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Original Article

METHOD DEVELOPMENT FOR QUANTIFICATION OF OXIDATION COMPLEXES OF NADOLOL AND RESVERATROL BY VISIBLE SPECTROPHOTOMETRY

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ABSTRACT

Objective: The present work is a report on two colorimetric methods based on oxidation and complexation of nadolol and resveratrol with ferric chloride and 2, 2'-bipyridyl.

Methods: The methods were developed for Perkin Elmer LAMBDA 25 UV –VIS spectrophotometer with 1 cm matched quartz cells. The functional groups vulnerable to oxidation were oxidized by ferric chloride and the reduced ferrous salts were complexed with 2, 2'-bipyridyl. The reaction conditions were optimized and validated to achieve maximum color intensity.

Results: The colored complexes show maximum absorbance measured at 424 nm for NAD and 522 nm for RES. The absorbance was found to increase linearly with an increase in concentration which was corroborated by the calculated regression coefficients (0.9998-0.9999). Linearity was obeyed in the range 5-25 and 20-100 µg/ml for RES and NAD, respectively. The molar absorptivity, sandell's sensitivity, LOD, LOQ and other validation parameters have been evaluated extensively as per ICH guidelines and all the parameters were found within the acceptance criteria for all the methods.

Conclusion: The proposed methods were proven to be more accurate, simple, precise and rapid by statistical validation and recovery studies; hence can be used for routine laboratory analysis.

Keywords: Nadolol (NAD), Resveratrol (RES), 2, 2'-bipyridyl and ferric chloride.

INTRODUCTION

Nadolol, chemically is (2R,3S)-5-{[(2R)-3-(tert-butylamino)-2-hydroxypropyl]oxy}-1,2,3,4-tetrahydro naphthalene-2,3-diol (fig. 1) is a non-selective beta blocker used in the treatment of high blood pressure and chest pain. It has a preference for beta-1 receptors, which are predominantly located in the heart, thereby inhibiting the effects of catechol amines and causing a decrease in heart rate and blood pressure; inhibition of beta-2 receptors, which are mainly located in the bronchial smooth muscle of the airways leads to airway constriction similar to that seen in asthma. Resveratrol chemically is a trans-3,5,4'-Trihydroxystilbene, which suppresses NF-kappa B (NF-kappa B) activation in HSV infected cells (fig. 2). Reports have indicated that HSV activates NF-kappa B during productive infection and this may be an essential aspect of its replication scheme.

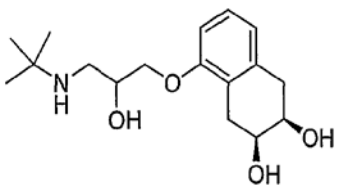


Fig. 1: Structure of NAD

Literature is enriched with several techniques for the determination of NAD [1-10] and RES [11-13]. NAD is official in BP [14] and USP [15]. RES is not official in any pharmacopoeia. By the official methods, NAD has been assayed by non-aqueous titration. Visible spectrophotometric methods of analysis are supposed to be more specific and accurate than titrimetric method of analysis. The other reported methods suffer from one or more disadvantages such as narrow linear response, lack of sensitivity and selectivity and usage of expensive reagents. The need for sensitive, cost effective and reliable spectrophotometric methods for the selected drugs is thus obviously recognized. Spectrophotometry is by far the instrumental technique of choice in the laboratories of underdeveloped and developing nations for the quantification of drugs owing mainly to its simplicity, high sensitivity and selectivity and often demanding low cost equipment. To overcome the difficulties by the reported methods the present work was aimed to explore the specificity of oxidation complexes with ferric salts and 2, 2' bipyridyl which was not reported earlier for the quantitative analysis of RES/NAD and to validate the methods according with ICH guidelines.

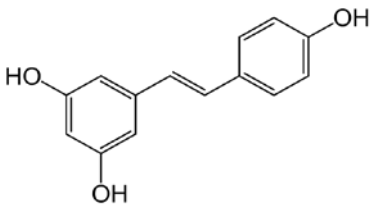


Fig. 2: Structure of RES

MATERIALS AND METHODS

Equipment

Double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer with 1 cm matched quartz cells were used for spectral measurements. Kemi KW 220 model water baths were used to control the temperature for color development. Samples were weighed using Sartorius electronic balance.

Chemicals

Pharmaceutical grade NAD and RES were graciously donated by Aurobindo Pharma Ltd, Hyderabad. 2, 2'-bipyridyl and ferric chloride of AR grade were used as chromogens for the experimental

work. Double distilled water/ethanol was used in the preparation of solutions. All the preparations were prepared afresh daily.

Preparation of 0.5% 2, 2'-bipyridyl

500 mg of 2, 2'-bipyridyl was dissolved in 5 ml 0.1M HCl and the final volume was made up to 100 ml with distilled water.

Preparation of 0.5% ferric chloride

500 mg of ferric chloride was dissolved in distilled water and made with 100 ml with the same.

Preparation of stock solution for estimation of NAD

25 mg of NAD was weighed and transferred to a 25 ml volumetric flask, 2 ml of methanol was added to dissolve and diluted to final volume with water. The resulting solution has a concentration of 1mg/ml.

Preparation of stock solution for estimation of RES

25 mg of RES was weighed and transferred to a 25 ml volumetric flask, dissolved and diluted to final volume with ethanol. The resulting solution has a concentration of 1mg/ml.

Procedure for calibration plot of NAD

In a series of 10 ml volumetric flasks, 0.2-1.0 ml (1 ml=1 mg/ml) of working standard solution of NAD was pipetted out and 0.8 ml of (0.5%) ferric chloride solution and 0.2 ml of (0.5%) 2,2'-bipyridyl were added, kept in the water bath at 70°C for 5 min, cooled and the final volume was made with 10 ml with water. The absorbance of the red colored chromogen was measured at 424 nm against the reagent blank. The amount of NAD present in the sample solution was computed from its calibration curve.

Procedure for calibration plot of RES

In a series of 10 ml volumetric flasks, 0.5-1.0 ml (1 ml=1 mg/ml) of working standard solution of RES was pipetted out and 0.2 ml of (0.5%) ferric chloride solution and 0.5 ml of (0.5%) 2,2'-bipyridyl were added and the final volume was made with 10 ml with water. The absorbance of the red colored chromogen was measured at 522 nm against the reagent blank. The amount of RES present in the sample solution was computed from its calibration curve.

Assay procedure for NAD

Twenty tablets of commercial samples (Corgard 80mg) of NAD were accurately weighed and powdered. Tablet powder equivalent to 100 mg of NAD was dissolved in water and the final volume was made up to 100 ml with water and the assay was carried out by the above procedure.

Assay procedure for RES

Twenty capsules of commercial samples (Resveratrol 50 mg) of RES were emptied and accurately weighed. Capsule powder equivalent to 50 mg of RES was dissolved in ethanol and the final volume was made up to 50 ml with ethanol. The assay was carried out by the above procedure.

RESULTS AND DISCUSSION

Optimization of the method

The method was optimized by selecting the proper solvent, chromogen, concentration of the reagent, order of an addition, a selection of the wavelength and stability of the colored product.

Solvent selection

Several solvents were used for the solubility of the drugs like water, HCl, sodium hydroxide, ethanol, methanol, DMF etc., and found that NAD is soluble in methanol and RES with ethanol. So finally these selected solvents were used as diluents throughout the procedure.

Selection of the chromogenic reagent

Ferric salts play a prominent role in the colorimetric determination of organic compounds. RES, or NAD hold the oxidisable centers, which could be oxidized by ferric salts and complexes with 2, 2'-bipyridyl, hence ferric salts and 2, 2' bipyridyl were selected as the chromogens.

Effect of ferric chloride concentration.

It was studied by treating the fixed volume of RES/NAD and bipyridyl concentration and in-turn varying the volume of ferric chloride from 0.2-1.0 ml. The results for both methods were depicted in Table 1.

Effect of 2, 2' bipyridyl concentration.

It was studied by treating the fixed volume of RES/NAD and ferric chloride concentration and in-turn varying the volume of 2, 2' bipyridyl from 0.1-2.0 ml. The results for both methods were depicted in Table 1.

Effect of time/temperature on reaction.

The effect of time and temperature on the formation of the colored complex was studied for all the methods. The complex formation was complete in 5-7 min time interval at room temperature for RES. NAD formed the colored complex with the chromogen at 70ºC in 7 min time interval. Heating above 70ºC and standing time above 8 min doesn't increase the color intensity of the complex. Fig. 3 and 4 represent an absorption spectrum of NAD and RES, respectively.

Table 1: Order of addition/concentration of reagents for the proposed methods.

0.8 ml (0.5% w/v) $FeCl_3$ + NAD + 0.2 ml (0.5 % w/v) 2, 2'-bipyridyl
0.2 ml (0.5% w/v) $FeCl_3$ + RES + 0.5 ml (0.5 % w/v) 2,2'-bipyridyl

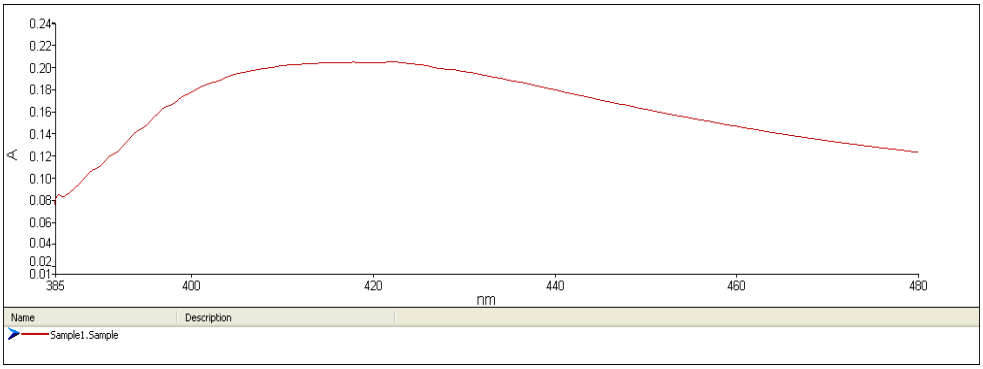


Fig. 3: Absorption spectrum of NAD by proposed method

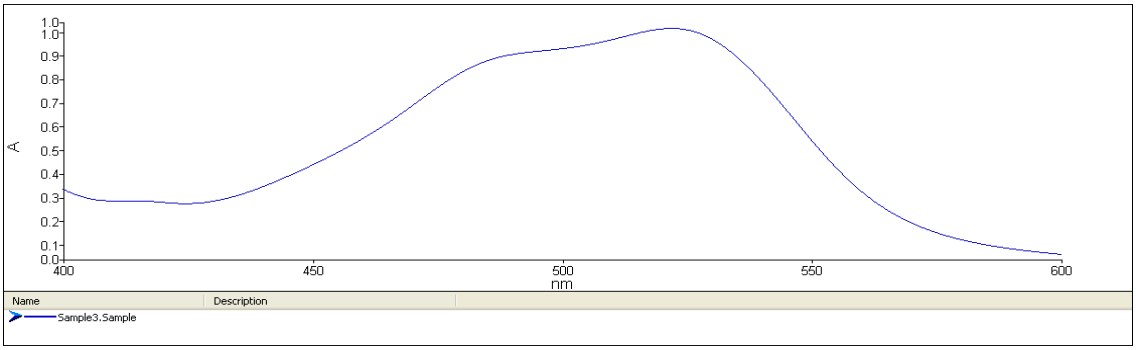


Fig. 4: Absorption spectrum of RES by proposed method

Method validation

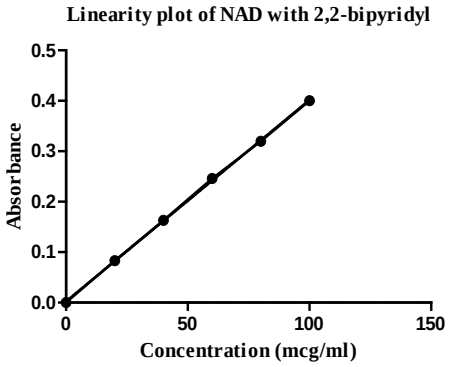
All the methods were validated for accuracy, precision, linearity, LOD, LOQ, ruggedness and robustness and the results were found to be satisfactory.

Linearity and range

At the described experimental conditions for RES/NAD standard calibration curves were constructed by plotting an increase in absorbance with concentration (fig. 5 and 6). A linear correlation was found between absorbance and concentration of RES/NAD and all the parameters regarding linearity were given in Table 2. The statistical parameters given in the regression equation were calculated from the calibration graphs. The high values of the regression coefficients and low values of y-intercepts of the regression equations, proved the linearity of the calibration curves.

Precision

The precision of the proposed methods was assessed by determining the relative standard deviation (RSD) of six replicate analyses on the same solution containing a fixed concentration of RES/NAD (within Beer's law limit). The low % RSD of the intraday and interday repeatability studies corroborates precision of the method. Table 3 represents the results of precision studies.



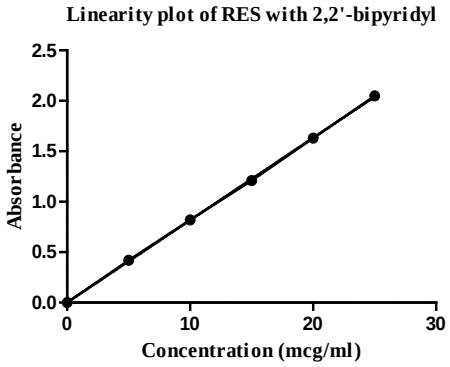


Fig. 5: Linearity plot of NAD fig. 6: Linearity plot of RES

Table 2: Optical and regression parameters

Parameters	NAD	RES
λ max, nm	424	522
Beer's law range (µg/ml)	20-100	5-25
Molar absorptivity (L. mole^{-1}. cm^{-1})	4.1×10^5	8.06×10^6
Sandell's sensitivity (µg/cm^2)/0.001 absorbance unit)	6×10^4	1.5×10^4
LOD, µg/ml	2.24	0.42
LOQ, µg/ml	6.8	1.28
Slope(m)	0.003991	0.08154
Intercept(b)	0.002429	0.002381
Correlation coefficient(r)	0.9998	0.9999

Table 3: Results of precision studies

Parameter	NAD		RES	
	Intra day n=6	Inter day n=6	Intra day n=6	Inter day n=6
Conc	60 µg/ml		15 µg/ml	
Mean abs	0.238	0.241	1.211	1.21
SD	0.0044	0.0016	0.0075	0.0089
% RSD	1.84	0.67	0.621	0.739

Table 4: Results of ruggedness studies

Parameter	NAD		RES	
	Analyst 1	Analyst 2	Analyst	Analyst 2
Conc	60 µg/ml(n=6)		15 µg/ml(n=6)	
Mean abs	0.243	0.251	1.02	1.01
SD	0.054	0.026	0.035	0.059
% RSD	0.84	0.17	0.261	0.432

Ruggedness

Ruggedness was checked by carryout the analysis by two different analysts and the absorbance was noted and % RSD was found to be satisfactory. Data was given in Table 4.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined by analyzing progressively lower concentrations of standard solution using optimized conditions and the results were presented in Table 2.

Accuracy

The validity and accuracy of the proposed methods were further assessed by recovery studies using the standard addition technique. For this purpose, a known amount of pure drug at three different levels was spiked to the fixed and known quantity of pre analyzed formulation samples and the nominal value of the drug was estimated by the proposed methods. The results given in Table 5 establish that the methods were reproducible by low SD and %RSD. No interference was evidenced from the commonly encountered formulation excipients.

Table 5: Results of accuracy studies of NAD by proposed methods.

Drug	Drug in Formulation (µg)	Std added (µg)	Amt Found (µg)	%Recovered	%RSD N=3
	60	40	98.75	99.09	0.791
NAD	60	60	118.37	99.21	1.365
	60	80	138.64	99.41	0.856
	15	5	19.83	99.39	0.258
RES	15	15	29.78	99.43	0.433
	15	25	39.57	99.02	0.614

Table 6: Assay results of NAD/RES

Formulations	Label claim (mg)	The amount found (mg)	% Recovery N=3
Corgard (tablet)	80	79.8	99.75
Resveratrol (capsule)	50	48.5	97.0

Application of the proposed methods to formulations

To evaluate the proposed methods, they were applied to the determination of RES/NAD in commercial formulations. The recoveries are close to 100%, indicating that there is no serious interference in samples. The good agreement between these results and known values indicates the successful application of the proposed methods for the determination of RES/NAD in formulations. The results are given in Table 6.

CONCLUSION

Two new, cost effective, simple and sensitive visible spectrophotometric methods, using 2, 2'-bipyridyl as reagent, were developed for determination of RES and NAD in bulk and in pharmaceutical formulations. The developed methods were also validated. From the statistical data, it was found that the proposed methods were accurate, precise and reproducible and can be successfully applied to the analysis of the same and could make a better alternative to the existing methods.

REFERENCES

1. Camila T, Pedro LG, Fabio P, Erika Rosa M, Maria SR. Quantitative determination of nadolol in tablets by HPLC and UV derivative spectrophotometry. Anal Lett 2008;41:424-36.
2. Lacroix PM, Curran NM, Lovering EG. Nadolol: High-pressure liquid chromatographic methods for assay, racemate composition and related compounds. J Pharm Biomed Anal 1992;10:917-24.
3. Perlman S, Szyper M, Kirschbaum JJ. High-performance liquid chromatographic analysis of nadolol and bendroflumethiazide combination tablet formulations. J Pharm Sci 1984;73:259-61.
4. Ram Gupta N, Brian Haynes R, Alexander Logan G, Donald Lorry MA, Achber C. Liquid chromatographic determination of Nadolol in plasma. Clin Chem 1983;29:1085-7.
5. Srinivas NR, Shyu WC, Vinod Shah R, Campbell DA, Barbhaiya RH. High-performance liquid chromatographic assay for the quantitation of nadolol in human plasma using fluorescence detection. Biomed Chromatogr 1995;9:75-9.
6. Frank Belas J, Mark Phillips A, Nuggehally Srinivas R, Rashmi Barbhaiya H, Ian Blair A. Simultaneous determination of nadolol enantiomers in human plasma by high-performance liquid chromatography using fluorescence-detection. Biomed Chromatogr 2005;9:140–45.
7. Al-Ghannam SM. A simple spectrophotometric method for the determination of nadolol in beta-blockers in dosage forms. J Pharm Biomed Anal 2006;40:151-6.
8. Kiranmai K, Sundar VD, Dhanaraju MD, Vijayalakshmi R. Spectrophotometric determination of Nadolol in bulk and pharmaceutical formulations. Asian J Res Chem 2010;3:1038-40.
9. Adegoke OA, Idowu OS, Olaniyi AA. A new spectrophotometric method for the determination of nadolol. J Iranian Chem Soc 2006;3:277-84.
10. Amin AS, Ragab GH, Saleh H. Colorimetric determination of beta-blockers in pharmaceutical formulations. J Pharm Biomed Anal 2002;30:1347-53.
11. Mark L, Martin SP, Peter A, Robert O. A validated hplc method for the quantitative analysis of trans-resveratrol and trans-piceid in hungarian wines. J Chromatogr Sci 2005;43:445-9.
12. Lopez R, Dugo P, Mondello L. Determination of trans-resveratrol in wine by micro-HPLC with fluorescence detection. J Sep Sci 2007;30:669-72.
13. Camont L, Cottart CH, Rhayem Y, Nivet-Antoine V, Djelidi R, Collin F, *et al*. Simple spectrophotometric assessment of the trans-/cis-resveratrol ratio in aqueous solutions. Anal Chim Acta 2009;634:121-8.
14. British Pharmacopoeia, The Department of Health and Social Services and Public Safety, British Pharmacopoeial Commission 2008;2:1504.
15. United States Pharmacopeia and National Formulary (USP 30-NF 25). Rockville, MD: United States Pharmacopeia Convention; 2007;2:2694.