ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CARBIDOPA, LEVODOPA AND ENTACAPONE IN ITS BULK AND TABLET DOSAGE FORM BY UPLC

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ABSTRACT

The study was conducted with the purpose to method develop and validate a Ultra Performance Liquid Chromatography method UV detector to determine Levodopa, Carbidopa and Entacapone content in table preparation using MeOH : Acetonitrile : phosphate buffer (pH 2.8, 30:40:30 v/v/v). Column is BEH C₁₈ (2.1 x 100 mm, 5 µm), flow rate 0.3 ml per minute and run time is 4 minutes. The method was validated toward parameters of accuracy, precision, linearity, LOD, LOQ, flow rate, robustness of the Levodopa, Carbidopa, and Entacapone tablet in the form of LOD respectively 0.0060, 0.0064, and 0.35 and LOQ respectively 1.30, 1.44 and 1.96

Keywords: UPLC, UV, MeOH, BEH, LOD, LOQ

INTRODUCTION

Combination of Levodopa, Carbidopa, and Entacapone are an anti-Parkinson drug. Levodopa is a Dopamine precursor and it affects brain dopaminergic system, Carbidopa is peripheral decarboxylase inhibitors, Entacapone is a selective, reversible catechol-o-methyl and these Carbidopa, levodopa and entacapone drugs are respectively soluble in dilute solutions of mineral acids, 1M HCl, acetone, Ultra performance liquid chromatography (UPLC) is often used to validate method of analysis by first optimizing various parameters. The optimization is important for the effectiveness and efficiency of instrument. The validation parameters reported in this paper are accuracy, precision, selectivity, detection limit, linearity, and robustness of validated for the estimation of Levodopa, Carbidopa, Entacapone standard as well as tablet dosage form.

Experimental

Ultra performance liquid chromatography equipped with Auto Sampler and PDA or UV detector type Acquity waters brand with Empower software; BEH (Ethylene Bridged Hybrid) C₁₈ (2.1 x 100 mm, 5 µm) and spectrophotometer UV-Vis [Shimadzu type 1601]. Levodopa, Carbidopa, and Entacapone tablet 200 mg from the factory sample phosphate buffer pH = 2.8 and 3.0 with degree of pro analysis reaction, methanol 99.99 % gradient grade for liquid 16 chromatography.

Preparation of Levodopa Standard Solution

About 10 mg Levodopa standard was weighed, then dissolved with methanol in flask of 110 ml up to the sign

limit, and homogenized to obtain concentration of Levodopa standard solution 1000 ppm.

Preparation of Carbidopa Standard Solution

About 10 mg Carbidopa standard was weighed, then dissolved with methanol in flask of 10 ml up to the sign limit, and homogenized to obtain concentration of Carbidopa standard solution 1000 ppm.

Preparation of Entacapone Standard Solution

About 10 mg Entacapone standard was weighed, then dissolved with methanol in flask of 10 ml up to the sign limit, and homogenized to obtain concentration of Entacapone standard solution 1000 ppm.

Preparation of Mobile Phase

Mix a mixture of above buffer 300 ml (30 %), 400 ml of Acetonitrile UPLC (40 %) and 300 ml of methanol UPLC (30 %) degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Accuracy

The method applied was the addition of sample standard and then analyzed. The difference of result was compared with added standard content as percentage of re-planning. The accuracy method is said to be good if the range of regain 98-102 % (Table 1). The recovery according to data was arranged with concentration 50 %, 100 % and 150 % each was replicated 5 times and the average obtained is 99.8 % - 98.65 % (Levodopa), 98.88 % - 100.8 % (Carbidopa) and 98.8 % 101.37 % (Entacapone).

Table 1: Accuracy of Test Result Method of Standard Additions of Levodopa, Carbidopa and Entacapone in the Sample (Conc 50 %, 100 %, 150 %) in Methanol Acetonitrile Phosphate Buffer Mobile Phase pH 2.8

S. No.	Drug	% Concentration	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1	Levodopa	50 %	261623	5	4.92	99.8 %	99.57 %
		100 %	541558	10	10.17	99.8 %	
		150 %	787629	15	14.80	98.65 %	
2	Carbidopa	50 %	82602	5	4.94	98.88 %	99.81 %
		100 %	168414	10	10.08	100.8 %	
		150 %	247145	15	14.79	98.62 %	
3	Entacapone	50 %	1484216	5	4.99	99.84 %	100.03 %
		100 %	2939922	10	9.89	98.8 %	
		150 %	4520858	15	15.20	101.37 %	

Precision

The test was done by examining the concentration 100 ppm each was replicated 5 times in method range. By using the result of standard dissolution in different concentration, the size of area can be known and peak height provided by UPLC

shows that the method used to determine Levodopa, Carbidopa and Entacapone content is precise since the Average Deviation Standard re-examination < 20 % as can be seen in Table 2.

Table 2: Precision of Test Result Method of Standard Additions of Levodopa, Carbidopa and Entacapone in Capsule Dosage Form

Injection	Area of Levodopa	Area of Carbidopa	Area of Entacapone
Injection-1	314786	38638	1649019
Injection-2	319029	39462	1703317
Injection-3	322118	38213	1723742
Injection-4	317893	39664	1731245
Injection-5	314258	38801	1708750
Average	317616.7	38955.5	1703214.7
Standard Deviation	3225.9	598.7	32306.4
% RSD	1.0	1.5	1.9

Linearity

Figure 1: Calibration curve by Levodopa by UPLC **Figure 2: Calibration curve by Carbidopa by UPLC**

Figure 3: Calibration curve by Entacapone by UPLC

Correlation Coefficient is 0.999. In order to determine linearity between concentration and size of area of Levodopa, Carbidopa and Entacapone (LCE), the solution series were prepared: 5, 10, 15, 20 and 25 ppm from LCE standard

solution 1000 ppm. The correlation value indicates that there is a good correlation between concentration and the size of area of Levodopa, Carbidopa and Entacapone (LCE) (Figure 1, 2 and 3).

Table 3: The test results assay of Levodopa, Carbidopa and Entacapone

Drug	No. of injections	Sample area	Standard area	Avg. sample area	Avg. Std area	Assay
Levodopa	Injection 1	291280	295310	290806	293757	98.80 %
	Injection 2	290885	292110			
	Injection 3	290254	293850			
Carbidopa	Injection 1	33562	33569	33674	33538	99.76 %
	Injection 2	33706	33432			
	Injection 3	33754	33612			
Entacapone	Injection 1	1554256	1524778	1552287	1518721	101.55 %
	Injection 2	1547440	1501216			
	Injection 3	1555166	1530170			

(A Concentration of 10 ppm) in Methanol Acetonitrile Phosphate Buffer (pH 2.8) [30 %:40 %:30 % v/v/v]

Figure 4: Standard Chromatogram for Carbidopa, Levodopa and Entacapone

Figure 5: Sample (tablet) Chromatogram for Carbidopa, Levodopa and Entacapone

RESULTS AND DISCUSSION

The result of Levodopa, Carbidopa and Entacapone content determination in tablet with several replications can be seen in Table 3. The results obtained for recovery at 50 %, 100 % and 150 % within the limits. The accuracy of Levodopa, Carbidopa and Entacapone mean recovery at 50 %, 100 % and 150 % respectively 99.57 %, 99.81 % and 100.3 % can be seen in Table 1. Hence method is accurate. The test of precision provides the repetitive value less than 2 % that is % RSD for each drug concentration tested 1.0 %, 1.5 % and 1.9 % can be seen in Table 2. The test of linearity provides equation line (linearity reports can be seen in Figure 1, 2 and 3) with the value of detection limit (LOD of Levodopa, Carbidopa and Entacapone respectively 0.006 ppm, 0.0064 ppm, and 0.35 ppm) and limit of quantity (LOQ of Levodopa, Carbidopa and Entacapone respectively 1.3 ppm, 0.0022 ppm, 0.019 ppm) can be seen in Table 3. The procedure of determining of Levodopa, Carbidopa and entacapone content is found I tablet preparation using UPLC Acquity waters brand with Empower software with Methanol : Acetonitrile : Phosphate buffer pH 2.8 with comparison 30:40:30 (v/v/v), speed flow 0.3 ml/min, using column BEH C₁₈ (2.1 x 100 mm, 5 µm) at room temperature 27°C and automatic 60 column pressure.

CONCLUSION

Validation levels of Levodopa, Carbidopa and Entacapone in tablet dosage form using 62 newly developed method is reliable and can be used routinely.

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REFERENCES

1. Ana Vemica, Biljana Janci C, Stojanovi C, Ivana Stamenkovi C and Elija Malenovic Chaotropic agents in liquid chromatographic method development for the simultaneous analysis of levodopa, carbidopa, entacapone and their impurities Journal of Pharmaceutical and Biomedical Analysis 2013; 77: 9-15. <http://dx.doi.org/10.1016/j.jpba.2013.01.007> PMID:23354116

2. Nadejda Soukhova, Zarema Kassymbek, Sanford Bradby, Amanda Martin Esker, Patricia White, Samir Wahab. Development of characterization methods for Entacapone in a pharmaceutical bulk, Journal of Pharmaceutical and Biomedical Analysis 2011; 54: 860-865. <http://dx.doi.org/10.1016/j.jpba.2010.10.019> PMID:21112716
3. Y Michotte, M Moors, D Deleu, P Herregodts and G Ebinger. Simultaneous determination of Levodopa, Carbidopa, 3-O-methyldopa and dopamine in plasma using high-performance liquid chromatography with electrochemical detection, Journal of Pharmaceutical and Biomedical Analysis 1987; 5: 659-664. [http://dx.doi.org/10.1016/0731-7085\(87\)80078-X](http://dx.doi.org/10.1016/0731-7085(87)80078-X)
4. Shu Fang Li, Hai Long Wu, Yong Jie Yu, Yuan Na Li, Jin Fang Nie, Hai Yan Fu, Ru Qin Yu. Quantitative analysis of levodopa, carbidopa and methyldopa in human plasma samples using HPLC-DAD combined with second-order calibration based on alternating trilinear decomposition algorithm, Talanta 2010; 81: 805-812. <http://dx.doi.org/10.1016/j.talanta.2010.01.019> PMID:20298857
5. F Bugamelli, C Marcheselli, E Barba, MA Raggi. Determination of l-dopa, carbidopa, 3-O-methyldopa and entacapone in human plasma by HPLC-ED, Journal of Pharmaceutical and Biomedical Analysis 2011; 54: 562-567. <http://dx.doi.org/10.1016/j.jpba.2010.09.042> PMID:21035976
6. Yadav Manish, Dixit Pramod, Trivedi Vikas, Gandhi Abhishek. Chromatographic separation of (E)- and (Z)-isomers of entacapone and their simultaneous quantitation in human plasma by LC-ESI-MS/MS Journal of Chromatography B 2009; (87): 533-540.
7. C Zapata Urzua, M Perez Ortiza, M Bravo, AC Olivieri, A Alvarez Lueje. Simultaneous voltammetric determination of levodopa, carbidopa and benserazide in pharmaceuticals using multivariate calibration Talanta 2010; 82: 962-968. <http://dx.doi.org/10.1016/j.talanta.2010.05.071> PMID:20678653
8. Hubert H Fernandez, Arvydas Vanagunas, Per Odin, Alberto J Espay, Robert A Hauser. Levodopa/carbidopa intestinal gel in advanced Parkinson's disease open-label study: Interim results Parkinsonism and Related Disorders 2012; (1): 1-7.
9. Paolo Solla, Antonino Cannas, Socrate Congia, Gianluca Floris, Rosa Aste, Paolo Tacconi, Maria Giovanna Marrosu. Levodopa / Carbidopa / entacapone-induced acute Pisa syndrome in a Parkinson's disease patient, Journal of the Neurological Sciences 2008; (275): 154-156. <http://dx.doi.org/10.1016/j.jns.2008.07.017> PMID:18814889
10. World Health Organization. Validation of analytical Procedures Used in The examination of Pharmaceutical Materials. WHO Technical Report Series No. 823; 2007.

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