Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

NOVEL SPECTROPHOTOMETRIC DETERMINATION OF LEVODOPA WITH NINHYDRIN

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Received: 31 Dec 2011, Revised and Accepted: 21 Feb 2012

ABSTRACT

An accurate, simple and fast Spectrophotometric method has been developed for the determination of Levodopa in bulk drugs. This method is based on the reaction of ninhydrin with primary amine present in the Levodopa in the presence of dimethyl formamide. This reaction produces a Ruhemann's purple colour product which absorbs maximally at about 624 nm. Beer's law was obeyed in the range of 10-50 µg/ml with good regression value 0.9940. The effects of variables such as temperature, heating time, concentration of colour producing reagent and stability of colour were investigated to optimize the procedure. The results are validated statistically. The value of LOD and LOQ was found to be 3.99µg/ml and 5.19µg/ml for Levodopa that indicates good sensitivity of proposed method.

Keywords: Levodopa, Ninhydrin, Dimethyl formamide, Spectrophotometry.

INTRODUCTION

Parkinson's disease is one of the most difficult problems in the medical field. The main cause of this disease is a significant depletion of dopamine due to the death of neurons which can produce dopamine in brain. It leads to tremor, muscle stiffness, bradykinesia and so on[1]. Levodopa ((-)-3-(3,4-dihydroxylphenyl)-l-alanine), a precursor of dopamine, is an important neurotransmitter which is used for the medication of neural disorders such as Parkinson's disease. After administration, Levodopa is converted into dopamine through enzymatic reaction catalyzed by dopadecarboxylase[2]. However, some side effects of systemic dopamine can appear if Levodopa is taken at high dosages because of the metabolism of Levodopa being extracerebral[3]. Therefore, a simple, highly sensitive and selective method that can be established for the determination of Levodopa is significant in the medical and life sciences. At present, different methods to determine levodopa have been employed: high-performance liquid chromatography,[4] fluorescence spectrometry,[5] electrochemistry method,[6,7] chemiluminescence,[8] flow injection analysis,[9] UV-Visible spectrophotometric analysis[10] and H^1- NMR analysis[11].

MATERIALS AND METHODS

Instrumentation

A SHIMADZU 1800 UV-VISIBLE spectrophotometer with 1.0 cm matching quartz cells were used for absorbance measurements. The UV spectra were recorded over the wavelength 400-800 nm.

Chemicals and Reagents

All reagents and chemicals used were of Analytical Grade. Gift sample of Levodopa was supplied by Wockhradt Pvt. Ltd, Aurangabad, and Maharashtra. Stock solution of Levodopa was prepared by dissolving 100 mg powder in 100 ml of Dimethyl formamide (DMF). Standard Levodopa solutions were prepared from stock solution A by appropriate dilution with DMF. 0.2% Ninhydrin (E. Merck) solution was also prepared in DMF.

Procedure for Levodopa in Bulk Drugs

Weigh accurately 100 mg of Levodopa, add 60 ml of DMF and stirred. It was filtered with Whatman filter paper # 1 and washed with DMF. The filtrate was diluted to 100 ml with DMF (Stock solution A). To 5 ml of this solution, 2 ml of 0.2% ninhydrin solution was added. The mixture was heated on a water bath at 80-85^{0}C for 5 minutes. The mixture was cooled to room temperature (25^{0}C) and then transferred to a 100 ml measuring flask. Final volume was made up to 100 ml with DMF and scanned in the UV-visible range at 800-400nm for the determination of λ max of Levodopa by using blank solution. The λ max of Levodopa was found to be 624 nm (Fig. 1).

Make a dilution from Stock solution A (1000 µg/ml) to get Stock solution B to have a concentration of 100 µg/ml. Add 2 ml of 0.2% ninhydrin solution to different aliquots of levodopa 1-5 ml of stock solution B (10-50 µg/ml) in heating tubes. Heat the mixture for 5 minutes at 80-□5^{0}C on a water bath. Cool and transfer it to 10 ml volumetric flask. Makeup the volume upto the mark with DMF. Measure the absorbance of solution at 624 nm against reagent blank. The absorbance of resulting solutions was measured at λ max 624nm and a calibration curve was plotted to get the linearity in the concentration range 10-50 µg/ml and regression values Fig. 2.

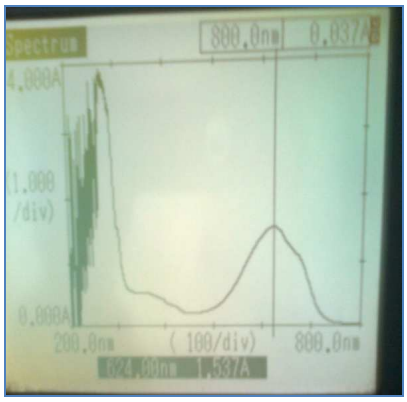


Fig. 1: Spectra of Levodopa

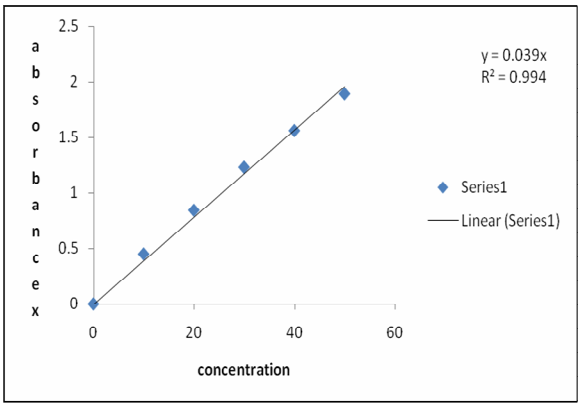


Fig. 2: Calibration curve of Levodopa at 624nm

METHOD VALIDATION

Linearity, Detection and Quantification Limits

A linear correlation was found between absorbance and concentration of Levodopa. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and the correlation coefficient (r) (Table 1). The graph shows negligible intercept and is described by the regression equation y = a + bx, where y is the absorbance and x is concentration in µg/ml. The limits of detection (LOD) and quantification (LOQ), sensitivity parameters such as molar absorptivity and Sandell sensitivity are also contained in Table 2.

Table 1: Observation table for calibration curve of Levodopa

Concentration range (µg/ml)	Absorbance (nm)
0	0
10	0.45
20	0.843
30	1.23
40	1.56
50	1.89

Accuracy

Accuracy of an analytical method is the closeness between the reference value and the found value[17]. The accuracy of the method was assessed by determination of the recovery of the method at 3 different concentrations (80%, 100% and 120% concentration) by addition of known amount of standard to the placebo. For each concentration three sets were prepared (Table 2).

Table 2: Optical characteristics and validation parameters of Levodopa

Parameters	Analytical data
Linearity Range (µg/ml)	0-50
λ max (nm)	624
ε (L/mol/cm)	7.4537×10^{3}
Sandell sensitivity (µg/cm²)	0.0264
Slope (b)	0.039
Intercept (a)	0.00
Standard deviation about regression (Sy)	±0.0607
Standard deviation of Slope (Sb)	±0.0014
Standard deviation of intercept (Sa)	±0.0439
Correlation co-efficient (r)	0.9940
Limit of detection (LOD,µg/ml)	3.99µg/ml
Limit of quantification (LOQ,µg/ml)	5.19µg/ml
Accuracy (%)	98.23-99.45

Precision

Precision of the method was calculated in terms of intermediate precision (intra-day and inter-day).[17] Three different concentration of Levodopa were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed good precision (Table 3).

Table 3: Evaluation of intra-day and inter-day accuracy and precision

L-dopa taken (µg/ml)	Intraday accuracy and precision L-dopa found (µg/ml)	RE %	RSD %	Interaday accuracy and precision L-dopa found (µg/ml)	RE %	RSD %
20	20.15	0.11	1.42	20.25	0.29	3.53
30	29.86	0.17	1.46	30.11	0.21	1.72
40	40.13	0.20	1.25	40.35	0.21	1.29

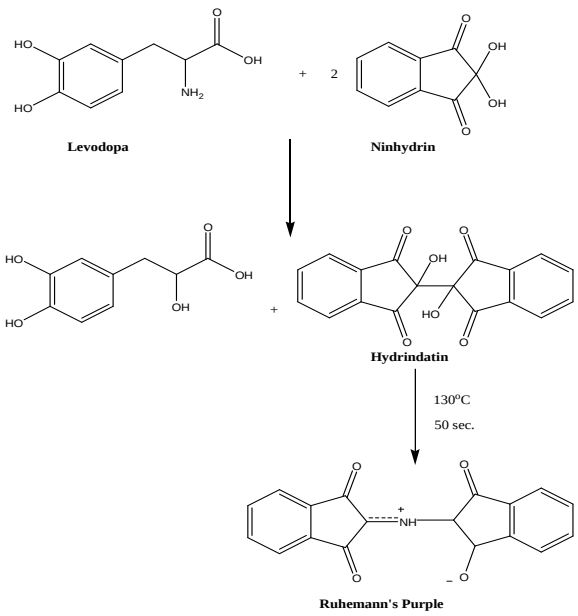
L-dopa- Levodopa; RE- Relative error; RSD- Relative standard deviation

RESULTS AND DISCUSSION

The possible use of ninhydrin for the detection and quantitative estimation of amino acids and imino acids depends on the formation of Ruhemann's purple[12]. The ninhydrin reagent is used for the determination of primary amines and amino acids[13,14]. Tzen-son L. Et al. used ninhydrin for the assay and kinetic studies on the enzymatic proteolysis. Levodopa contains a primary aliphatic amino group which reacts with ninhydrin reagent in DMF medium. It was reported that in alkaline medium, ninhydrin is converted to o-carboxyphenyl glyoxal which would reduce ninhydrin to 2-hydroxyindan-1,3-dione. The primary amino group of Levodopa reacted with ninhydrin to give diketohydrindylidene diketohydrindamine.

Levodopa reacts with ninhydrin via oxidation deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the colored Ruhemann's purple[15], which absorbs a maximum at 624 nm as shown in Fig. 2. The proposed reaction between Levodopa and ninhydrin is shown in the Scheme I.

To optimize the reaction conditions, different parameters have been investigated such as temperature, heating time, reagent concentration, and color stability. It was observed that complete colour development was attained at 80-85 ^{0}C. The optimum reaction time was determined by heating the reaction mixture on a water bath at 80-85 ^{0}C. It was noted that complete colour development was attained in five minutes. The effect of ninhydrin concentration on the colour development was investigated. 2 ml of 0.2% ninhydrin reagent produced maximum colour intensity.



Scheme I

CONCLUSION

This paper presents a spectrophotometric evaluation of Levodopa with ninhydrin in presence of dimethyl formamide produces a Ruhemann's purple colored product which absorbs maximally at about 624 nm. The proposed method is simple, precise and accurate for the determination of Levodopa.

ACKNOWLEDGEMENT

The authors are gratefully acknowledging the receipt of pure Levodopa as gift sample from Wockhardt Pharmaceuticals Ltd. (Aurangabad, India). Authors express their gratitude to Yash Institute of Pharmacy, Aurangabad for providing the Instrumental and Chemicals facility.

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