International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2014; 4(1):32-39

Research Article

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LEVODOPA, CARBIDOPA AND ENTACAPONE IN COMBINED DOSAGE FORM

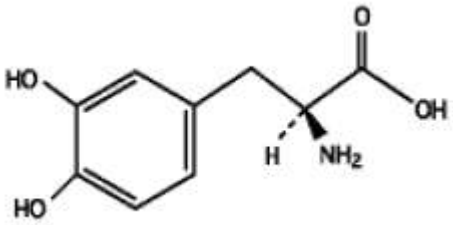
Aliya Thahaseen*, Dr. Yeluri Rama Chandra Reddy
Pharmaceutical analysis and Quality assurance.

*Corresponding Author: Aliya Thahaseen; Email: twinkletwilight12@gmail.com

Abstract: An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of levodopa and carbidopa and entacapone in combined-dosage form. Agilent zorbax sb-C18 (250mm*4.6mm*5µ) column with mobile phase containing water pH 4.0 adjusted with sodium dihydrogen orthophosphate: methanol in the ratio of (600: 400, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 25°C and effluents were monitored at 284 nm. The retention times of levodopa and carbidopa and entacapone were 1.4min and 2.2min and 4.4min, respectively. The correlation co-efficient for levodopa and carbidopa and entacapone were found to be 0.99 and 0.99 and 0.99, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of levodopa and carbidopa and entacapone in formulations was found to be 100% and 100% and 100% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of levodopa and carbidopa and entacapone in combined dosage form.
Key words: RP-HPLC, Levodopa, Carbidopa, And Entacapone

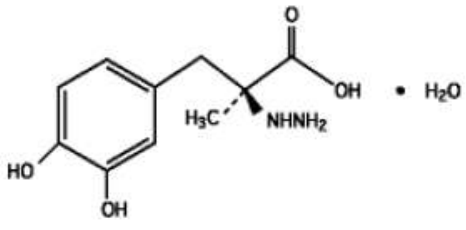
INTRODUCTION

Levodopa, an aromatic amino acid, is a white, crystalline compound, slightly soluble in water, with a molecular weight of 197.2. It is designated chemically as (-)-L-α-amino-β-(3,4-dihydroxybenzene) propanoic acid. Its empirical formula is $C_9H_{11}NO_4$, and levodopa is an intermediate in the dopamine biosynthesis clinically. levodopa is one of the main drugs used to treat Parkinson's symptoms. It can be used at all stages of the disease. levodopa is a chemical building-block that your body converts into dopamine. It replaces the dopamine that is lost in Parkinson's. levodopa is the most effective medicine for relieving symptoms of Parkinson's disease. It helps reduce tremor, stiffness, and slowness and helps improve muscle control, balance, and walking. It does not affect freezing, dementia, or problems with involuntary (autonomic) functions, such as constipation, urinary problems, impotence,or pain.



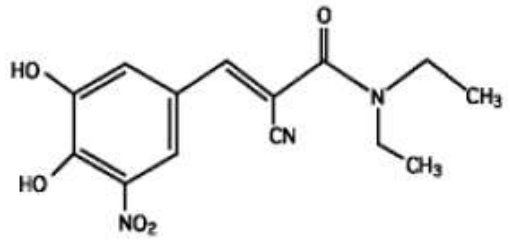
Levodopa

Carbidopa, an inhibitor of aromatic amino acid decarboxylation, is a white, crystalline compound, slightly soluble in water, with a molecular weight of 244.3. monohydrate.Its empirical formula is $C_{10}H_{14}N_2O_4 \bullet H_2O$. It is designated chemically as (-)-L-α-hydrazino-α-methyl-β-(3,4-dihydroxybenzene) propanoic acid carbidopa is a drug given to people with Parkinson's disease in order to inhibit peripheral metabolism of levodopa. This property is significant in that it allows a greater proportion of peripheral levodopa to cross the blood brain barrier for central nervous system effect.carbidopa is used with levodopa to treat Parkinson's disease. Parkinson's disease is believed to be related to low levels of a chemical called dopamine in the brain. levodopa (Dopar, Larodopa) is turned into dopamine in the body. Carbidopa is used with levodopa to prevent the breakdown (metabolism) of levodopa before it can reach the brain and take effect. Carbidopa is only effective if it is taken with levodopa. It has no effect if it is used alone.carbidopa is used with levodopa to treat the stiffness, tremors, spasms, and poor muscle control of Parkinson's disease. These medications are also used to treat the same muscular conditions when they are caused by drugs such as chlorpromazine (Thorazine), fluphenazine (Prolixin), perphenazine (Trilafon), and others.



Carbidopa

Entacapone, an inhibitor of catechol-O-methyltransferase (COMT), is a nitro-catechol-structured compound with a molecular weight of 305.3. The chemical name of entacapone is (E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethyl-2-propenamide. Its empirical formula is $C_{14}H_{15}N_3O_5$. entacapone is used in the treatment of Parkinson's disease. It is used in combination with levodopa and carbidopa to treat the end-of-dose 'wearing-off' symptoms of Parkinson's disease. Entacapone helps the levodopa and carbidopa work better by allowing more of it to reach the brain, where it has its effects. Many people taking levodopa for Parkinson's have problems with the effects of the levodopa wearing off between scheduled doses, causing symptoms to return or worsen. Entacapone blocks a certain natural substance (COMT enzyme) that breaks down the levodopa in the body. This effect allows the levodopa to last longer in the system so that it doesn't wear off before the next dose.



Entacapone

MATERIAL AND METHODS

Instrumentation: The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and Agilent zorbax sb-c18 column (250mmx4.6mm, particle size 5µm).

Chemicals and Reagents: levodopa and carbidopa and entacapone was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E. Merck (India) Ltd.,sodium dihydrogen orthophosphate of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions: The mobile phase consisting of water (pH 4.0 adjusted with sodium dihydrogen orthophosphate) and methanol (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of (600: 400, v/v) was pumped into the column at a flow rate of 1.0ml/min. The monitored at 284nm and the run time was 6min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system

PREPARATION OF STANDARD SOLUTION

Levodopa: Accurately weighed quantity, 200 mg of levodopa was transferred into 50ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15 min. make up the volume with mobile phase. From the above solution take 5ml into 25ml volumetric flask and make up the volume with mobile phase

Carbidopa: Accurately weighed quantity, 50 mg of carbidopa was transferred into 50ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15 min. make up the volume with mobile phase. From the above solution take 5ml into 25ml volumetric flask and make up the volume with mobile phase

Entacapone: Accurately weighed quantity, 400 mg of Entacapone was transferred into 50ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15 min. make up the volume with mobile phase. From the above solution take 5ml into 25ml volumetric flask and make up the volume with mobile phase

PREPARATION OF SAMPLE SOLUTION

Take accurately sample powder equivalent to two tablets and transfer it into 50ml of volumetric flask dissolve and diluted to volume with mobile phase and sonicate for 10 min.From the above solution take 5ml into 25ml volumetric flask make up the volume with mobile phase.

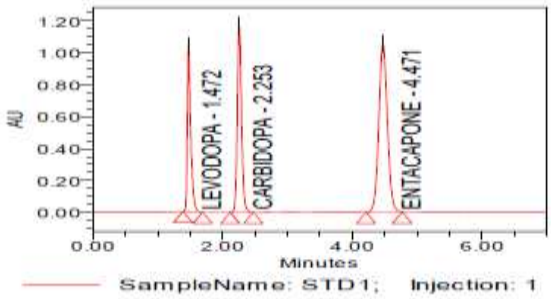


Fig.1:Standard chromatogram for levodopa and carbidopa and entacapone

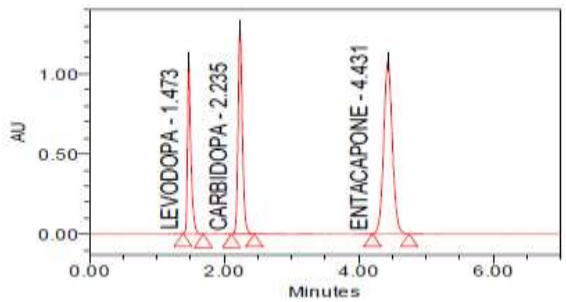


Fig.2: Formulation chromatogram for levodopa and carbidopa and entacapone

METHOD VALIDATION

System Suitability Studies: The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Table1: System Suitability Parameters

Parameters	levodopa	carbidopa	entacapone
Correlation coefficient	0.99	0.99	0.99
Regression equation	y =16616x	y=19288x	y =16616x
LOD	2.909	2.4896	2.938
LOQ	9.697	8.2988	9.792
Theoritical plates	4158	5880	5544
Tailing	1.645	1.181	1.068

Specificity: The specificity was established by preparing a levodopa and carbidopa and entacapone standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure

ACCURACY AND PRECISION:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table-3&4).

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Fig 3, 4 &5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2).

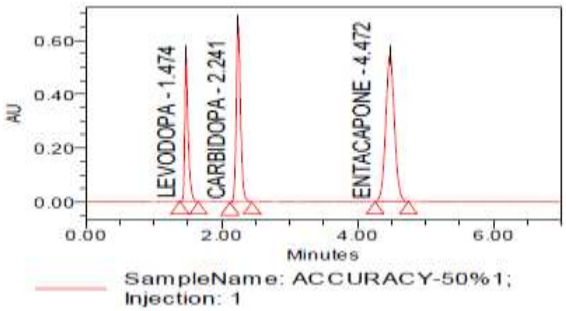


Fig.3: Accuracy Chromatograms-50% of levodopa and carbidopa and entacapone

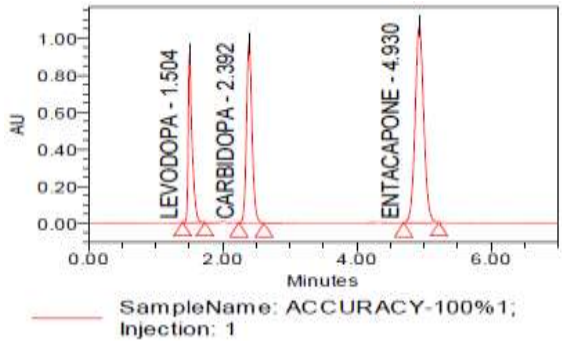


Fig.4: AccuracyChromatograms100%of levodopa and carbidopa entacapone

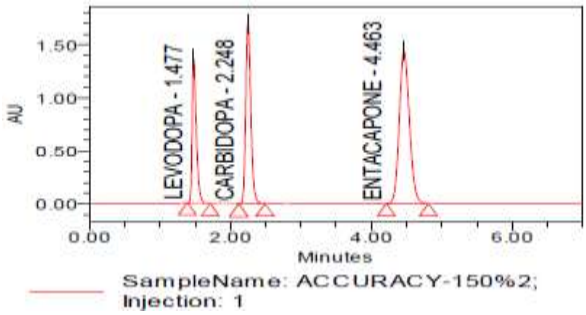


Fig.5: Accuracy Chromatograms-150% of levodopa and carbidopa and entacapone

Table 2 : Precision Studies

S.No.	Sample weight	Area (levodopa)	Area ((carbidopa)	Area (entacapone)	%Assay (levodopa)	%Assay (carbidopa)	%Assay (entacapone)
1	543.10	3760333	5720550	9701769	99	100	99
2	543.10	3767607	5720569	9704352	99	100	99
3	543.10	3769784	5721107	9700001	99	100	99
4	543.10	3767642	5721519	9709453	99	100	99
5	543.10	3762966	5722813	9702223	99	100	99
6	543.10	3760039	5728259	9702614	99	100	99

Table 3: Accuracy for Levodopa

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	271.50	1888874	395.927	398.17	101	101
50%	271.50	1884501	395.927	397.25	100	
50%	271.50	1889527	395.927	398.31	101	
50%	271.50	1886911	395.927	397.76	100	
50%	271.50	1889358	395.927	398.28	101	
50%	271.50	1886880	395.927	397.75	100	
100%	543.10	3760039	792.000	792.62	100	100
100%	543.10	3768341	792.000	794.37	100	
100%	543.10	3761290	792.000	792.88	100	
150%	814.60	5647628	1187.927	1190.52	100	100
150%	814.60	5644340	1187.927	1189.83	100	
150%	814.60	5643380	1187.927	1189.63	100	
150%	814.60	5647480	1187.927	1190.49	100	
150%	814.60	5642172	1187.927	1189.37	100	
150%	814.60	5646405	1187.927	1190.26	100	

Table 4: Accuracy for carbidopa

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	271.50	2867279	100.000	99.91	100	100
50%	271.50	2865628	100.000	99.85	100	
50%	271.50	2865786	100.000	99.86	100	
0%	271.50	2860400	100.000	99.67	100	
50%	271.50	2865843	100.000	99.86	100	
50%	271.50	2869763	100.000	100.00	100	
100%	543.10	5728259.00	200.037	199.60	100	100
100%	543.10	5722340.00	200.037	199.39	100	
100%	543.10	5728743.00	200.037	199.62	100	
150%	814.60	8584632	300.037	299.13	100	100
150%	814.60	8583988	300.037	299.11	100	
150%	814.60	8582357	300.037	299.05	100	
150%	814.60	8586364	300.037	299.19	100	
150%	814.60	8581955	300.037	299.04	100	
150%	814.60	8588807	300.037	299.27	100	

Table 5: Accuracy for Entacapone

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	271.50	4858893	792.800	794.05	100	100
50%	271.50	4854096	792.800	793.27	100	
50%	271.50	4850153	792.800	792.62	100	
0%	271.50	4853423	792.800	793.16	100	
50%	271.50	4859595	792.800	792.66	100	
50%	271.50	4850381	792.800	793.02	100	

100%	543.10	9702614	1585.892	1585.62	100	100
100%	543.10	9706466	1585.892	1586.25	100	
100%	543.10	9707498	1585.892	1586.42	100	
150%	814.60	14540738	2378.692	2376.28	100	100
150%	814.60	14597106	2378.692	2385.49	100	
150%	814.60	14585606	2378.692	2383.61	100	
150%	814.60	14564095	2378.692	2380.10	100	
150%	814.60	14517935	2378.692	2372.55	100	
150%	814.60	14597708	2378.692	2385.59	100	

LINEARITY AND RANGE

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616x (R^2=0.99) for levodopa and y = 19288x (R^2=0.99) for carbidopa and y =16616x (R^2=0.99) for entacapone. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above.The overlay chromatograms of Linearity for levodopa and carbidopa and entacapone shows in Fig 6 and the results for calibration curves are given Fig7,8&9.

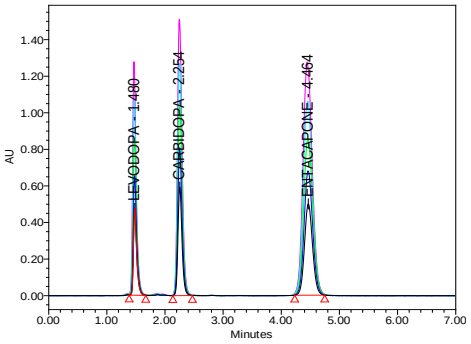


Fig.6: Overlay chromatograms of Linearity for levodopa and carbidopa and entacapone

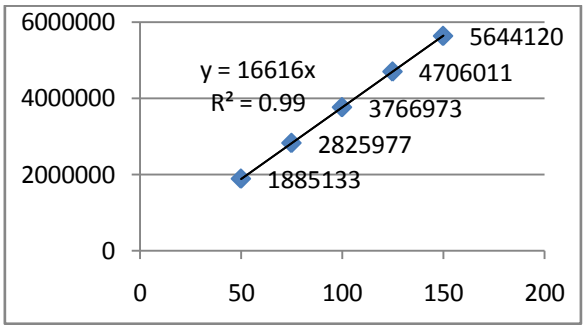


Fig. 7: Linearity Curve for levodopa

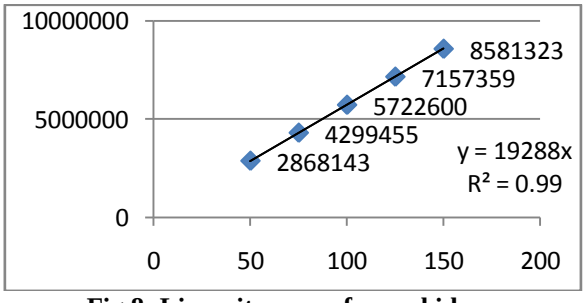


Fig.8: Linearity curve for carbidopa

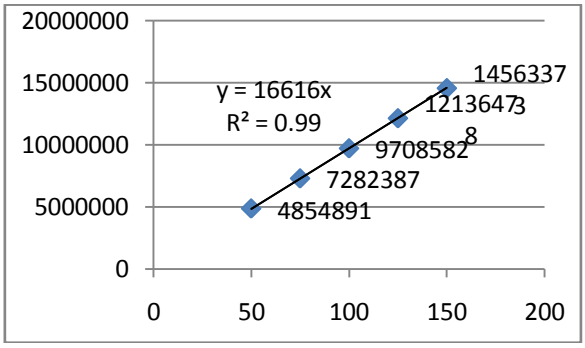


Fig. 9: Linearity Curve for entacapone

Limit of detection & Limit of quantifications (LOD & LOQ):

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of levodopa and carbidopa and entacapone. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels and the results are tabulated in Table 02.

(a) LOQ = 10 σ / S

(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response

S = slope of the calibration curve

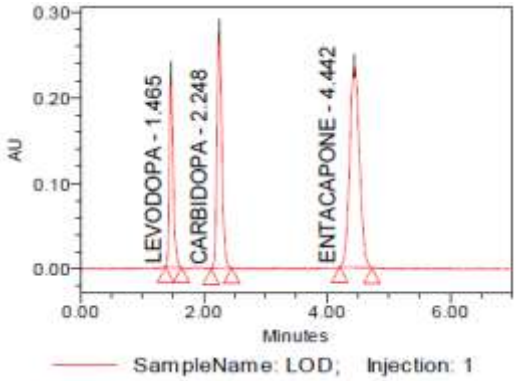


Fig.10: LOD Chromatograms for levodopa and carbidopa and entacapone

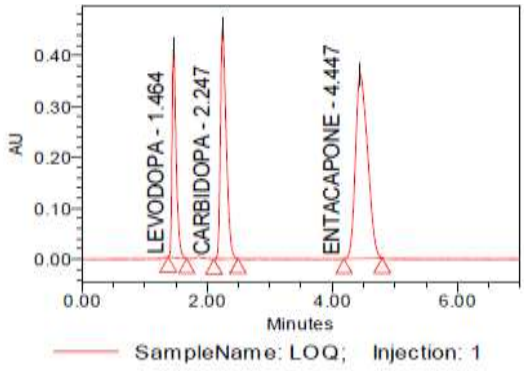


Fig.11: LOQ Chromatograms for levodopa and carbidopa and entacapone

ROBUSTNESS

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are rugged and robust (Table-6,7&8)

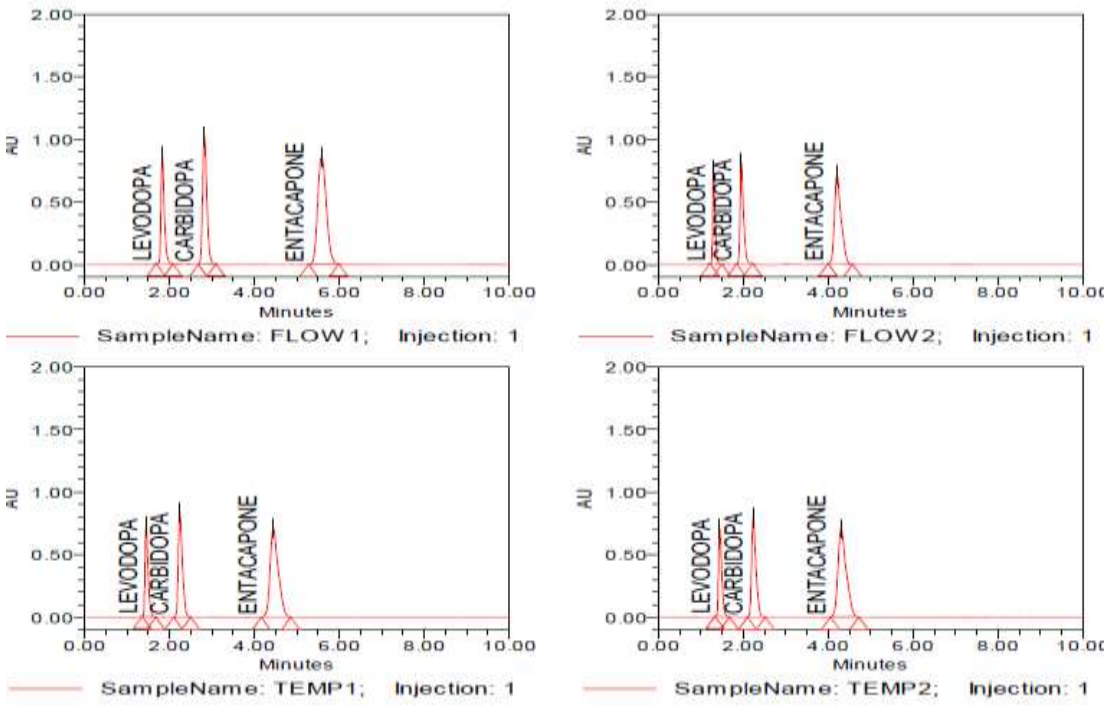


Fig.12: Robustness Chromatograms for levodopa and carbidopa and entacapone

Table 6: Robustness for levodopa

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	levodopa	1.827	4662438	1.501	4720
2	Temp1	25°C	levodopa	1.444	3664680	1.581	4808
3	Temp2	35°C	levodopa	1.439	3707608	1.566	4642
4	Flow2	1.2ml/min	levodopa	1.294	2976611	1.545	4944

Table 7: Robustness for carbidopa

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	carbidopa	2.817	7152995	1.311	4738
2	Temp1	25°C	carbidopa	2.239	5643179	1.441	4397
3	Temp2	35°C	carbidopa	2.229	5632398	1.464	4215
4	Flow2	1.2ml/min	carbidopa	1.949	4477567	1.498	4712

Table 8: Robustness for Entacapone

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Entacapone	5.580	12100300	1.165	4658
2	Temp1	25°C	Entacapone	4.443	9537349	1.327	4271
3	Temp2	35°C	Entacapone	4.298	9576725	1.384	4110
4	Flow2	1.2ml/min	Entacapone	4.205	7663185	1.406	4314

RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are levodopa and carbidopa and entacapone standard chromatograms and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for levodopa and carbidopa and entacapone studies. The method accuracy was evaluated by recovery studies. levodopa and carbidopa and entacapone recovery was founded 100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate shown in table 3,4&5. Linearity calibration curve was given below fig: 7,8&9 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation coefficient. Linear correlation was found to be y = 16616x for levodopa and y = 19288x for carbidopa and y = 16616x for entacapone. The intra day and inter day variations was calculated in terms of %RSD and results was found to be intra day and inter day respectively. Method robustness results were given by table 6,7&8.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of levodopa and carbidopa and entacapone in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of levodopa and carbidopa and entacapone in pure and its pharmaceutical dosage forms.

ACKNOWLEDGEMENT

Author thank full to department of Pharmaceutical analysis and Quality assurance and Rainbow pharma training lab, Kukatpally, for providing instruments and analytical support

REFERENCES

1. Bala Ramesh Chary, R and Madhusudhan Rao, *Drug Development and Industrial Pharmacy*. **2000**; 26(8): 901-906.
2. KrishnaVeni, Y., Jayasagar, G and Madhusudhan Rao, *Drug Development and Industrial Pharmacy*. **2000**; 27(8): 161-168.
3. Stocchi, F., Vacca, L., Ruggieri, S. and Olanow, C. W. A clinical and pharmacokinetic study. *Arch Neurol.* **2005**; 62: 905-910.
4. Block, G., Liss, C., Reines, S., Irr, J. and Nibbelink, D. *Eur Neurol.* **1997**; 37: 23-27.
5. Sharif et al., Stability indicating RP-HPLC and TLC methods for the determination of ziprasidone in bulk powder and in pharmaceutical formulations. *Biomed chromatogram.* **2004**; 18:143.
6. Srinu babu et al., .Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design. *Talanta*. **2007**; 71:1424.
7. ICH, Q1A (R2) Stability testing of new drug substances and products, international conference on Harmonization. **1996**.
8. ICH, Q2 (R1) validation of analytical procedures; Text and methodology, international conference on Harmonization. **1996**.
9. Levodopa and Carbidopa website- http://www.rxlist.com/sinemet-drug.htm
10. Simultaneous spectrophotometric determination of carbidopa and levodopa by partial least squares regression, principal component regression and least squares support vector machine methods , *Thai J. Pharm. Sci*. **2009;**33; 123-136 123.
11. J.M. Cedarbaum. Clinical pharmacokinetics of antiparkinsonian drugs, *Clin. Pharmacokinet*. **1987**;13: 141-178.
12. R.M. Pinder, R.N. Brogden, P.R. Sawyer, T.M. Speight,and G.S. Avery. Levodopa and decarboxylase inhibitors: areview of their clinical pharmacology and use in the treatmentof Parkinsonism, *Drugs*, **1976;** 11: 329-377.
13. Bell, C. E.; Somerill, A. R. *Biochemistry Sect. J.* **1966,** 98, C 1.
14. Imai, K. J. Chromatogr. **1975**, 105, 135.
15. Sharma, C.; Mohanty, S.; Kumar, S.; Rao, N. G. *Analyst* **1996**,121, 1963.
16. Lee, H. B.; Hong-you, R. L.; Fowlie, P. J. A. J. *Assoc. Off. Anal.Chem*. **1989**, 72, 979.
17. Zhu, A. Z.; Liu, J.; Fu, C. G. Fenxl. Ceshi, Xuebao **1997**, 16, 47.
18. Parsons, L. H.; Kerr, T. M.; Weiss, F. *J. Chromatogr. B, Biomed.Appl.* **1998**, 709, 35.
19. Ricebery, L. J.; Vanuis, H. V.; Levin, L. *Anal. Biochem.* **1974**, 60,551.
20. Nozaki, O.; Iwaeda, T.; Kato, Y. J. Biolumin. *Chemilumin.* **1996**, 11, 309.
21. Martindale. 32nd Ed. Great Britain: Council of Royal Pharmaceutical Society of Great Britain; **1999**. The Complete Drug Reference; p. 1168.