



Simultaneous estimation of levodopa and carbidopa in bulk, pharmaceutical dosage forms and dissolution sample analysis by RP-HPLC-PDA method

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ABSTRACT

A simple, specific, and accurate reverse phase liquid chromatographic method was developed for the simultaneous estimation of Levodopa (LD) and Carbidopa (CD) in bulk and pharmaceutical dosage forms. A Phenomenex C₁₈-RP Aqueous reverse phase column (250 x 4.6mm, 5 μm) with mobile phase containing 0.05%(v/v) o-phosphoric acid: acetonitrile (96:4%(v/v)) was used at 1mL/min flow rate in isocratic mode and the eluents were monitored at 220 nm. The retention times of LD and CD were 4.2 and 7.4 min respectively and showed a good linearity in the concentration range of 20-100 μg/mL for LD and 10-50μg/mL for CD with a correlation coefficient (R) of 0.9999 and 0.9998. The percentage assays for controlled matrix tablets (SYNDOPA) were found to be 101.98 and 100.47 respectively for LD and CD. The proposed method was validated as per ICH guidelines and successfully applied for the simultaneous estimation of LD and CD in tablet formulations and dissolution sample analysis.

Keywords: Levodopa, Carbidopa, Phenomenex column, ICH guidelines, SYNDOPA.

INTRODUCTION

LD is an anti-Parkinsonian drug used in the treatment of Parkinson's disease. LD is a prodrug of dopamine. A conventional oral dopa medication controls the evolution of Parkinson's disease adequately for about 5 years [1]. Co-administration of LD with inhibitors of extra cerebral dopa decarboxylase (IEDD) such as CD allows a marked reduction in LD dosage without compromising the therapeutic effect. CD diminishes the optimum dose of LD by about 70-80%, decreasing the plasma concentration fluctuations. This kind of combination also reduces the time to onset of the therapeutic benefit due to an increase in the bioavailability of LD and to a decrease of the incidence and the severity of the side-effects [2].

Literature survey revealed that few analytical methods like HPLC [3-9], LC-MS/MS [10], NMR [11], HPTLC [12], simultaneous UV spectrophotometric methods [13-15] and electrophoresis [16] have been reported for the estimation of LD either alone or in combination with other drugs like CD, Entacapone, Methyl dopa. However, the reported HPLC method for the simultaneous estimation of LD and CD used mobile phases like phosphate buffers (which are not LC-MS compatible) and with ion pairing agents. All these methods were developed with UV detector. So far there was also no validated HPLC method reported for the simultaneous analysis of LD and CD in dissolution studies. Hence, the present investigation was aimed at developing a simple, rapid, sensitive and economic RP-HPLC-PDA method for simultaneous estimation of LD and CD in bulk, dosage forms and in dissolution samples.

EXPERIMENTAL SECTION

Chemicals

LD and CD were a gift samples from Divi's Laboratories, Hyderabad, India. Acetonitrile, water and o-phosphoric acid were purchased from E. Merck, Mumbai, India. All the solvents and reagents were of HPLC grade.

SYNDOPA® (Sun Pharma Ltd, Mumbai) tablets containing LD (200 mg) and CD (50 mg) were commercially purchased.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Phenomenex- RP C₁₈ column (250 × 4.6mm, 5μ).

Chromatographic Conditions

Mobile phase consisting of 0.05% (v/v) *o*-phosphoric acid: acetonitrile (96:4 v/v) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45μm (Millipore) and sonicated for 3 min in ultrasonic bath before use. The flow rate was 1 mL/min and the injection volume was 20 μL. PDA detection was performed at 220 nm and the separation was achieved at ambient temperature.

Preparation of stock and standard solutions

The stock solution of LD and CD strength 1mg/mL were prepared by dissolving 10 mg of each drug in 10mL of methanol in a volumetric flask. Appropriate volumes of these stock solutions were then further diluted with 0.1N HCl (Diluent) to get the required concentrations of standard solutions at a concentration range of 20-100 μg/mL and 10-50μg/mL.

VALIDATION

Linearity

A linear relationship should be evaluated across the range of analytical procedure which may be demonstrated directly on the drug substance by dilution of a standard stock solution. The linearity of LD and CD responses were determined by preparing and injecting standard solutions in the range of 20-100μg/mL and 10-50ug/mL. The data was given in Table 1.

Precision

Precision was measured in terms of repeatability of application and measurement.

System Precision

Repeatability of standard application was carried out using six replicates of the same standard concentration (40μg/mL, 20μg/mL). The data was given in Table 1.

Method Precision

The method precision was determined by preparing a sample solution of single batch Levodopa and Carbidopa Tablet six times and analysing as per the proposed method. Repeatability was carried out using six replicates of the same concentration (40μg/mL, 20μg/mL). The data was given in Table 1.

Accuracy

The accuracy of the method was determined through recovery studies by the standard addition method by spiking 80%, 100%, 120% of the known quantities of standard within the range of linearity to the synthetic solution of drug product with 40μg/mL of LD and 20μg/mL of CD these solutions were analyzed in triplicate, the data was given in Table 1.

LOD and LOQ

LOD and LOQ were determined by calibration curve method. Standard solutions of LD and CD were prepared in the range of 20-100μg/mL and 10-50μg/mL and injected (20μL) in triplicate. Average peak area of three drugs was plotted against concentration. LOD and LOQ were calculated by using following equations: $LOD = (3.3 \times \sigma)/m$; $LOQ = (10.0 \times \sigma)/m$ (Where, σ is the standard deviation of the responses and m is mean of the slopes of the calibration curves).

System Suitability

System suitability studies were carried out by injecting a 60μg/mL and 30μg/mL standard of LD and CD at different injection volumes. The data was given in Table 2.

Specificity

Specificity studies were carried for both pure drug and drug product by comparing the 3D plots with blank (diluent) and placebo. Peak purity tests were also carried out to show that the analyte chromatographic peak is not

attributable to more than one component as the impurities are not available by analyzing the peak purity index data. The data was shown in Fig 2 & 3.

Assay

Twenty tablets were weighed individually, finely powdered and 16mg of powder blend equivalent to 10mg of LD and 2.5mg of CD was accurately weighed and transferred to a 10 mL volumetric flask and 5 mL of methanol was added to the same. The flask was sonicated for 5 min and volume was made up to the mark with methanol. The above solution was filtered using Nylon disposable Syringe Filter (0.45 μm) and the 1mL of the filtrate was diluted to 10 mL with diluent in 10 mL volumetric flask. The amount present in the each tablet was calculated by comparing the area of standard LD and CD with that of the tablet sample.

Dissolution Analysis

In-vitro dissolution studies for SYNDOPA tablets were carried in 900 mL of 0.1N HCl as dissolution medium using USP type II (Paddle method) Dissolution Rate Test Apparatus (DISSO 8000, LAB INDIA) at 50 rpm. The temperature of medium was maintained constant at $37^{\circ} \pm 0.5^{\circ}\text{C}$. Dissolution samples were collected manually at 0, 0.5, 1, 2, 3, 4 hrs. At each time point, 5mL sample was removed and filtered through a nylon filter (0.45 μm); an aliquot of filtrate was suitably diluted and analyzed by HPLC. The amount of LD and CD in the test samples was calculated by comparing test the peak area with that of the standard.

Filter compatibility study

In this study nylon filter (0.45 μm) and PVDF filter compatibility was evaluated. Sample solution was prepared and the solution was filtered using 0.45 μm nylon filter and PVDF filter. Filtered samples were injected and chromatograms were observed. The data was given in Table-3.

RESULTS AND DISCUSSION

The present investigation was carried out with a view to develop a rapid and economical RP- HPLC-PDA method for the simultaneous estimation of LD and CD in bulk, dosage forms and dissolution sample analysis. In the present investigation, different mobile phase combinations were tested to develop a highly sensitive LC method, for the simultaneous analysis of LD and CD in bulk and formulations. Initial trials were carried with Devlosil RP Aqueous column (250x4.6mm, 5 μm) using 15 mM phosphate buffer and methanol (90:10%w/v) as mobile phase with 1.0mL/min flow rate with methanol as diluent. LD and CD were eluted but the peaks were broad and peak splitting was observed. In the next trail the mobile phase used was 0.1% w/v octane sulphonic acid and acetonitrile (90:10) with acetonitrile as diluent, the peaks eluted at almost same retention time (LD-3.1min, CD-3.3min) along with the solvent front. In another trial the mobile phase was changed using 0.1% w/v octane sulphonic acid and acetonitrile with 10% methanol used as diluent, the resolution was good (LD-4.2 min, CD-7.8 min) but peaks were broad with a band width of 1.8min.

The trials were continued by changing the column to Phenomenex C₁₈ (250x4.6mm) and also the mobile phase, 0.05% *o*-phosphoric acid and acetonitrile (90:10% v/v) with 0.1N HCl as diluent. Under these conditions the LD was eluted along with the solvent front and both peak shapes were good. Further trails were carried out by changing the mobile phase composition, 0.05% *o*-phosphoric acid (pH 2.2) and acetonitrile (96:4 v/v) at a flow rate of 1 mL/min. Under these conditions a good resolution between the peaks was observed and peaks were symmetrical, tailing factor was within the limits and both the LD and CD peaks were eluted within 10 min run time. The retention times were 4.2 and 7.4min respectively for LD and CD. For quantitative analytical purpose wavelength was set at 220 nm, which provided better reproducibility without interference. The method was validated as per ICH guidelines. A sample chromatogram of the standard peaks along with diluent was shown in Fig 1. The peak purity indices were also found to be greater than 0.9999 and this indicates absence of the impurities in pure LD, CD and EC samples, peak purity indices of LD and CD are shown in Fig 1 along with UV spectra.

Linearity

A linear relationship was evaluated across a concentration range of 20-100 $\mu\text{g/mL}$ for LD and 10-50 $\mu\text{g/mL}$ for CD which was analysed in triplicate. The range of concentrations was selected based on 80-120 % of the test concentration (for assay). Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient (R^2) was found to be 0.997 and 0.995 and shows good linearity. The data of the calibration curve was given in Table 1.

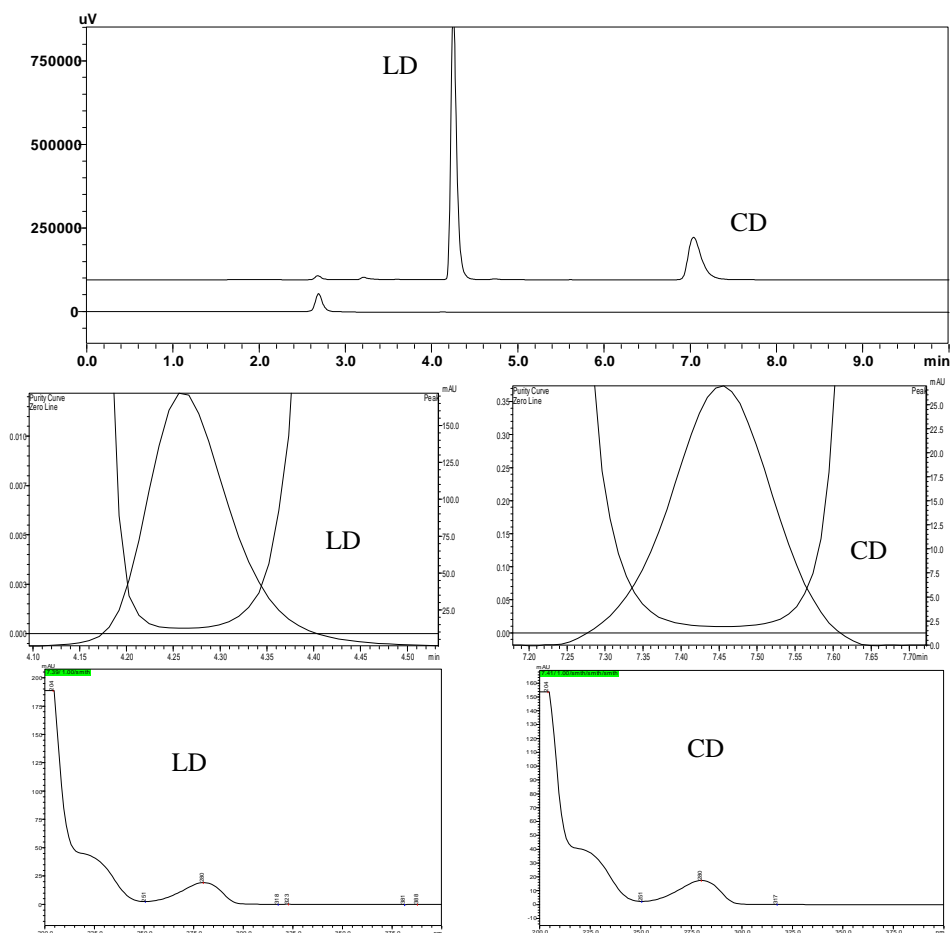


Figure 1: Overlay chromatograms of Diluent, LC and CD with Peak purity index curves and UV spectra

System precision

Precision studies were carried out in terms of repeatability. Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration (40µg/mL of LD and 20µg/mL of CD) and the data given in Table 1. The % RSD was found to be below 2.

Method precision

The method precision was determined by preparing a sample solution from a single batch of Levodopa and Carbidopa Tablet. Repeatability was carried out using six replicates of 40µg/mL of LD and 20µg/mL of CD. The data was given in Table 1. The % RSD was found to be below 2 and fulfilled the ICH guidelines criteria.

Table 1: Validation data for LD and CD

Validation data of LD and CD			
Parameters		LD	CD
Linearity (n=3)	Concentration	20-100ug/mL	10-50ug/mL
	Regression equation	y =39004x-14742	y =303776x-13581
	Regression Coefficient (R ²)	R ² = 0.997	R ² = 0.995
	Correlation Coefficient(R)	R = 0.9999	R = 0.9998
Accuracy (n=3)	% Level of Addition	Mean Percent Recovery(%RSD)	Mean Percent Recovery(%RSD)
	80	99.85 (0.80)	99.98 (0.72)
	100	100.14 (0.54)	100.64 (0.38)
	120	100.07 (1.01)	100.99 (0.68)
Precision (n=6)			
System Precision	Average peak area of the standard sample (%RSD)	1386865 (0.21)	455295.7 (0.57)
Method Precision	Average peak area of the assay sample (%RSD)	1595216.3 (0.51)	532984.2 (0.12)

Accuracy

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product as the exact components are unknown and for drug substance the analyte peak is evaluated by 3D plot of the chromatogram in order to confirm the existence of only LD, CD drug component at 4.2, 7.4 min as shown in Figure 3. As the impurities are not available, the recovery of the added standard to the drug product sample was calculated and it was found to be in the range of 99.13-101.23% and 99.52-101.46% for LD and CD respectively. These results indicate a good accuracy of the method to that of the labelled claim. The obtained recovery results were given in Table 1.

Limit of detection (LOD) & Limit of quantification (LOQ)

LOD and LOQ were calculated from the average slope and standard deviation of the calibration curve. LOD for LD and CD was found to be 0.217, 0.095 $\mu\text{g/mL}$ whereas LOQ for LD and CD was calculated to be 0.657, 0.289 $\mu\text{g/mL}$ respectively. These results indicate that the method is sensitive enough to carry out the routine analysis of LD and CD combination dosage forms.

System suitability

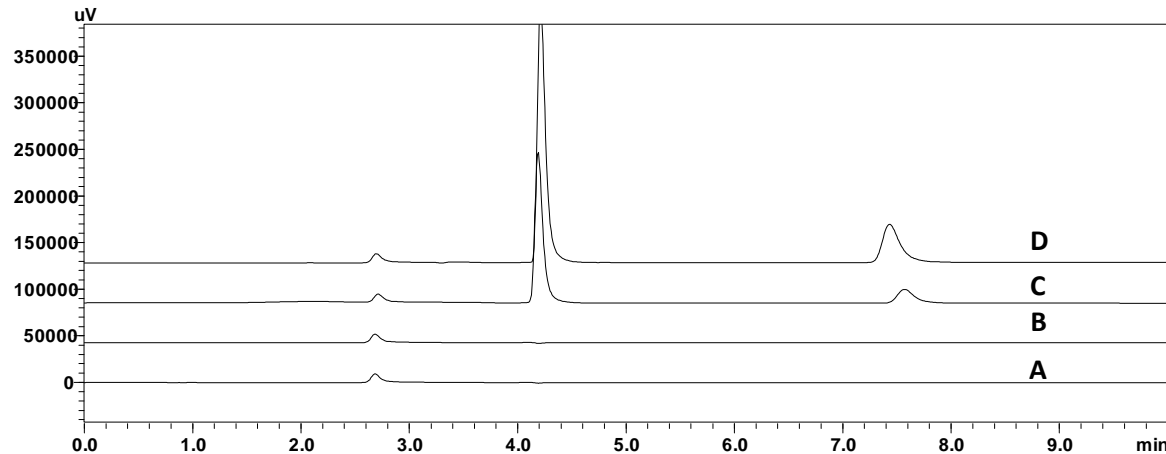
System suitability studies were carried out by injecting a 60 $\mu\text{g/mL}$ and 30 $\mu\text{g/mL}$ standard of LD and CD at different injection volumes. The data was given in Table 2.

Table 2: System suitability parameters

Parameters	LD(%RSD)	CD (%RSD)
Retention Time (min)	4.20 (0.57)	7.39 (0.79)
Tailing Factor	1.47 (1.25)	1.47 (1.68)
Theoretical Plates	15275.62(1.41)	10685.77 (1.17)

Specificity

The specificity of the method was established by spiking with diluent solution of commonly used excipients in the tablet and showed no peaks within the retention time of two drugs and also over the range of 10.0min as shown in Figures 2 and 3.

**Figure 2: Chromatograms of A: Placebo, B: Blank, C: Sample, D: Standard samples****Assay**

The amount present in the each tablet was calculated by comparing the area of standard with that of tablet sample. The assay was found to be within the limits and the present LC conditions can be used for the assay of LD and CD in different commercially available formulations.

Dissolution analysis of modified release dosage form

The validated method was used for the *in vitro* dissolution analysis of SYNDOPA tablets. The % drug release was found to be NLT 85% at the end of dissolution, proving that the developed method can be successfully applied for the routine *in vitro* dissolution sample analysis of LD and CD. The dissolution profile was shown in Figure-3.

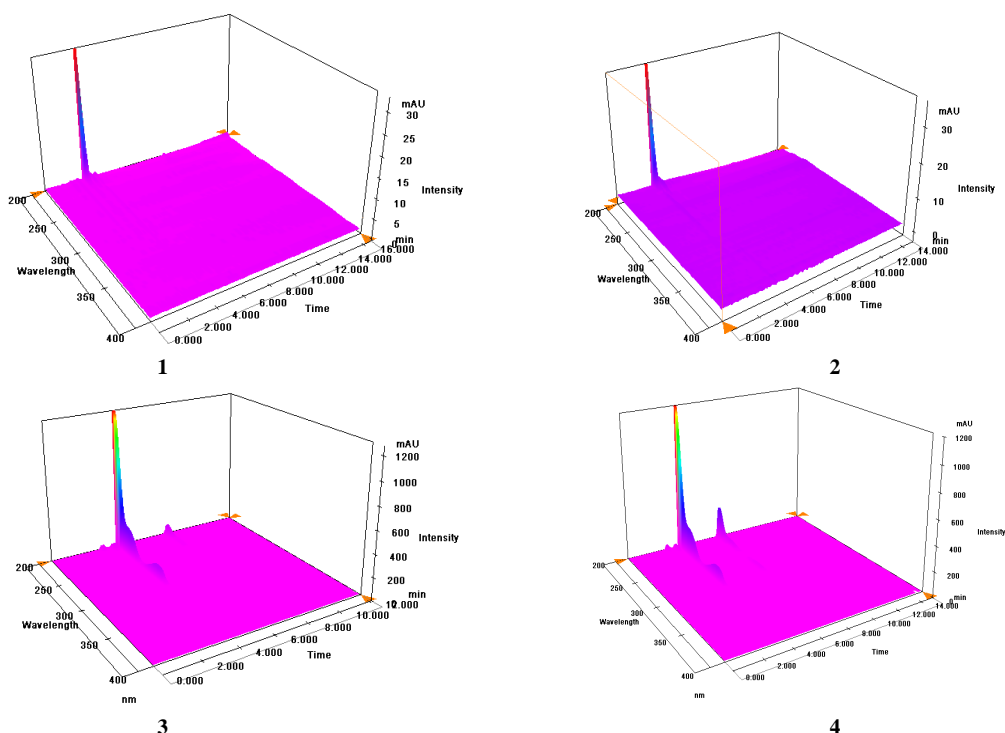


Figure 3: 3D Plots of diluent (1), Placebo (2), Sample (3), Standard (4) chromatograms

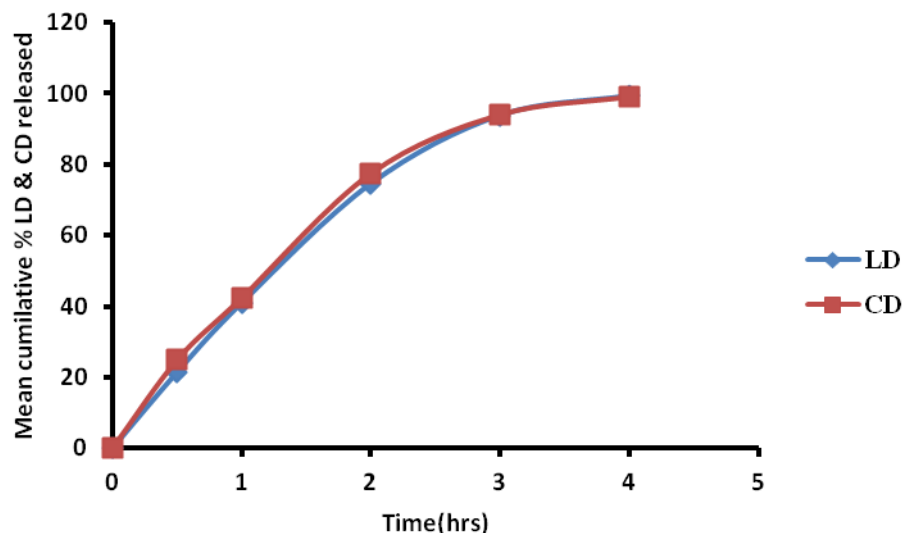


Figure 4: Dissolution profiles of LD and CD in 0.1N HCl

Filter compatibility study

Compatibility of dissolution samples with 0.45µm nylon & PVDF disposable filters were studied. Standard sample solution and filtered dissolution medium samples were analyzed and the variation in the assay value when compared to unfiltered standard sample was calculated and data was tabulated in Table-3. After the analysis it was found that nylon filters are suitable for filtration.

Table 3: Filter compatibility study

Sample name	Peak area of		% Difference
	LD	CD	
Standard sample(LD 40µg/mL, CD 20µg/mL,)	1389203	454225	- -
Samples filtered through 0.45µm nylon filter	1380569	453058	0.621 0.256
Samples filtered through 0.45µm PVDF filter	1346580	441894	3.068 2.714

CONCLUSION

The proposed RP-HPLC - PDA method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of LD and CD in combination and for dissolution sample analysis using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of LD and CD without interference from diluent and placebo. The proposed method is highly sensitive, economical, reproducible, reliable, rapid and specific and also has the unique advantage of LC conditions. Therefore, this method can be employed in quality control to estimate the amount of LD and CD in bulk, dosage forms and for dissolution sample analysis.

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