International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(1): January-February 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 07-12-2011; Accepted: 25-12-2011

SIMULTANEOUS ESTIMATION OF LEVODOPA, CARBIDOPA AND ENTACAPONE IN PHARMACEUTICAL DOSAGE BY VALIDATED REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Keywords:

RP-HPLC, Levodopa

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ABSTRACT

The reverse phase high performance liquid chromatography (RP-HPLC) method of levodopa and carbidopa is available in United State of Pharmacopoeia-27 (USP-27) but no reference is available for combined estimation of levodopa, carbidopa and entacapone in tablets formulation. The aim of our present work is to develop a precise and validated RP-HPLC method for the simultaneous determination of levodopa, carbidopa and entacapone in tablets formulation. The quantification was carried out by using Waters X-Terra RP18 (250X4.6 mm), 5µm column in gradient mode with mobile phase, buffer: acetonitrile (65:35). The flow rate was 1.5 ml/min. The peak purity of levodopa, carbidopa and entacapone were 0.99990, 0.99946 and 0.99990 respectively. Ruggedness and robustness of method were performed and the percentage relative standard deviation (RSD) was found below 2.0%. The percentage recovery was found in the range of 98% to 102% at three different levels. Calibration curves were linear over studies ranges with correlation co-efficient found between the range of 0.99 to 1.00. Sample and standard solution stability study was performed over 12 h at room temperature and found stable. The percentage deviation was below 2.0%.

INTRODUCTION: Levodopa, carbidopa and entacapone tablets contain three active substances in one film coated tablet. Levodopa is used to treat Parkinson's disease[1,2] but plus carbidopa and entacapone, which improve the antiparkinson effects of levodopa. The elimination half-life of levodopa, the active moiety of antiparkinsonian activity, was 1.7 h (1.1-3.2 h). Levodopa is extensively metabolized to various metabolites. Two major pathways are decaroxylation by dopa decarboxylase (DDC) and O-methylation by catechol-O-methyltransferase (COMT). Current evidence indicates that systems of Parkinson's disease are related to depletion of dopamine in the corpus striatum, Administration of dopamine is ineffective in the treatment of Parkinson's disease apparently because it does not cross the blood-brain barrier[3]. However, levodopa, the metabolic precursor of dopamine, does cross the blood-brain barrier, and presumably is converting to dopamine in the brain. This is thought to be the mechanism whereby levodopa relieves systems of Parkinson's disease. When levodopa is administrated orally it is rapidly decarboxylated to dopamine in extra cerebral tissues so that only a small portion of a given does is transported unchanged to the central nervous system. Carbidopa inhibits the decarboxylation of peripheral levodopa, making more levodopa available for transport to the brain. When co-administered with levodopa, carbidopa increases plasma levels of levodopa and reduced the amount of levodopa required to produce a given response by about 75%[4]. Carbidopa prolonged the plasma half-life of levodopa from 50 min to 1.5 h and decrease plasma and urinary dopamine and its major metabolite, homovanillic acid. Entacapone is a selective and reversible inhibitor of catechol-O-methyltransferase (COMT) [5]. When entacapone is given in conjunction with levodopa and carbidopa, plasma levels of levodopa are greater and more sustained than after administration of levodpa and carbidopa alone. It is believed that at a given frequency of levodopa administration, these more sustained plasma levels of levodopa result in more constant dopaminergic stimulation in the brain, leading to greater effects on the signs and symptoms of Parkinson's disease [6,7].

MATERIAL AND METHODS

Chemicals and Materials:

Levodopa, caridopa and entacapone were supplied by Zydus Cadila Limited India. Acetonitrile, ortho phosphoric acid, Di sodium hydrogen ortho phosphate and 1-decane sulfonate sodium salts were used of HPLC grade (Spectrochem and E-Merck Limited).

Instrumentation:

Shimadzu 2010C integrated high performance liquid chromatographic system was used for this experiment. Shimadzu 2010C system equipped with quaternary gradient pump, 2010C UV-VIS

detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The waters X-Terra RP18 (250X4.6 mm), 5 µm was used as a stationary phase.

HPLC Condition:

Column	X-Terra RP18 (250X4.6 mm), 5µm
Detector	280 nm
Injection volume	10 µl
Flow rate	1.5 ml/min
Temperature	30˚
Run time	30 min
Mobile phase	Buffer: Acetonitrile (65:35)

Gradient programme:

Time (min)	Buffer (Unit)	Acetonitrile (Unit)
00.01	100	00
10.00	100	00
11.00	065	35
24.00	065	35
25.00	100	00
30.00	100	00

Buffer preparation:

Weigh 8.75 g Di sodium hydrogen ortho phosphate in to 1.0 l volumetric flask. Then add 1.802 mg 1-decane sulfonate sodium salt, shake well and make volume up to mark with HPLC grade water. Adjust pH 3.0 with dilute ortho phosphoric acid solution.

Diluent:

0.1N HCl: Acetonitrile (1:1)

Standard preparation:

Standard stock solutions were prepared in diluent and further for further dilution, dilute it with 0.1N HCl to make final concentration levodopa 150 µg, carbidopa 40 µg and entacapone 200 µg respectively.

Sample preparation:

Weigh accurately tablets powdered equivalent to about 75 mg levodopa, 10 mg of carbidopa and 49 mg of entacapone in to 100 ml volumetric flask. Add about 50 ml diluent and sonicate it for 20 minute to dissolve. Filtered it through 0.45 µ HVLP nylon filter and made further dilution 10 ml to 50 ml with 0.1N HCl.

RESULTS AND DISCUSSION

The detection wavelength was chosen at 280 nm because the levodopa, carbidopa and entacapone in tablet dosage form have better absorption and sensitivity at this wavelength. As per USP-29 the pH of mobile phase was 2.8 for the combination of levodopa and carbidopa tablets[8]. However, to achieve the better separation of levodopa and carbidopa in the present combination, the mobile phase pH was kept at 3.0. A chromatogram was shown in Fig. 1, 2, and 3, which illustrate the separation of all three active ingredients in this system. The gradient program throughout HPLC method was adopted to analyze all the three components in a single run.

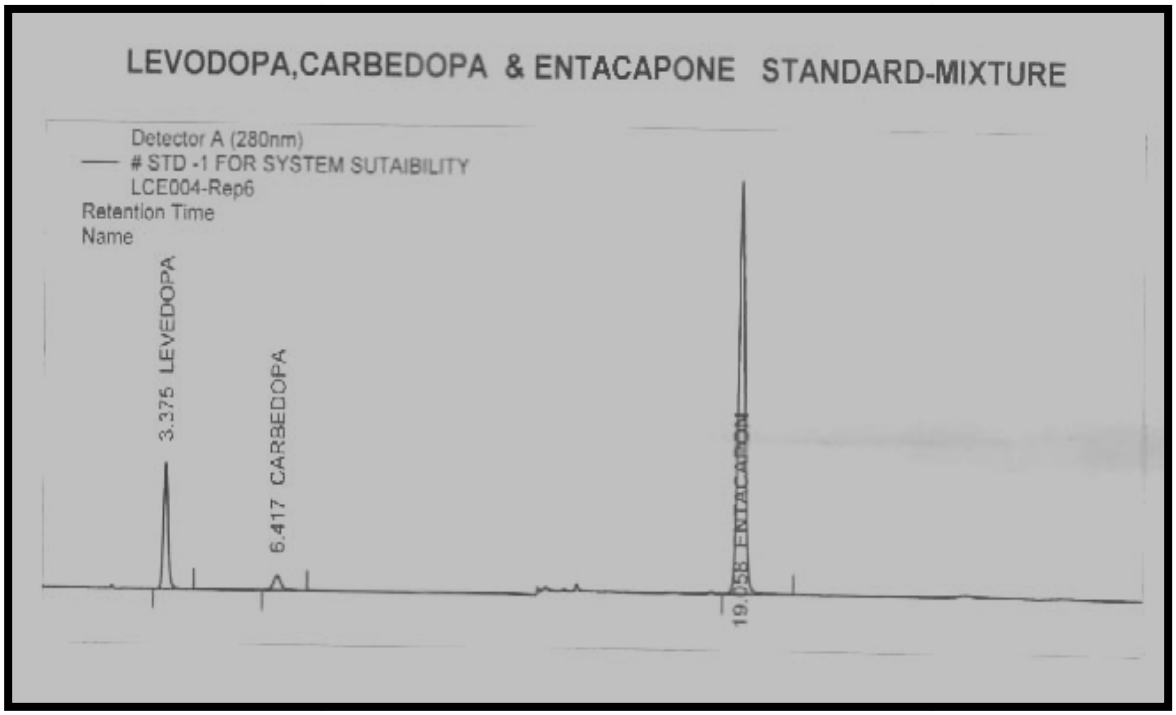

Fig 1 Levodopa, carbedopa and entacopone standard-mixture

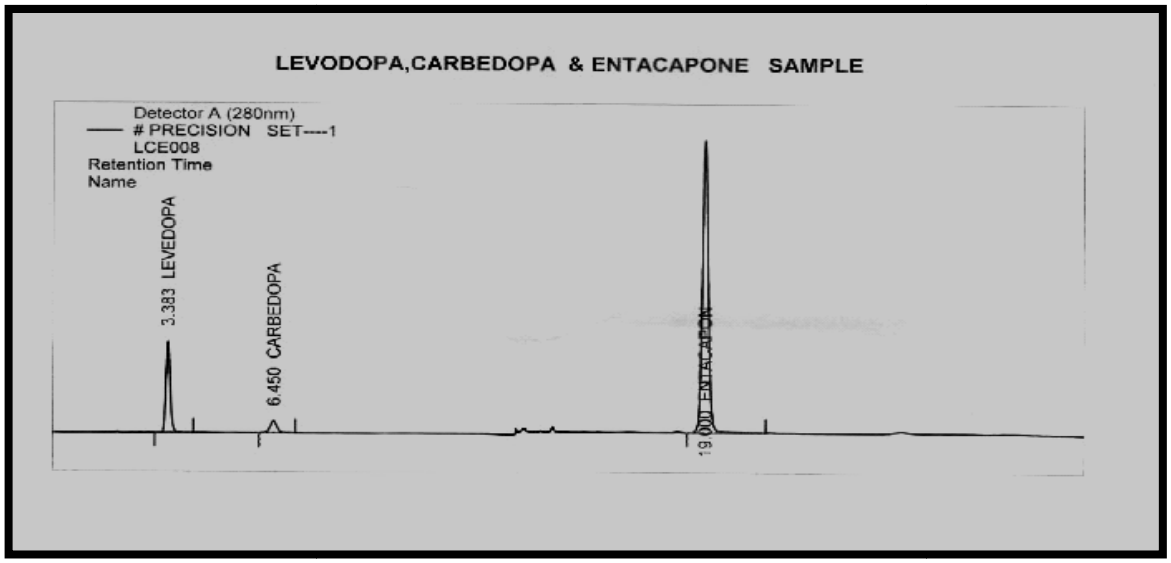

Fig 2 Levodopa, carbedopa and entacopone sample

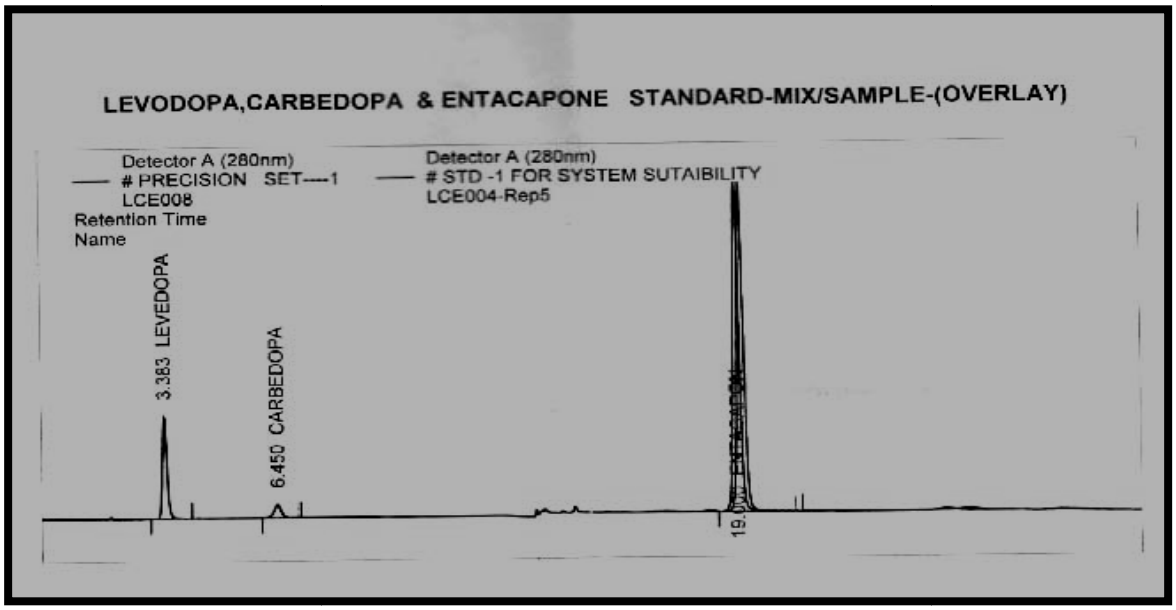

Fig 3 Levodopa, carbedopa and entacopone standard-mixture (Overlay)

System suitability and system precision:

System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented in table 1.

Linearity and calibration curve:

The linearity of the calibration curve was determined by weighed (1/c) least square regression analysis. The correlation coefficient was found to be 0.99 to 1.00. A linear relationship was found for all components. The results of linearity, limit of detection and limit of quantification were presented in table 2.

Specificity:

There was no interference from sample placebo and peak purity of levodopa, carbidopa and entacapone were 0.99934, 0.99960 and 0.99999. It showed that developed analytical method was specific for the analysis of levodopa, carbidopa and entacapone in tablet dosage form.

Standard and sample solution stability:

Standard and sample solution stability was evaluated at room temperature for 12 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 12 h at room temperature.

Method precision:

The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained were presented in table 3.

Method accuracy:

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were presented in table 4.

Method robustness:

Robustness of the method was determined by small deliberate changes in pH, flow rate and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 5.

Method Ruggedness:

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in table 6.

Table 1 System Suitability and System Precision

Compound	Retention time (Mean ± SEM)	Theoretical plates	Capacity Factor-k'	Resolution R	Asymmetry T	Selectivity α
Levodopa	03.34 ± 0.0022	3514.19	0.69	-	1.19	-
Carbidopa	06.51 ± 0.0020	5264.42	2.21	10.46	1.01	03.20
Entacapone	19.25 ± 0.0032	58822.38	8.60	38.12	1.04	12.46

Table 2 Characteristics of the Analytical Method Derived From the Standard Calibration Curve

Compound	LOD µg/ml	LOQ µg/ml	Linearity range n=(5)	Correlation co-efficient µg/ml	Residual std. regression σ	Slope of regression S
Levodopa	0.715	2.17	30 to 240	0.99992	6226.32	28699.99
Carbidopa	0.525	1.59	7.5 to 60	0.99918	3773.05	23704.79
Entacapone	7.615	23.08	40 to 320	0.99512	201624.54	87368.55

LOD=Limit of detection, LOQ=Limit of quantification

Table 3 Method Precision

Compound	Concentration µg/ml (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD of Assay
Levodopa	150	03.46 ± 0.0022	98.13 ± 0.1606	0.4008
Carbidopa	37.5	06.52 ± 0.0020	99.02 ± 0.1424	0.3533
Entacapone	200	19.23 ± 0.0151	95.40 ± 0.1633	0.4193

Table 4 Method Accuracy

Level	Drug Added (mg)	Drug recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
For Levodopa				
50%	075.20	075.89	100.93 ± 0.4910	0.8426
100%	150.25	151.17	100.60 ± 0.7000	1.2052
150%	225.03	228.01	101.33 ± 0.2603	0.4450
For Carbidopa				
50%	17.33	17.30	99.70 ± 0.5860	1.0179
100%	35.10	35.46	101.00 ± 0.5508	0.9445
150%	53.29	54.26	101.83 ± 0.6085	1.0315
For Entacapone				
50%	100.75	101.98	101.23 ± 0.2404	0.4413
100%	200.75	201.81	100.53 ± 0.5175	0.8915
150%	300.27	298.82	99.50 ± 0.6174	1.0743

Table 5 Method Robustness

Compound	% RSD in Normal and Changed condition (n=5)		
For Temperature	**% RSD Normal**	**% RSD (+ 5°C)**	**% RSD (- 5°C)**
Levodopa	0.1	0.0	0.1
Carbidopa	1.1	0.8	0.4
Entacapone	0.2	0.6	0.4
For pH	**% RSD Normal**	**% RSD (+ 0.2 unit)**	**% RSD (- 0.2 unit)**
Levodopa	0.1	0.1	0.2
Carbidopa	1.1	0.1	0.5
Entacapone	0.2	0.2	0.3
For Flow Rate	**% RSD Normal**	**% RSD (+ 10%)**	**% RSD (- 10%)**
Levodopa	0.1	0.0	0.0
Carbidopa	1.1	0.0	0.1
Entacapone	0.2	0.2	0.3

Table 6 Method Ruggedness

Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 1	**Analyst-1, Instrument-1 & Column-1**	
Levodopa	100.17 ± 0.2963	0.7257
Carbidopa	99.50 ± 0.2066	0.5085
Entacapone	96.68 ± 0.1991	0.5042
Day 2	**Analyst-2, Instrument-2 & Column-2**	
Levodopa	99.63 ± 0.1116	0.2743
Carbidopa	98.75 ± 0.1785	0.4426
Entacapone	95.67 ± 0.1202	0.77

CONCLUSION

The method described enables to the quantification of levodopa, carbidopa and entacapone in film-coated tablets. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility.

ACKNOWLEDGEMENTS

The authors are thankful to Zydus Cadila Healthcare Limited, Ahmedabad, India for providing reference standards and all facilities to complete this research work.

REFERENCES

1. Calne D. A definition of Parkinson's disease. In: Parkinsonism.Relat Disord 2005;11:S39-40.
2. David G.S., Anne B.Y. Treatment of Central Nervous System Degenerative Disorders. In: Joet G Hardman, LeeE. Limbard. The Pharmacological Basis of Therapeutics. 10th ed. New York, Mc Graw Hill, 2001; p.552-560.
3. Rajput A., Rajput A.H, Parkinson's disease management strategies. Expert Rev Neurother. 2000; 6(1): 91-96.
4. John H. Parkinson's disease: New Treatments Slow Onslaught of Symptoms. FDA Consumer. 1998; 32: 34.
5. Castro A., Valldeoriola F., Linazasoro G., Rodriguez-Oroz M.C., Stochi F., Marin C. Optimization of use of levodopa-carbidopa-entacapone combination. Neurologia. 2005; 20(4): 180-188.
6. Lyons K.E, Pahwa R. Conversion from sustained release carbidopa/levodopa to carbidopa/levodopa/entacapone (Stalevo) in Parkinson disease patients. Clin Neuropharmacol. 2006; 29(2): 73-76.
7. Brooks D.J, Agid Y., Eggert K., Widner H., Ostergaard K., Holopainem A. TC-INIT Study Group. Treatment of end-of-dose wearing-off in parkinsons's disease. Eur Neurol. 2005; 53(4): 197-202.
8. Anonymous.United States Pharmacopoeia.Asian. Toronto: Webcom Limited. 2006; 377-378.