ISSN 2277-2685

IJPSL/Oct . 2020/ Vol-10/Issue-4/1-7

G.Ratnakumari et. al International Journal of Pharmacetical Sciences Letters

Better Colorimetric Reserpine Determination in Tablets with 4-Caboxyl-2,6-dintrobenzene diazonium ion (CDNBD) Utilization

G.Ratnakumari [1],V.Raju [2],Divya [3],Mariya Sultana [4,]
Assistant professor [1,2,3,4],
Department of Pharmacy,
Samskruti College of Pharmacy,
Kondapur (V), Ghatkesar (M) Medchal Dist, Telangana, India.

Abstract

Goal: Creating a quick, easy, and enhanced colorimetric technique for reserpine tablet assay
Method: The procedure involves combining the aromatic rings of reserpine with the diazonium ion of 4-carboxyl-2,6-dinitrobenzene, which results in the creation of an azo adduct. The assay of reserpine in tablets was conducted by means of method application and optimization of reaction conditions and validation.
Result: Reserpine and CDNBD bonded easily, and when the experimental conditions were optimized, the reaction was finished in 10 minutes at room temperature. For the azo adduct that formed, a 1:1 drug to reagent stoichiometric ratio was found. In relation to the medication, the adduct showed a bathochromic shift, while in relation to the reagent, it showed a clear hyperchromic shift. 470 nm colorimeters were used for sample analysis. The tests demonstrated linearity and reproducibility within the 2.25 - 24 µg/mL concentration range. The analysis of reserpine in tablets was effectively conducted using the novel approach, with results that were comparable to those of the official (USP) spectrophotometric method (p > 0.05). When compared to the previously published colorimetric technique for reserpine, this approach offers a significant improvement.
In conclusion, the devised approach is quick and may be used for serpine in-process quality control.
KEYWORDS: diazo coupling, 4-Caboxyl-2,6-dintrobenzene diazonium ion (CDNBD), colorimetric, and serpine

INTRODUCTION

Reserpine is an alkaloid that is synthesized or extracted from the roots of several Rauwolfia (Apocyanaceae) species, namely R. serpentina and R. vomitoria. It's possible that the substance derived from natural sources contains similarly comparable alkaloids. 1. Ancient Hindu Ayurvedic texts describe the therapeutic use of the root of the climbing plant Rauwolfia serpentina (Benth.), which is native to India2. Reserpine has been used to treat schizophrenia and other chronic psychoses as well as hypertension. Additionally, it has been used to treat Raynaud's syndrome. Chemically speaking, reserpine is 3,4,5-trimethoxybenzoyl methyl reserpate3 or (3β, 16β, 17α, 18β, 20α)-11, 17-dimethoxy-18 [(3, 4, 5-trimethoxybenzoyl)-oxyl] yohimban-16-carboxylic acid methyl ester. The only recognized component of the BP 2002 4 is the active pharmaceutical ingredient, which is identified by a UV process after nitrosation. All Rauwolfia preparations according to USP 24/NF 195 are UV-treated after a thorough and prolonged solvent extraction process. In the USP 24/NF 19, assays for further multi-ingredient formulations containing serpine are conducted using HPLC techniques. It has been reported that reserpine and other indole alkaloids from Rauwolfia vomitoria and serpentina were determined using HPLC and HPTLC. The optimal HPLC separation was obtained using 10% CH3CN and 0.1% trifloroacetic acid in water.6. A two-step HPLC analysis, both qualitative and quantitative, of a reserpinechlorothiazide combination has also been reported7. There are also descriptions of other chromatographic techniques8. Numerous flourimetric methods for reserpine in dosage forms, bulk, or biological fluids have been reported. Hydrogen peroxide, selenious acid, p-toluenesulphonic acid, vanadium pentoxide, hexa-amine cobalt (III) tricarbanato cobaltate, and 2-iodoxybenzoate in aqueous acetic acid are some of the agents that have been used. In addition, a flow-injection assembly was implemented after fluorescence derivatization11. The measurement of reserpine and two more Rauwolfia alkaloids, yohimbine and rescinnamine, using chemiluminometric analysis based on a reaction with KMnO4/polyphosphoric acid, has been reported12.

There have also been reports of reserpine being extracted into chloroform from a pH 4.0 phosphate buffer, followed by the formation of ion pairs with either methyl orange or bromocresol purple9.
The most widely used colorimetric method for reserpine entailed using sodium nitrite to oxidize the molecule to 3,4-didehydroreserpine and measuring the absorbance of the oxidation product at a wavelength of around 390 nm. Reserpine has also been oxidized with nitrite in acetic acid and then extracted into chloroform. Reserpine has also undergone colorimetric analysis by reactions with sodium glyoxalate, phenylisocyanate, xanthydrol (50–500µg), vanillin, and aminopyrimidine9.
Many of the aforementioned processes have the drawbacks of being time-consuming, having complicated methods, and requiring substantial solvent extraction. Following up on our work on the creation of comparatively straightforward colorimetric techniques for the evaluation of organic compounds of pharmaceutical significance13–17, we present a novel colorimetric method utilizing the recently created 4-carboxyl-2, 6-dinitrobenzene diazonium ion (CDNBD) 18–20 for the determination of reserpine in tablet and bulk dosage form.

EXPERIMENTAL

Chemicals and Reagents
Pre-coated thin layer chromatographic plates GF254, 0.2mm (Merck, Germany), 4-amino-3,5-dinitrobenzoic acid (ADBA) synthesized in our laboratory; brindine (Novartis Pharma SPA Torre Annunziata Italy for Novartis Pharma AG Basle, Switzerland); Regroton (Norvatis Pharma AG Levent-Istanbul); reserpine (CRS number 92808, Sandoz Pharma); ethanol, ethylacetate, glacial acetic acid, concentrated sulphuric acid, sodium nitrite), glacial acetic acid, concentrated sulphuric acid, and sodium nitrite (all analytical reagents from BDH, Poole, England); and sodium nitrite (CRS number 92808, Sandoz Pharma).
Equipment includes: an analytical balance H80 (Mettler, UK), an ultrasonic bath (Langford Electronics, UK), a vortex mixer (Griffins and George Ltd, UK), a UV/VIS spectrophotometer (Unicam Aurora, Helios Scan Software v1.1, Pye Unicam, England), and a digital colorimeter (model 6051, Jenway, U.K.).

METHOD

Preparation of stock solutions
As previously described 20, an optimized procedure was used to prepare the CDNBD reagent solution utilizing ADBA in concentrated sulfuric acid. 6 mg of serpine was dissolved in 10 milliliters of glacial acetic acid to create the stock solution. It produced a 0.6 mg/mL solution.

Studies on optimization
Steepest climb method21 was used to optimize temperature and response time. In a test tube, an aliquot of the reserpine stock solution (100 µL) was added to the reagent solution (500 µL). The reaction mixture was then stirred for 10 seconds in a vortex mixer, and it was then incubated for 5, 20, and 30 minutes at different temperatures. Every decision was made in two copies. The reaction mixture was placed in an ice bath and 5 milliliters of ice-cold water was added to stop the reaction.
As previously optimized13, 5 mL of ethylacetate was used to extract the aqueous solution, which was then stored in an aluminum foil-wrapped vial. Similar steps were used to generate a blank reagent solution, but glacial acetic acid was used in place of the reserpine stock solution. Using a UV/VIS spectrophotometer, the absorption spectra of the reaction mixture extract was compared to that of the blank reagent extract. For sample determination on the colorimeter, the optimum difference in absorbtivity between the adduct and the reagent was found at an absorption wavelength of 470 nm.
An aliquot of the reserpine stock solution (100 µL) was added in turn to the reagent solution (500 µL) in eight test tubes to establish the ideal reaction time. The coupling reaction was conducted by incubating for 0, 2, 5, 10, 20, 25, and 30 minutes at 60 degrees Celsius. Following each reaction period, ethylacetate extracts of the reaction mixture were made as normal, and the colorimeter was used to detect the absorbance at 470 nm. The time that matched the samples' maximum absorption was then identified as the ideal reaction time. Every decision was made twice.

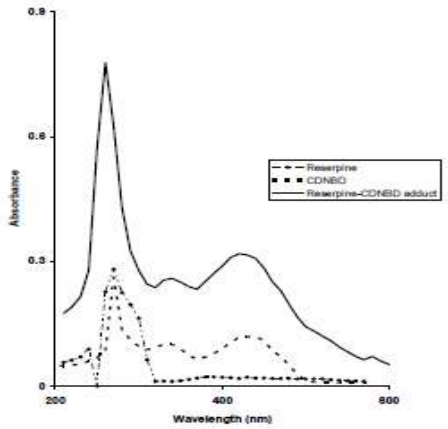
Stoichiometric ratio of drug-reagent adduct formation: The above-described process was used to create equimolar solutions (0.918 mM) of the reagent and the drug stock solution. The reagent solution was added in increments of 0.25, 0.33, 0.50, 0.67, 0.75, and 1.0 mL to seven distinct test tubes. The medication stock solution was then added to each tube until it reached 1.0 mL. The drug stock solution was replaced with glacial acetic acid for the same kind of blank assays. After 10 seconds of vortex mixing and 10 minutes of refrigeration at 30 degrees Celsius, the mixtures were extracted into 5 milliliters of ethylacetate. Plotting the acquired absorbance values against the mole fraction of the reagent solution, the absorbance was measured at 470 nm in comparison to the blank. Every decision was made in two copies. A test for weight homogeneity was performed on the two brands of tablets that were acquired. A quantity of powdered Brinerdine(R) tablet equal to 0.4 mg of reserpine was weighed out and dissolved in 20 milliliters of chloroform. Following dissolving, 2 mL aliquots of the solution were dried in test tubes and cleared. After being reconstituted in 0.25 milliliters of glacial acetic acid, the sample was diazotized in 0.5 milliliters of CDNBD reagent solution. As previously, the sample was handled.
A quantity equal to 0.5 mg of Regroton® was dissolved in 25 milliliters of chloroform and handled as Brinerdine®. The reference protocol for reserpine and hydrochlorothiazide was adapted from the USP 2000 spectrophotometric procedure.

RESULTS

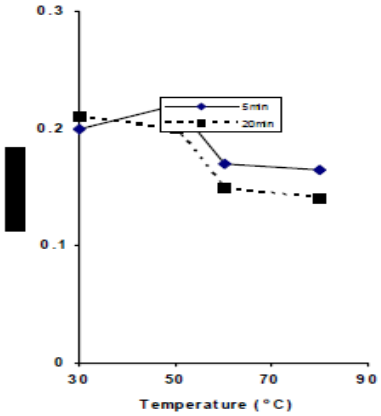
It was discovered that serpine and CDNBD paired immediately, and the color was consistent for days at room temperature. When the TLC plate containing the adduct and a pure sample of reserpine was inspected under the UV light at 365 nm, the adduct, however, lacked the fluorescence that is typically inherent in reserpine. This suggests that a new chemical is forming.

Figure 1 shows the azo adduct, the blank reagent, and the absorption of reserpine between them. It was discovered that reserpine had a strong peak at 290 nm and no discernible light absorption in the visible spectrum. However, the adduct's spectra showed the presence of a new λmax at 420 nm in the visible spectrum. At 470 nm, the adduct and reagent's absorptivity differences were determined to be the greatest. One unusual characteristic of the adduct's absorption spectra is that, in contrast to the reagent's (λmax 255, 340, and 430 nm), it shows more of a hyperchromic shift. The adduct's color, which ranges from reddish-brown to brilliant orange, differs from the reagent's yellow hue. The adduct's absorbance peaked at 30 °C and a 10-minute reaction time. For the reagent solution, the maximum absorbance of the adduct was reached at a mole fraction of 0.5. It was discovered that the absorbance decreased at both lower and higher mole fractions. Wrapped for three hours, the azo adduct created by the coupling process remained stable. The reserpine content of the tablets assayed by the new method and the USP method did not differ significantly, as demonstrated in Table 1 ($p>0.05$). Under ideal conditions, the calibration line's linear regression equation is $y = 0.007x + 0.1012$, with a correlation coefficient of 0.9974 ($r2 = 0.9948$). The test of serpine utilizing CDNBD yielded a detection limit of 2.245µg/mL. For the slope and intercept, the 95% confidence limits are 0.007±0.001 and 0.1012±0.0091, respectively. The assay of reserpine using the CDNBD technique was conducted with recovery experiments conducted at concentration levels of 6, 12, and 18µg/ml. Table 2 presents the results of the three-day evaluation of the accuracy and repeatability. The accuracy overall was determined to be 101.17±3.16 with a 3.12% coefficient of variation. When samples were exposed to the laboratory environment, the photostability of the reserpine azo adduct to diffuse light in ethylacetate was tested over the course of three hours. The results showed that the absorbance of the samples progressively decreased to a constant value of 0.16 between 90 and 180 minutes.

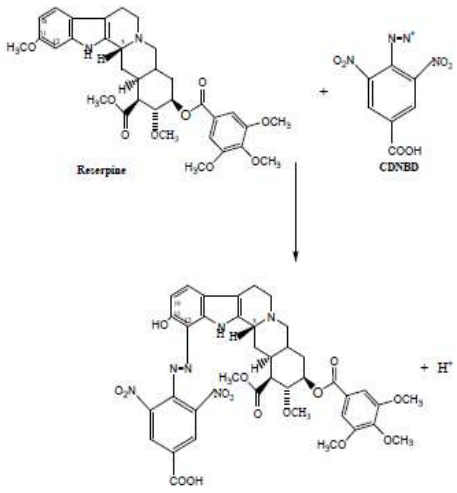
Over the course of the three hours, the wrapped samples' absorbance remained consistent.



(1) Overlaid absorption spectra of Reserpine, CDNBD and Reserpine-CDNBD azo adduct



'2) Optimization of coupling reaction temperature at two time levels



(3) Coupling reaction between CDNBD and reserpine

DISCUSSION

The first of these techniques to be used is the colorimetric determination of reserpine by diazo coupling reaction utilizing CDNBD. Although the majority of previously documented techniques rely on measuring reserpine's fluorescence, a more accessible and easily adjustable colorimetric approach would be preferable. Investigations were done on coupling reaction temperature optimization at 30, 50, 60, and 80 degrees Celsius for five and twenty minutes. Figure 2 shows the optimization of reaction temperature. One noteworthy finding is that the absorbance of the adduct progressively decreases after 50oC at 5 and 20 minutes. A very little rise (0.02 absorbance units) was seen at 5 minutes when the temperature was raised by 20oC (30oC to 50oC). After that, the azo adduct's absorbance decreased. Parallel to this, the absorbance at 30 °C for 20 minutes is just 0.01 more than at 30 °C for 5 minutes. The interaction of reserpine with CDNBD was chosen to occur at room temperature (30 oC) since using the most convenient temperature would result in an increase in analysis time. Ten minutes was shown to be the ideal coupling time via optimization.

The reserpine azo adduct's maximum absorbance was noted at 0.5 mole fraction of reagent. This suggests that all that was needed for the dye synthesis to occur was one CDNBD molecule for each reserpine molecule. Following a slow rise from lower mole fractions of the reagent, the absorbance peaked at 0.5 and then began to decline. The proton on the indole ortho (C-12) to the methoxyl group is the most protected, according to reserpine's 1H NMR24. Therefore, CDNBD will target this site electrophilically. The structure of the reserpine-CDNBD adduct is suggested by analyzing the spectra of indomethacin16, which has the same indole nucleus as reserpine (Figure 3). This is supported even more by the found 1:1 mole ratio between CDNBD and reserpine. When compared to other previously published spectrophotometric techniques that used non-specific reactions for reserpine separation and determination, the recovery experiments' findings demonstrated a significant improvement. This high recovery rate is similar to certain other flourimetric techniques' results, including Walash et al. 10's, which reported a recovery rate of 100.8±1.4%.

It was discovered that the findings obtained with a lower concentration value of 6 µg/mL were the least accurate. The reason for this is because it is almost within the limit of quantitation, which is 6.7335 µg/mL (LOQ = 3 times LOD). Better recoveries, however, were seen for analyte sizes of 12 and 18 µg/ml. Selecting 12 µg/mL as the working concentration for further investigation was justified by the fact that it is the calibration line's midway and yields precision findings that are near to 18 µg/mL concentration levels. According to the photostability study's

findings, all sample solutions should be kept out of direct sunlight. This is a feature that the novel approach, CDNBD, shares with all other methods—including the BP and USP methods—used to analyze reserpine. On the other hand, just 0.03 absorbance loss (from 0.21 at 0 minutes to 0.18 at 30 minutes) would be seen if the analysis using the CDNBD technique could be completed in thirty minutes. Therefore, when sample solutions in ethylacetate are covered in aluminum foil, the azo adduct of serpine is stable for up to three hours.

Table 1: Reserpine assay in Regroton® and Brinerdin®

Tablet brand	CDNBD method[a]	USP method[b]	p-value	
			F test	t test
Brinerdin®	104.3±1.5	101.2±1.7	0.32	0.07
Regroton®	102.8± 2.5	101.5± 1.4	0.18	0.37

USP requirement: content of reserpine = 90-110% of labeled amount
a; n =5 b; n =4

Table 2: Assessment of accuracy and repeatability of the new method of assay of reserpine

Concentration (µg/mL)	Day 1		Day 2		Day 3	
	Mean± S.D*.	RSD%	Mean±S.D*	RSD%	Mean ± S.D*	RSD%
6	95.0± 6.0	6.3	100.9± 6.0	5.9	98.0± 6.9	7.0
12	103.9± 3.0	2.9	102.4± 3.4	3.4	103.9 ±3.0	2.9
18	104.8± 3.8	3.6	101.8± 0	0	99.9±2.3	2.3

Between-day statistics = 101.2 ± 2.2% (Mean ± s.e.m), RSD (of s.e.m) = 3.1%
** n=9, Regression equation: y = 0.007x + 0.1012 (r^2 = 0.9948)*

Pinotsis et al.'s chemiluminometric measurement yielded worse precision than this novel approach using azo dye production. 12. The CDNBD approach is more sensitive than using xanthydrol and vanillin as derivatizing reagents. An additional benefit of this procedure over the standard USP method is the degree of selectivity. In the USP procedure, any residual or contaminated aromatic skeleton will likewise react when nitrous acid is created in situ for nitrosation.
Nevertheless, CDNBD can only respond to an active skeleton.

CONCLUSION

This novel reserpine technique is an enhanced way for determining its dosage. It has the benefits of simplicity, speed, and selection. Colorimeters are also reasonably priced and widely accessible. It has accuracy comparable to that of the official approach and may be used as an analytical method for reserpine during processing. In our lab, we are looking into using CDNBD as a pre-column derivatization reagent for reserpine HPLC analysis.

REFERENCES

1. Partiff K Martindale: The Complete drug reference Ed 32. The Pharmaceutical Press, London, 1999, p. 942.
2. Oates JA and Brown NJ. Antihypertensive agents and the drug therapy of hypertension In: Hardman JG and Limbird LE (eds). Goodman and Gilman's The pharmacological basis of Therapeutics Ed 10. McGraw-Hill Medical Publishing Division N.Y., 2001, pp. 882-884.
3. Budavari S (ed.). Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals. Ed 12. Merck Research laboratories, Division of Merck and Co. Inc. N.J., 1996, 1400-1401.
4. British Pharmacopoeia. The Pharmaceutical Press, London, 2002; p. 1487
5. United states Pharmacopoeia USP 24 /NF 19. United States Pharmacopoeial Convention Inc. Rockville, USA, 2000, pp. 1466-1468, 1470-1478
6. Klyushnichenko VE, Yakimov SA, Tuzova TP, Syagailo Ya V, Kuzovkina IN, Wulfson AN, Miroshnikov AI. Determination of indole alkaloids from R. serpentina and R. vomitoria by highperformance liquid chromatography and highperformance thin-layer chromatography. J. Chromatogr. A 1995; 704: 357-362.