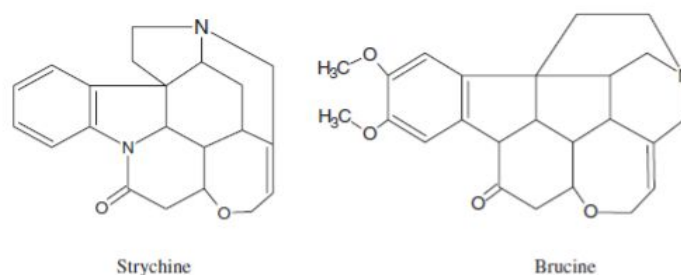


Fig. 1 Structures of strychnine and brucine

development of a new UV derivative spectro photometric method for the simultaneous estimation of strychnine and brucine in herbal formulation and validation of the method was done as per ICH guidelines (International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use).

Materials and Methods

Materials

Authentic strychnine and brucine were purchased from Sigma Aldrich, USA. Tentex Forte Tablets (Himalaya Health Care) were procured from local market. Chloroform of A. R. grade, was obtained from Merck India.

Apparatus

The absorbance of all the solutions was measured using UV-VIS Double Beam Spectrophotometer (Lab India, Mumbai). Drying and concentration steps were performed using rotavapor (Buchi, Switzerland).

Standard solutions

Strychnine and brucine stock solutions (1000 $\mu\text{g/mL}$) were prepared by transferring accurately 50 mg of strychnine and brucine separately in to two 50 mL volumetric flasks, dissolved in chloroform and the volume was made up to the mark.

Selection of wavelength

Standard solutions of strychnine and brucine were diluted separately with chloroform to obtain solutions containing 10 $\mu\text{g/mL}$ of strychnine and brucine respectively. The diluted solutions were scanned in the spectrum mode between 400 nm and 200 nm with a bandwidth of 2 nm against chloroform as blank. These zero order spectra of strychnine and brucine were treated to obtain corresponding first order derivative spectra with an inter-point distance of 10 nm in the range of 400-200 nm.

Derivative conditions

The first-order derivative spectra of strychnine and brucine were overlapped. The zero-crossing point (ZCP) values of strychnine at which the brucine showed some derivative response were recorded. The wavelength 265.4 nm was selected for the quantitation of strychnine (where the derivative response of brucine was 0). Similarly 256.4 nm was selected for the

quantification of brucine (where the derivative response of strychnine was 0). Characteristic wavelengths (ZCPs) for strychnine and brucine were confirmed by varying the concentration of both drugs⁶⁻⁷.

Calibration curves

The standard solutions of strychnine and brucine (1000 $\mu\text{g/mL}$) were used to prepare 2 different sets of diluted standard.

Series A: This series consisted of strychnine solutions of various concentrations (10-50 $\mu\text{g/mL}$) prepared by pipetting appropriate volumes (0.1, 0.2, 0.3, 0.4, 0.5 mL) of strychnine standard solution into 10 mL volumetric flasks and diluting to volume with chloroform.

Series B: This series consisted of brucine solutions of various concentrations (10-50 $\mu\text{g/mL}$) prepared by pipetting appropriate volumes (0.1, 0.2, 0.3, 0.4, 0.5 mL) of brucine standard solution into 10 mL volumetric flasks and diluting to volume with chloroform.

Quantitation

About 20 tablets were taken and ground to coarse powder and average weight was calculated (645.54 mg). From the powder, the average weight (0.65 g) was weighed and extracted with 50 mL of methanol under reflux for one hour. The solution after extraction was filtered using Whatmann filter paper No.41 and was evaporated to obtain dry residue. The residue obtained was dissolved completely in chloroform and was then subjected to further UV analysis.

Results and Discussion

In the present study, a simple, precise, accurate and rapid UV derivative spectrophotometry method have been developed and validated for the determination of strychnine and brucine in herbal formulation. Quantitative determination of strychnine and brucine was done by the developed method. The developed method was validated in terms of precision, accuracy, stability, LOD and LOQ.

Linearity and range

For linearity, five different concentrations of strychnine and brucine were used in a working range of 10-50 $\mu\text{g/mL}$. Linear regression equations and

correlation coefficient (r) values for strychnine and brucine are presented in table 1⁸⁻¹⁰.

Precision

The intraday and interday precisions of the proposed method were determined by estimating the corresponding response 3 times on the same day and on 6 different days over a period of 1 week for three different concentrations of 20, 30 and 40 µg/mL of strychnine and brucine. The results are reported in terms of relative standard deviation (RSD) in table 1⁸⁻¹⁰.

Accuracy

Accuracy is nearness of a measured value to the true value. It provides an indication of any systematic error or bias in the method. The accuracy of the method was determined by calculating recoveries of strychnine and brucine by the method of standard additions. A known quantity of standard strychnine and brucine was added to a pre-quantified sample solution. The amount of strychnine and brucine was estimated by measuring response at appropriate wavelength of 265.4 nm and 256.4 nm. The recovery was verified by estimation of the markers in triplicate samples at each specified concentration level⁸⁻¹⁰.

Sensitivity

Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined by kSD/s where k is a constant (3 for LOD and 10 for LOQ), SD is the standard deviation of the analytical signal, and s is the slope of the concentration /response graph⁸⁻¹⁰.

Stability

Solutions of both of the drugs in chloroform were studied for their stability at ambient temperature for 2 h.

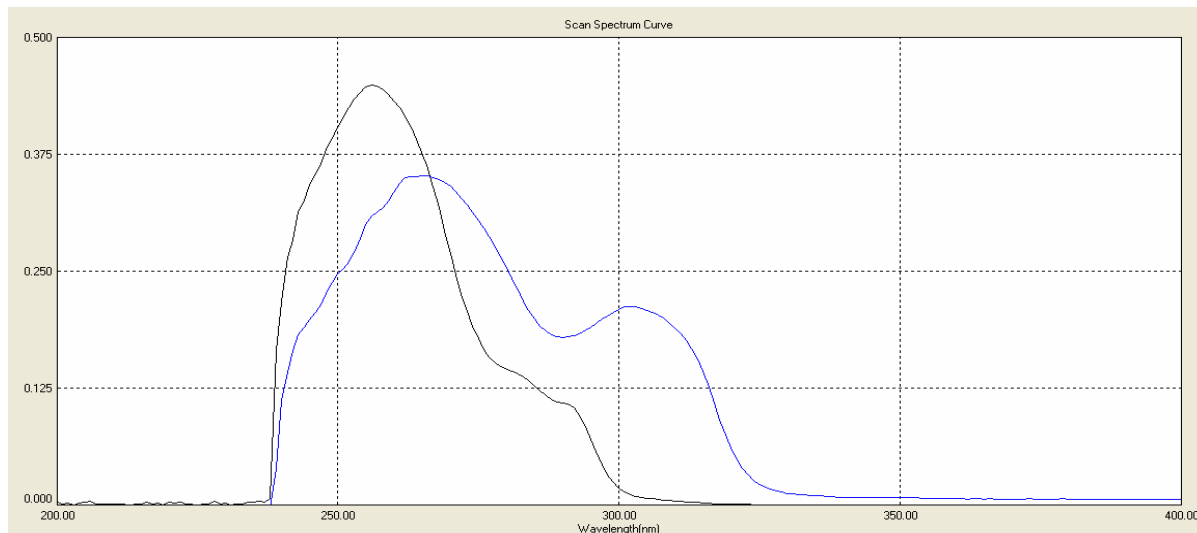
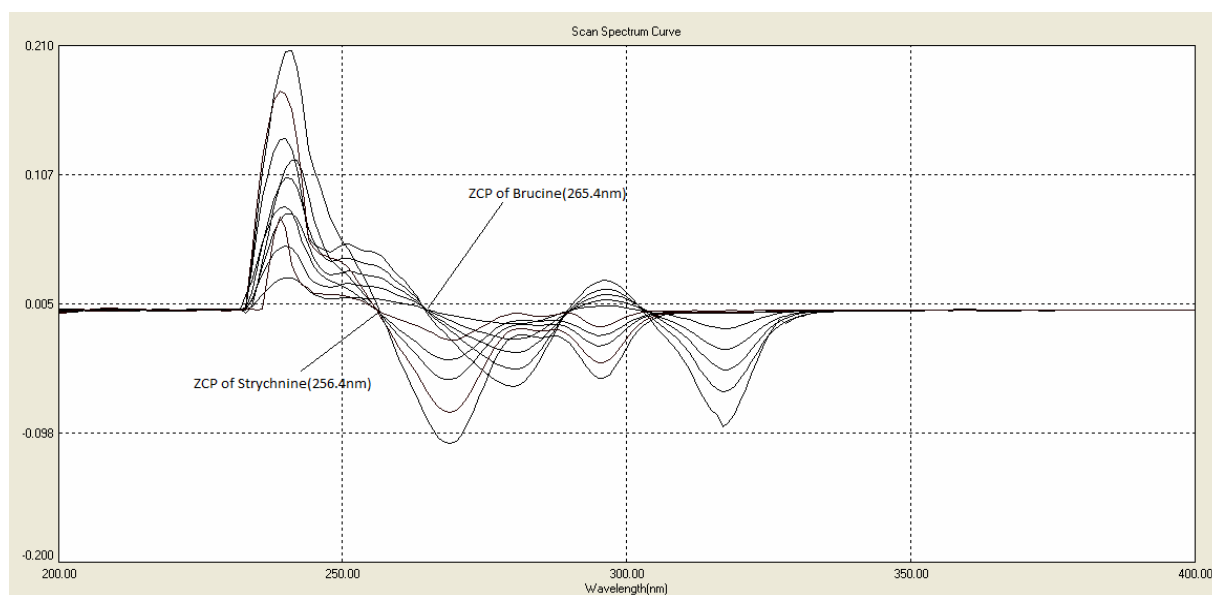
The zero order overlaid spectrum of strychnine and brucine was shown in (Figure 2). The overlaid first derivative spectra (d-1) of strychnine and brucine (Figure 3) were found to be appropriate for the determination of strychnine and brucine by having separated ZCP in chloroform. Also strychnine significantly contributes to the absorbance of brucine at the maximum absorbance wavelengths. The d-1 spectrum of brucine has zero absorbance at 265.4 nm, where strychnine gives a significant derivative response, while the d-1 spectrum of strychnine has zero absorbance at 256.4 nm, where brucine gives significant derivative response. Therefore, 265.4 nm was selected for the estimation of strychnine and 256.4 nm for estimation of brucine. The proposed first derivative zero crossing method showed linearity in the concentration range of 10-50 µg/mL for strychnine and brucine with correlation coefficient, 0.995 and 0.999 for strychnine and brucine respectively. The regression analysis of the calibration curves is shown in Table 1. The precision result of the solutions showed the % RSD values less than 2% both for intra-day assay and inter-day assay precision. The LOD values for strychnine and brucine were 0.113 and 0.275 µg/mL respectively. The LOQ values for strychnine and brucine were 0.377 and 0.905 µg/mL respectively (Table 2). The average % recovery of strychnine and brucine was found to be 97.88% and 98.36 % respectively, which shows that the method does not suffer from any interference due to other constituents. The result shows that the presence of other constituents in the formulation did not interfere with the final determination of active components (Table 2).

Table 1 Summary of validation parameters of strychnine and brucine

Parameters	Results	
	Strychnine	Brucine
Linearity		
Range	10-50	10-50
Linear equation	$Y = mx + C$	$Y = mx + C$
Slope (m)	-0.0151	0.0062
Intercept (C)	-0.0004	-0.0004
Correlation coefficient (r)	0.9995	0.999
Standard deviation (SD)	0.35	0.25
Precision (% RSD)		
Intraday precision (n=3)	% RSD = -1.069	% RSD = 1.433
Interday precision (n=3)	% RSD = -0.753	% RSD = 1.842
Accuracy (% Recovery)	97.88 %	98.36%
Limit of Detection (LOD)	0.113 µg/mL	0.275 µg/mL
Limit of Quantification (LOQ)	0.377µg/mL	0.905 µg/mL

Table 2 Assay results of herbal formulation

Formulation	Strychnine (mg/tablet)	Brucine (mg/tablet)
Tentex Forte Tablets	2.54	1.97

**Figure 2 Overlaid zero order absorption spectra of strychnine and brucine****Figure 3 Overlaid first derivative absorption spectra of strychnine and brucine**

Conclusion

In the present study, a simple and reproducible method for the simultaneous determination of strychnine and brucine in herbal formulations by UV derivative spectroscopy is developed. The strychnine and brucine content in Tentex forte herbal tablet was evaluated. The proposed method being precise and sensitive can be used for quantitative determination of strychnine and brucine in crude drugs and formulations. The

advantage of this method lies in the simplicity of the sample preparation, rapidity, and the low costs of reagents used. The validated parameters show that the developed method is quick, selective and cheap. Since other methods are giving very less resolution, the developed method is more suitable for the simultaneous determination of strychnine and brucine in crude drug as well as in multi-component herbal formulation.

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