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Original Article

Method Development and Determination of Levodopa in Pharmaceutical Dosage Forms by Visible Spectrophotometry

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ABSTRACT

Two simple, accurate and economic spectrophotometric methods A and B have been developed for the determination of levodopa in bulk and dosage forms. These methods are based on the formation of chloroform soluble ion-associates in the presence of acidic dyes namely Bromo Phenol Blue, BPB (Method A) and Bromo Cresol Purple, BCP (Method B) exhibiting λ_{max} at 418 nm each. These methods obey Beer's law in the concentration range of 2.0-10.0 µg/mL for both the methods. The molar absorptivities are found to be $8.77x10^3$ and $1.94x10^4$ L/mol.cm for methods A and B respectively. These methods are found to be suitable for the assay of levodopa in pharmaceutical formulations.

Key words: Levodopa, BPB, BCP, Spectrophotometry.

INTRODUCTION

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 [1,2]. However, some side effects of systemic dopamine can appear if levodopa is taken at high dosages because of the metabolism of levodopa being extra cerebral [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]. The present manuscript describes two simple, sensitive, accurate and rapid methods for the determination of levodopa.

EXPERIMENTAL

Instruments: All the spectral measurements were made on an ELICO SL-159 model, 2 nm high resolution, double beam spectrophotometer with 1cm length quartz coated optics and Wavelength range 190-800 nm. All the chemicals and reagents used were of analytical grade and freshly prepared solutions were always used in the investigations.

Chemicals and Reagents: Aqueous solutions of (0.2%, $3.203x10^{-3}$M) Bromo Phenol Blue (BPB), (0.2%, $7.16x10^{-4}$M) Bromo Cresol Purple (BCP) and 0.1M HCl were prepared by dissolving 8.6 mL of Conc.HCl and diluted to 1 liter. Chloroform was used in both methods A&B as solvent.

Preparation of standard drug solution: 1.0% stock solution of drug levodopa was freshly prepared by transferring accurately weighed 100 mg of the drug into 100 mL volumetric flask and dissolved in double distilled water, and then made up to the mark. Then, working standard solutions of $100\mu g.mL^{-1}$ are prepared by transferring 10.0 mL of the stock solutions into two 100 mL standard flasks and made up to the mark

Recommended procedures for the Methods A & B: Into a series of 125 mL separating funnels containing aliquots (0.5-2.5 mL) of standard drug solution, 6.0mL of 0.1 M HCl solution and 5.0 mL of 0.2% dye solution were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 15 mL with distilled water and organic layer to 10 mL with $CHCl_3$. The contents were shaken for 2 min. The two phases

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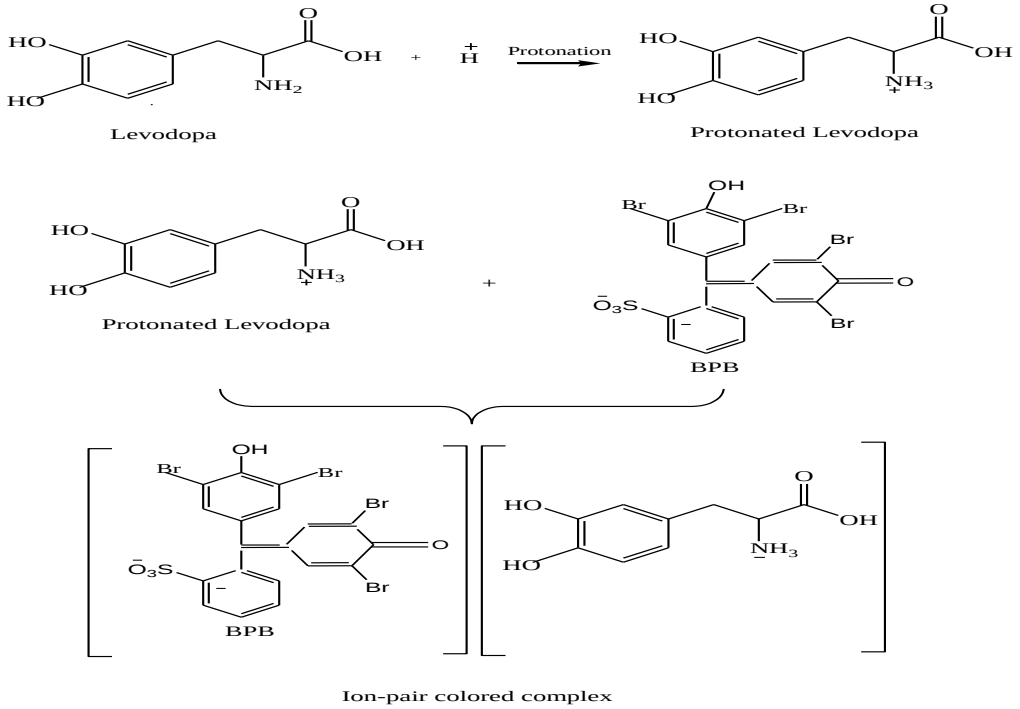
were allowed to separate and absorbance of the separated chloroform layer was measured at λ_{max} 418 nm (Method A & Method B) against a similar reagent blank. The amount of levodopa present was deduced from the appropriate calibration curves (Figure 1 & 2)

Based on the analogy, the following are the proposed reaction mechanisms for the formation of coloured products.

Method-A: With BPB



Method-B: With BCP

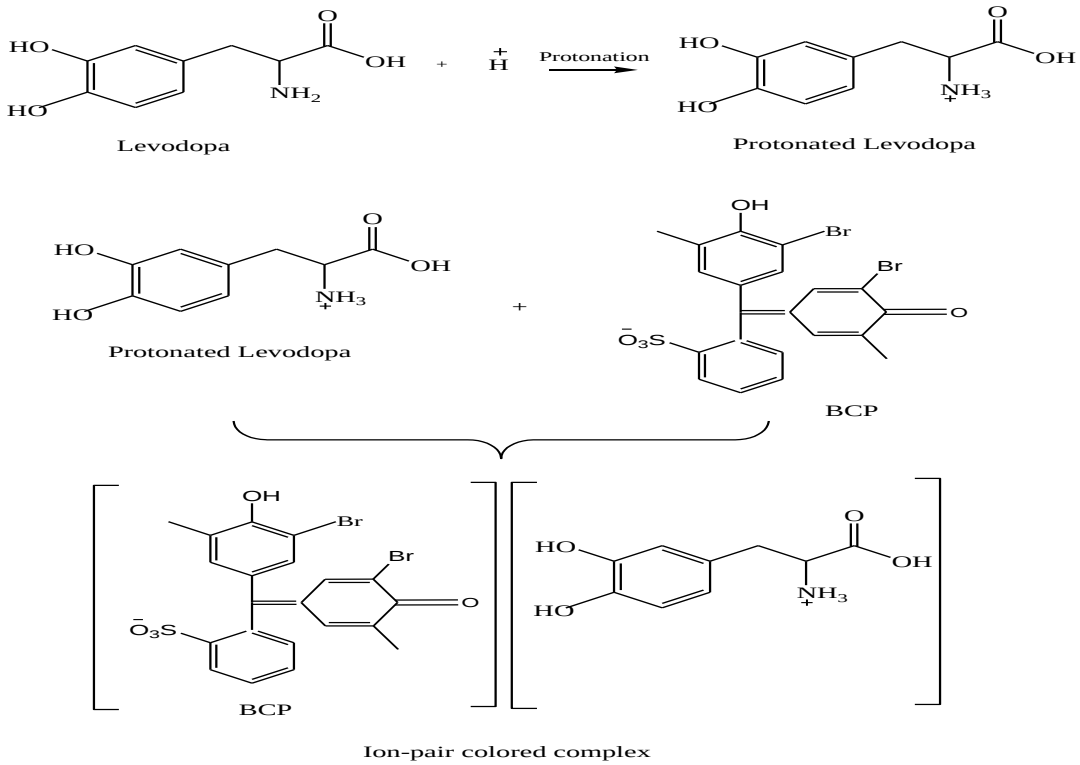


Fig. 1 : Beer's Law plot of levodopa with BPB (Method-A)

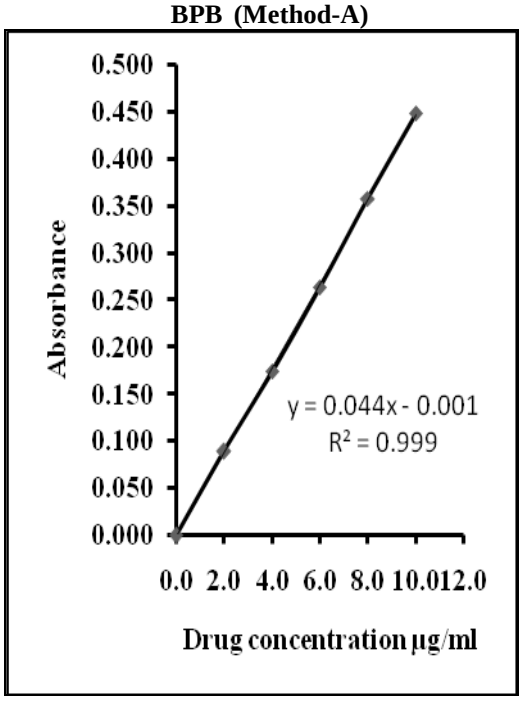


Fig. 2: Beer's Law plot of levodopa with BCP (Method-B)

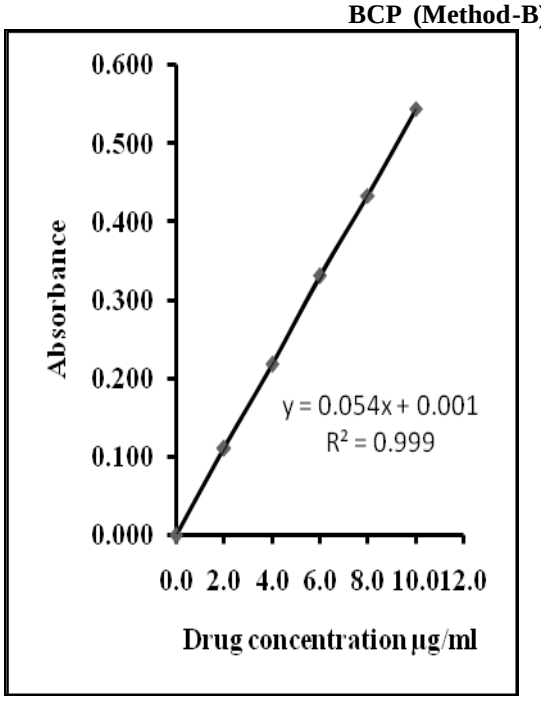


Table 1: Optical and regression characteristics of the proposed methods for levodopa

Name of the Parameter	Method A	Method B
Maximum wavelength (λ_{max})	418	418
Beer's law limits µg.mL^{-1}	2.0-10.0	2.0-10.0
Sandell's sensitivity(µg/cm^2 / 0.001 Absorbance)	2.25E-02	1.80E-02
Molar absorptivity (lit/mole/cm)	8.77E+03	1.94E+04
Slope (b)	4.51E-02	5.42E-02
Intercept(a)	3.90E-03	2.60E-03
Standard deviation on slope(S_b)	4.19E-04	3.79E-04
Standard deviation on intercept(S_a)	2.78E-03	2.51E-03
Standard error on estimation(S_e)	2.21E-02	2.21E-02
Correlation coefficient (r)	0.9999	0.9999
Limit of detection (LOD) µg.mL^{-1}	0.1848	0.1390
Limit of quantification (LOQ) µg.mL^{-1}	0.6161	0.4633

Table 2: Assay of levodopa in pharmaceutical formulations

Formulations*	Amount taken (mg)	Amount found by proposed methods**		Reference method	Percentage recovery by proposed methods**	
		Method A	Method B		Method A	Method B
Tablet	250mg	248.11±0.49 F=2.94 t=1.14	247.89±0.78 F=1.16 t=1.41	248.55±0.84	99.82±0.71	99.73±0.39

*** * Average of six determinations considered**

RESULTS AND DISCUSSION

The ion association complex is a special form of molecular complex resulting from two components extractable into organic solvent from aqueous phase at a suitable pH. In present methods A&B, the dyes produce stable anionic component in aqueous medium, which interact with the protonated nitrogen of the drug in acidic medium to form ion association complex. The complex thus formed is more stable due to electrostatic interactions. Ion-pair extractive spectrophotometry has attracted considerable attention for quantitative analysis of many pharmaceutically active compounds. Optimizations of the spectrophotometric conditions were intended to take into account of method development. The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar absorptivity, % relative standard deviation and regression characteristics like standard deviation of slope (Sb), standard deviation of intercept (Sa), standard error of estimation (S), and detection limit were calculated. Levodopa in formulations was successfully analyzed by the proposed methods. The values obtained by the proposed methods are presented in **Table 1.** The Beer's law was obeyed in the concentration ranges 2.0-10.0 µg/ml for both the methods. The values obtained for the determination of levodopa in tablet sample by the proposed and U.V method are compared in **Table 2** .The statistical parameters such as t and F values indicated that there is no significant difference between the reported and reference methods in terms of accuracy and precision. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods.

CONCLUSION

The methods reported here are found to be simple, sensitive, accurate and precise. The present methods involve the formation of highly stable colored species which makes it easier for the determination of levodopa from pharmaceutical dosage forms in a routine manner. The Beer's law was obeyed in the concentration ranges 2.0-10.0 µg/ml for both the methods .From the statistical parameters such as t and F values, we can infer that there is no significant difference between the reported methods indicating that the reproducibility and accuracy of the methods are good.

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