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Validated RP-HPLC method for estimation of L-dopa from bulk and formulations

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ABSTRACT

L-dopa is a naturally occurring amino acid L-3,4-dihydroxyphenylalanine, is most efficacious drug in treatment of Parkinson's disease. Various methods for analysis of the same are available but are time consuming and expensive. Here we have developed a new, precise and simple RP-HPLC method for estimation of L-dopa from bulk and formulation using a UV detector. The selected mobile phase was composed of 95:5 v/v of 25 mM ammonium acetate and methanol pH 4.0. The wavelength selected was 280 nm. This method was validated according to ICH Guidelines. There was no significant difference in the intraday and interday analysis of L-dopa determined for three different concentrations using this method. Linearity of the method was found to be 0-30μg/ml with regression coefficient of 0.99921. The detection limit and quantitation limit were 0.0024μg/ml and 0.0073μg/ml respectively. The method was found to be simple, accurate, precise, economical and robust.

KEYWORDS

L-dopa;
RP-HPLC;
Recovery.

INTRODUCTION

Parkinson's disease is believed to be related to low levels of the neurotransmitter dopamine in the brain. Therefore, the dopamine precursor levodopa[3-(3, 4-dihydroxyphenyl)- *L*-alanine] is employed for its treatment[1]. A number of methods like spectrophotometry [2-4], gas chromatography (GC)[5], high performance liquid chromatography (HPLC)[6], chemiluminescence (CL)[7,8], amperometric and voltammetric determination[9-11], potentiometry[12], radio-immunoassay[13] and flow injection analysis (FIA)[14-16] have been reported in literature for the determination of L-dopa in biological samples and pharmaceutical formulations. Each method has disadvantages of high cost, low selectivity, use of organic solvents, complex sample preparation procedures and long analysis time.

The main purpose of this investigation is to develop and validate reverse phase HPLC method which is simple, rapid, precise and sensitive method for estimation of L-dopa from formulation. This method could also be easily used in routine analytical work and for dissolution and diffusion studies at very low concentrations of L-dopa.

MATERIALS AND METHODS

Materials

L-dopa pure drug was obtained as gift sample from IPCA Pvt. Ltd. Methanol HPLC, Ammonium acetate

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AR, glacial acetic acid were procured from Merck Laboratories, All the reagents used were of analytical grade and the reagent solutions were prepared using double distilled water.

Instrument used

The HPLC system consisted of intelligent HPLC pump (model Jasco PU-2080 plus), intelligent UV-Vis detector model (Jasco UV-2075) and 20µl sample loop injector (#7725i, Rheodyne, USA). The equipment was operated through software Borwin version 1.5, LC-Net II/ADC system.

Methods

Preparation of mobile phase

The mobile phase of L-dopa analysis included 25 mM ammonium acetate in double distilled water (95%) and methanol (5%). The pH of the mobile phase was adjusted to 4.0 with glacial acetic acid. The mixture was sonicated for 15 min. and filtered the resultant mixture through 0.45µ nylon membrane filter (Whatman).

Determination of λmax

Weighed amount of L-dopa was dissolved in mobile phase to obtain a 100 µg/ml solution. This solution was subjected to scanning between 200 - 400 nm and an absorption maximum was determined.

Chromatographic condition

The separations were performed on Inertsil ODS, C_{18} column having dimensions 4.6mm∞ϕ×250mm, 5µm particle size (GL. Sciences Inc., Japan). The mobile phase consisted of 95:5v/v of 25 mM ammonium acetate and methanol. The pH of the binary solvent mixture was finally adjusted to 4.0 with glacial acetic acid. The wavelength selected was 280 nm. The flow rate was 1.0 ml/min.

Linearity and calibration

Primary stock solution of L-dopa was prepared by dissolving 50 mg of L-dopa in mobile phase to produce 50 ml solution. Secondary stock solution of 100 µg/ml was prepared by diluting the primary stock with mobile phase. The secondary stock solutions were diluted suitably to obtain calibration standards of 10, 20, 30.....upto 100 µg/ml. These solutions were injected into HPLC (20 µl) in triplicate and peak areas were noted. Calibration curve for L-dopa was then plotted between peak areas against concentration at 280 nm.

Method validation

The method was validated for accuracy and recovery, precision, detection limit, quantitation limit and robustness according to the ICH guidelines.

Accuracy

Recovery studies were performed to judge the accuracy of the method.. The recovery studies were performed by standard addition method. Known amount of standard (100 µg/ml) was taken in three 10 ml volumetric flask and to it added 10, 20, 30 µg/ml of working standard solution respectively and made the volume to mark. The amount of L-dopa added was determined from the peak area ratios by fitting these values in calibration curve and percent recovery was determined.

Precision

The intra day and inter day precision study of L-dopa was carried out by determining the corresponding responses. Different levels of drug concentration were prepared 3 times on the same day and on three different days and the responses were noted for evaluating the variability

Detection and quantitation limit

Based on the calibration curve plotted, the standard deviation of the y-intercepts regression line was determined and was placed in the following equation for determining detection limit and quantitation limit.

Detection limit = 3.3σ/s

Quantitation limit = 10σ/s

Where, 'σ' is standard deviation of the y-intercepts regression lines and's' is the slope of the calibration curve.

Robustness

Robustness was evaluated for determining the system suitability to ensure the validity of analytical procedure. This was done by varying the composition of organic phase by ± 3% and pH by ± 0.2, of the mobile phase.

Analysis of tablet formulation

Twenty levodopa tablets were ground and mixed well, and a quantity sufficient to yield 10 mg of levodopa

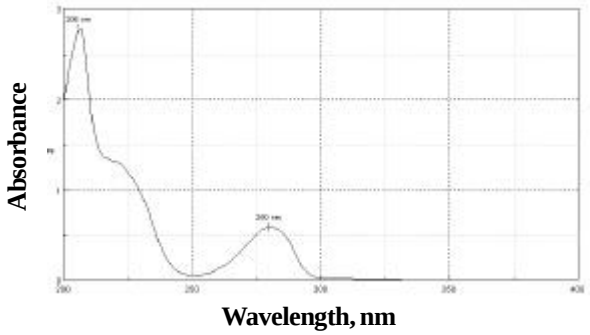

Figure 1: UV Scan of L-dopa in mobile phase

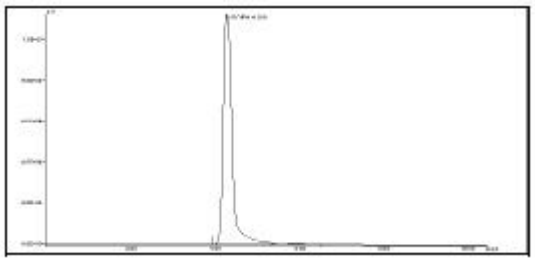

Figure 2: HPLC Chromatogram of L-dopa

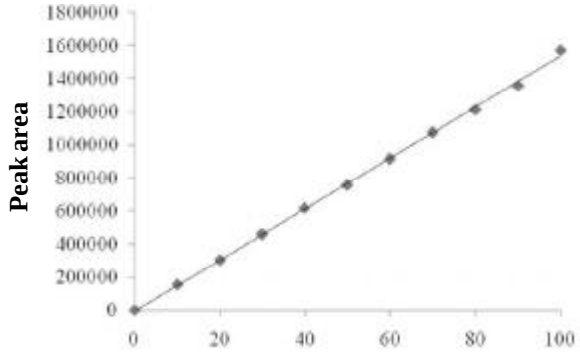

Figure 3: Calibration curve of L-dopa

was accurately weighed into a 100-ml volumetric flask. 100 milliliters of mobile phase was added, and the flask was sonicated for 30 min. The resulting mixture was filtered through 0.22µ nylon membrane filter (Whatman). The filtered solution was injected into HPLC and drug concentration was estimated from the calibration curve. The analysis was done in triplicate.

RESULTS AND DISCUSSION

For the optimisation of mobile phase comprising of ammonium acetate and methanol different pH values and different combinations were investigated. The mobile phase was optimised on the basis of asymmetry factor, peak area obtained, retention time and number of theoretical plates. Amongst the various compositions tried satisfactory separation, well resolved, short retention time, and good symmetrical peaks were obtained with the mobile phase comprising of 95:5 v/v of 25mm ammonium acetate and methanol (pH=4). The pH of the binary mixture was adjusted to 4.0 with glacial acetic acid. The UV-Vis scan of L-dopa in mobile phase revealed absorption maximum at 280 nm. Hence this wavelength was selected for the further analysis. The scan is shown in figure 1.

TABLE 1: L-dopa Calibration curve data

Concentration(µg/ml)	Peak area (mV-sec)*	% RSD
0	0	0
10	153823.29 ± 2306.16	1.49
20	305085.81 ± 0.00176	5.79E-07
30	458234.80 ± 0.0012	2.62E-07
40	616613.96 ± 1063.49	0.17
50	756142.35 ± 1189.14	0.15
60	915137.05 ± 2720.97	0.29
70	1075036.03 ± 1515.14	0.14
80	1214642.72 ± 3066.67	0.25
90	1360872.69 ± 7975.55	0.58
100	1575123.94 ± 10005.64	0.63

***Each value is average ± SD (n = 3)**

The peaks were highly resolved, the retention time was found to be 4.30 minute as depicted in figure 2. The asymmetry factor was 1.45 while number of theoretical plates was around 2797. All these factors revealed that the mobile phase was suitable for further analysis.

The calibration plot for L-dopa was obtained by plotting the peak areas vs the concentration and was found to be linear. Regression analysis showed very good correlation. The calibration plot is shown in figure 3. The peak areas for the corresponding concentration of the calibration curve are depicted in TABLE 1. The standard deviation for all concentration levels were low and the % RSD also did not exceed 0.25 %.

The statistical analysis of data obtained for the calibration curve of L-dopa in pure solution indicated a high level of precision for the proposed method, as evidenced by low value of coefficient of variation. The coefficient of correlation was highly significant. The linearity range was observed between 0-30µg/ml. The plot clearly showed a straight line (Y=15422X-4690).The accuracy of the method was judged by recovery studies. The recovery of L-dopa was found to

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TABLE 2: Recovery studies

Sr. no.	Standard Amount (µg/ml)	Amt of std. added (µg/ml)	Theoretical amount (µg/ml)	Amount recovered (µg/ml)	% Recovery
1.	100	10	110	109.66	99.69
2.	100	20	120	120.42	100.34
3.	100	30	130	129.89	99.91

TABLE 3: Intra-day variability of L-dopa

Conc. (µg/ml)	Peak areas*	% RSD
40	622666.08 ± 7495.50	1.20
60	918080.65 ± 1441.90	0.15
80	1219198.78 ± 3376.55	0.27

***Each value is average ± SD (n = 3)**

TABLE 4: Inter-day variability

Conc.(µg/ml)	Peak areas	% RSD
40	620398.04 ± 6597.22	1.06
60	916458.11 ± 2989.55	0.32
80	1216957.27 ± 4557.81	0.37

***Each value is average ± SD (n = 3)**

be 99.69 - 100.34. The results are showed in TABLE 2.

The precision of the method was evaluated by interday and intraday analysis which also showed good results with very low variations as revealed by very low %RSD values (< 0.25%). The results are shown in TABLES 3 and 4.

The detection limit for L-dopa was 0.0024µg/ml while quantitation limit was 0.0073µg/ml, which suggests that a nanogram quantity of L-dopa can be estimated accurately.

The drug L-dopa was estimated from tablet formulation using the method described. Three different tablet formulations were used. The estimated content varied from 99.84 - 101.69 with % RSD < 0.257%. The assay values of the formulated formulations were very close to the label claim. This indicated that the excipients didn't interfere analysis method by proposed method. The HPLC chromatogram also reveals that the retention time is similar to that of the standard drug sample.

CONCLUSIONS

From the results and discussion it can be concluded that the method described for the estimation of L-dopa from formulation is simple, accurate, sensitive and reproducible. The proposed method requires very low time for separation and hence utilizes less solvent for separation. The proposed method could be employed even for routine analysis in quality control laboratories for different type of formulations.

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