

FIRST-ORDER ULTRAVIOLET DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF RESERPINE IN ANTIHYPERTENSION TABLET

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ABSTRACT

A new ultraviolet derivative spectrophotometry (UVDS) method has been developed for determination of reserpine in antihypertension tablets. A first-order UVDS based on the measurement of the distance between peaks to baseline (D_z) at the wavelength of 312 nm was used. Evaluation of analytical performance showed that accuracy as percentage recovery was 99.18-101.13%, precision expressed as relative standard deviation (RSD) was 1.91% and linear correlation was also obtained 0.9998 in the range of 10-50 $\mu\text{g/mL}$. Estimation of limit of detection and limit of quantitation was 0.8868 $\mu\text{g/mL}$ and 2.6874 $\mu\text{g/mL}$, respectively. As a reference method, HPLC methods from United States Pharmacopoeia (USP) were used. Commercially tablets available were analyzed by the two methods. The content of reserpine in tablets was found 0.2260 \pm 0.0033 mg by UVDS and 0.2301 \pm 0.0051 mg by the USP methods. The result obtained from the two methods was compared statistically using F-test and t-test and showed no significant differences between the variance and mean values of the two methods at 95% confidence level. This method was faster, easier, low cost and gave result as well as the reference method published by USP.

Keywords: ultraviolet derivative spectrophotometry; first-order derivative spectrum; reserpine

ABSTRAK

Metode baru menggunakan spektrofotometri derivatif UV (SDUV) telah dikembangkan untuk penentuan reserpin dalam tablet antihipertensi. Pengukuran jarak antara puncak ke garis dasar (D_z) pada panjang gelombang 312 nm dari spektrum derivatif orde pertama digunakan untuk penentuan kuantitatif reserpina. Evaluasi kinerja analitik yang dilakukan menunjukkan akurasi sebagai persentase perolehan kembali sebesar 99,18-101,23%, presisi metode yang diekspresikan sebagai simpangan baku relatif (SBR) sebesar 1,91%, dan korelasi linear dengan nilai 0,9998 diperoleh pada kisaran konsentrasi 10-50 $\mu\text{g/mL}$. Estimasi limit deteksi dan limit kuantitasi diperoleh sebesar 0,8868 $\mu\text{g/mL}$ and 2,6874 $\mu\text{g/mL}$. Sebagai metode pembandingan digunakan metode KCKT dari United States Pharmacopoeia (USP). Sebagai sampel digunakan tablet komersial yang kemudian dianalisis menggunakan dua metode tersebut. Kadar reserpine dalam tablet diperoleh sebesar 0,2260 \pm 0,0033 mg dengan SDUV dan 0,2301 \pm 0,0051 mg dengan metode KCKT USP. Hasil yang diperoleh dari dua metode tersebut dibandingkan secara statistika menggunakan uji F dan uji t dan menunjukkan tidak berbeda nyata untuk ragam dan rerata dari dua metode pada selang kepercayaan. Metode SDUV memiliki keuntungan yaitu cepat, mudah, berbiaya rendah, dan memberikan hasil yang relatif sama dengan metode KCKT yang diterbitkan oleh USP.

Kata Kunci: spektrofotometri derivatif ultraviolet; spektrum derivatif orde pertama; reserpina

INTRODUCTION

Reserpine (Fig.1.) is an alkaloid derived from the roots of *Rauwolfia serpentina* which has been used for antihypertension and tranquilizer drugs. It also proposed has protective ability against neurodegenerative diseases, especially Alzheimer's disease [1]. Many analytical technique have been used for quantitative

analysis of reserpine such as spectrophotometry [2], liquid chromatography-mass spectrometry [3], spectrofluorometry [4], colorimetric [5], photochemically induced fluorescence [6] and high performance liquid chromatography [7].

Derivative spectrophotometry (DS) is an analytical technique which consists in the differentiating of normal spectrum by mathematical transformation of spectral

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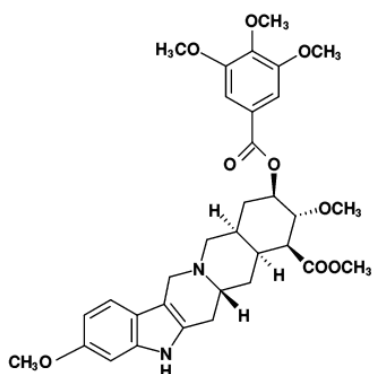


Fig 1. Reserpine

curve into a derivative. In DS, the information contained in the spectrum is presented in a potentially more useful form and greatly increasing the versatility of the technique [8]. This technique usually improves resolution bands, eliminates the influence of background or matrix and provides more defined fingerprints than traditional ordinary or direct absorbance spectrum, since it enhances the detectability of minor spectral features [9-10]. Although the use of derivative spectrum is not new, it has only become practical in recent years with the development of microcomputer technology, which allows the almost instantaneous generation of derivative spectrum.

As an alternative to the existing techniques, in this work we proposed a fast and simple method for determination of reserpine in tablets based on UVDS. This technique has several advantages such as fast, simple, and inexpensive [8]. It is useful for determining active compound in drugs [11-14]. Furthermore the proposed method was compared to HPLC method published by USP [7] as a reference method.

EXPERIMENTAL SECTION

Materials

Reserpine was purchased from Sigma-Aldrich (St Louis, USA), ethanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Ammonium chloride was obtained from Merck (Darmstadt, Germany). Serpasil® 0.25 mg tablets (Biochemie, Indonesia) obtained from local drugs store was used as samples.

Instrumentation

UVDS method was performed on UV-vis spectrophotometer U-2800 equipped with 10 mm quartz cells and connected to an IBM-PC computer, fitted with UV Solutions 2.0 software (Hitachi, Tokyo, Japan). HPLC analysis was performed on HPLC LaChrome Elite

L-2000 (Hitachi, Tokyo, Japan). LiChrosorb RP-18 column (250 mm × 4.6 mm i.d., 5 μm particle size) purchased from Merck (Darmstadt, Germany) was used.

Procedure

Preparation of standard and sample solutions

An accurately weighed 5 mg reserpine was dissolved in warm ethanol (60-65 °C) and stirred for 30 min in the range of temperature 45-50 °C. The solution was transferred to 50 mL volumetric flask and diluted with ethanol. The solution was used as stock standard solution. Serpasil® 0.25 mg tablets were ground and an amount equivalent to 1.25 mg reserpine was accurately weighed and dissolved in ethanol with the same procedure as the standard solution preparation.

Optimization of UVDS parameters

Scan speed, derivative order, smoothing and number of point window were optimized in order to get suitable setting for obtaining derivative spectrum in the quantitative analysis of reserpine by UVDS. Scan speed at 100, 200, 400 and 800 nm/min was evaluated. First, second, and third derivative order; smoothing using Savitzky-Golay method at the level 2, 3 and 4; and number of point window at 7 until 51 with an interval 2 were evaluated too under the optimum scan speed. All the parameters were evaluated using UV solutions 2.0 software.

Calibration curve and sample measurements by UVDS

Working standard solutions at 10, 20, 30, 40, and 50 μg/mL were prepared from the main standard solution. Absorbance of standard solutions was measured at the optimum UVDS condition. The calibration curve was then constructed by plotting the graphically measured amplitude (D_z at wavelength 312 nm) from the optimum condition vs. the corresponding reserpine concentration. The sample solution was measured too and concentration of the sample was determined from the calibration curve.

Determination of reserpine by HPLC

Quantitative analysis of reserpine by HPLC was carried out according to the procedures described in USP [7]. The HPLC was carried with a condition as follow: LiChrosorb RP-18 (250 mm × 4 mm i.d., 5 μm particle sizes) was used as separation column and acetonitrile and ammonium chloride 1% (1:1) at pH 5.6 as mobile phase with a flow rate 1.0 mL/min and injection volume of 20 μL. Before used, the mobile phase was sonicated and filtered through a 0.45 μm membrane filter (Millipore®) as well as for standards and

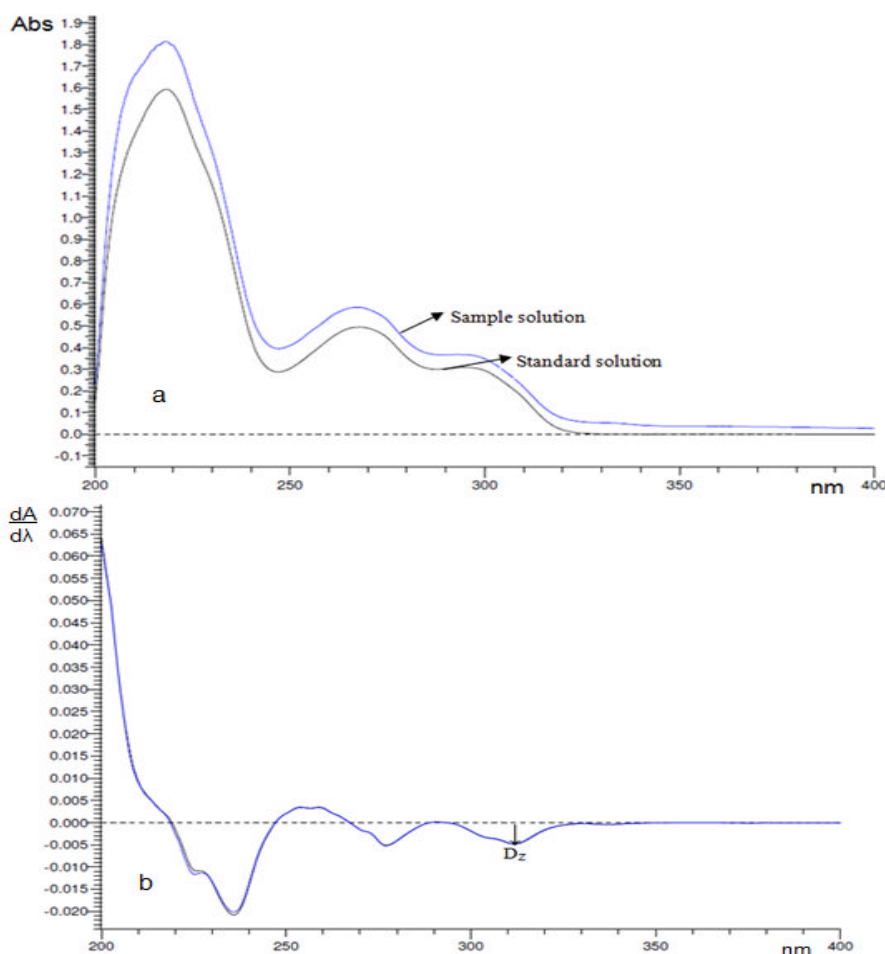


Fig 2. (a) Original spectrum, (b) $1D$ spectrum of reserpine and sample (concentration of reserpine = 25 $\mu\text{g/mL}$)

samples solutions. Detection wavelength for detection of the analyte was made at 268 nm.

Analytical performance test

The UVDS method was evaluated for its analytical performance by measuring:

Accuracy. The accuracy was determined by spiking method. Three samples (10, 15, and 17.5 mL) of standard reserpine (100 $\mu\text{g/mL}$) were transferred to individual 50 mL-volumetric flask, 10 mL of sample solution was added to each volumetric flask containing the standard solutions, and filled to the mark with ethanol. All solutions were prepared in three replicates and analyzed. Recovery of analysis represents the accuracy.

Precision. The precisions of the methods were determined by intraday repeatability, which was evaluated by analyzing 9 samples at the same concentration. Percentage of RSD was representative the precision.

Linearity. The calibration curve was obtained from five concentrations of standard solution in the range 10-50

$\mu\text{g/mL}$. Each concentration was made in 6 replicates and measured at the optimum UVDS condition. The linearity was evaluated by ordinary least square regression method.

Limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were determined based on standard deviation and slope of standard curves regression equations.

RESULT AND DISCUSSION

Ethanol 60-65 $^{\circ}\text{C}$ was used as extraction solvent in our work. Reserpine has low solubility in ethanol, but heating and stirring during dilution process improved its solubility. UV cut-off of ethanol is smaller than the maximum wavelength of reserpine. Therefore, absorption of the solvent does not interfere the UV spectrum of reserpine. UV cut-off of ethanol was 210 nm while maximum wavelength of reserpine was 268 nm. The UV spectrum of reserpine in ethanol was showed in Fig. 2.

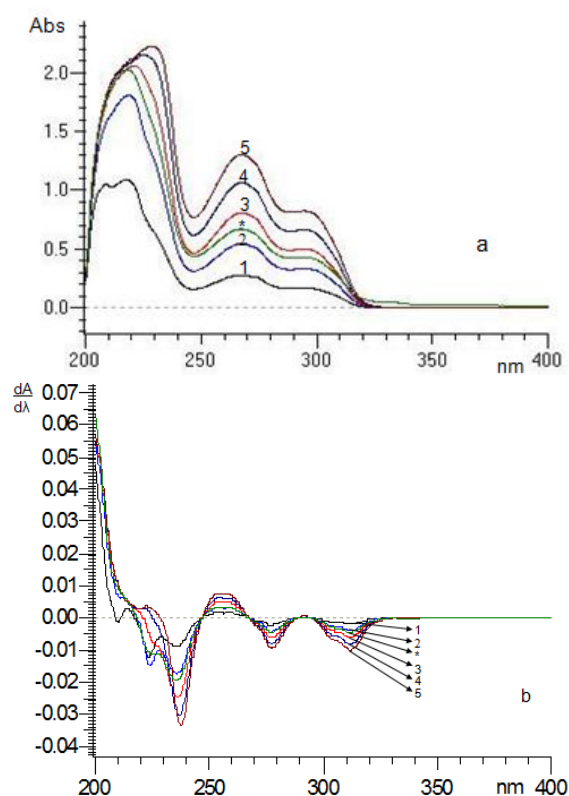


Fig 3. Overlay spectrum of reserpine standard solution with the concentration of 10 µg/mL (1), 20 µg/mL (2), 30 µg/mL (3), 40 µg/mL (4), 50 µg/mL (5), and sample solution (*) in original spectrum (a) and ¹D spectrum (b)

Table 1. Calibration data, LOD and LOQ for the determination of reserpine by ¹D UVDS method

| Parameters | ¹ D ₃₁₂ |
|--------------------------------------|-----------------------------------|
| Concentration range (µg/mL) | 10–50 |
| Correlation coefficient (r) | 0.9998 |
| Regression equation (Y) ^a | |
| Slope (b) | $0.0002 \pm 1.27 \times 10^{-6}$ |
| Intercept (a) | $0.00008 \pm 4.83 \times 10^{-5}$ |
| LOD (µg/mL) | 0.8868 |
| LOQ (µg/mL) | 2.6874 |

^a Average of six determinations

Table 2. Accuracy of the ¹D UVDS methods for the determination of reserpine

| Reserpine (mg) | | Percentage recovery (%) |
|----------------|--------------------|-------------------------|
| Added | Found ^a | |
| 1.0300 | 1.0417 ± 0.0358 | 101.13 |
| 1.6300 | 1.6167 ± 0.0358 | 99.18 |
| 2.0050 | 2.0258 ± 0.0036 | 101.04 |

^a Average from three determinations

UV-spectrophotometric method can not be used for the determination of reserpine directly at the maximum wavelength because matrix effect of UV-absorbing

substances in the samples will influence the determination of analyte. This effect is clearly seen in Fig. 2a, which show the absorption spectrum of sample have higher absorbance values than the spectrum of reserpine standard dissolved in ethanol at the same concentration as the sample solution. Derivatization was conducted to minimize the interference from absorption due to the matrix sample. The first-order derivative (¹D) spectrum of reserpine gave the best alignment between the standard and the sample spectrum in for determination of reserpine. It indicated that the matrix of sample did not disturb the measurement as shown in Fig. 2b.

From the optimization process, we obtained the optimum condition for measurement of reserpine by UVDS. The optimum scan speed was 200 nm/min, this scan speed provided the most narrow spectrum. ¹D was chosen for determination of reserpine because in some cases higher order derivatization can reduce signal-to-noise ratio [15] and also make the linearity and sensitivity much worse. Smoothing order and number of point window affected the amplitude and resolution of the spectrum. Small number of point causes narrow amplitude while high number of point gives broad amplitude. The optimum smoothing order was 3 while the optimum number of point window was 27.

Under the described optimum conditions, the calibration curve were prepared by plotting D_z (distance between peak to baseline) from the ¹D spectrum values against the concentration of reserpine at the wavelength 312 nm. As shown in Fig. 3, the values of D_z from each reserpine standard solution were increased linearly with the increasing concentration of reserpine standard solution, so D_z could be used as a signal to measure the concentration of reserpine in a sample. Excellent linearity was observed in the range concentration of 10–50 µg/mL with correlation coefficient (r) was 0.9998 and negligible intercept, confirming the mutual independence of the component derivative signals. The linearity of calibration curve and conformity of the ¹D measurements to Beer's law were proved by the high values of the correlation coefficient. The calibration data is summarized in Table 1.

Analytical performance of the UVDS method was performed by evaluating the accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). Accuracy was verified through recovery test by adding some known amount of standard to the sample solution. Three standardized concentrations were measured in three replicates, resulting percentage of recovery in the range of 99.18–101.13% (Table 2). These values indicated no considerable differences between the amounts of reserpine and the theoretical concentration value of sample dilutions. Precision was

Table 3. Determination of reserpine by ¹D UVDS and HPLC method and statistical analysis between the two methods

| Sample | Label claim | UVDS ^a | | HPLC ^b | |
|--------------------------------|-------------|-------------------|---------|-------------------|---------|
| | | mg/tablet | RSD (%) | mg/tablet | RSD (%) |
| Serpasil® | 0.25 mg | 0.2260 ± 0.0033 | 1.91 | 0.2301 ± 0.0051 | 2.10 |
| F _{calculated} | | | 1.299 | | |
| F _{table^c} | | | 3.687 | | |
| t _{calculated} | | | 1.714 | | |
| t _{table^c} | | | 2.160 | | |

^a Average of nine determinations^b Average of six determinations^c Theoretical value for F and t at 95% confidence level

examined from 9 determinations of reserpine in the sample within the same day. A satisfactory precision of the UVDS method expressed by relative small amount of RSD (%) as showed in Table 3. LOD and LOQ were estimated from the calibration curve and showed in Table 1. According to those values, UVDS method is capable to detect concentrations of reserpine in the concentrations above 0.8868 µg/mL, but not precise and accurately. Concentrations values higher than 2.6874 µg/mL of reserpine may be precise and accurate quantified by the proposed UVDS method.

The results obtained from UVDS method was compared with a HPLC method from USP statistically by the student t-test and the variance F-test to determine whether the means and variance of the two methods were significantly different. The student t-values and variance ratio F-values at the 95% confidence level did not exceed the theoretical values, indicating that there was no significant differences between the means and precisions of the UVDS method and HPLC method for determination of reserpine in the samples used (Table 3).

CONCLUSION

First-order UVDS method could be used for quantitation of reserpine in antihypertension tablet. The proposed method is simple and fast and doesn't require elaborate treatment and not time consuming method. Analytical performance of the method showed a good result, so this method can be used as an alternative method to HPLC for determination of reserpine.

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