Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 41 / Number 4 / December 2021 / pp. 221-227

Development of an HPLC method for the Simultaneous Determination of Levodopa and Carbidopa in Pharmaceutical Dosage Forms

Farmasötik Dozaj Formlarında Levodopa ve Karbidopa'nın Eşzamanlı Tayini için bir HPLC yönteminin geliştirilmesi

Osman A. O. Mohammed[1, 2]
ORCID:0000-0002-8188-8069
Shaza W. Shantier[1*]
ORCID:0000-0002-2543-0248
Imad O. Abureid[1]
ORCID:0000-0002-1381-1369
Elrasheed A. Gadkariem[1]
ORCID:0000-0002-7099-0068

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, Sudan, P.O.Box 1996

[2] National Drug Quality Control Laboratory, Khartoum, Sudan

Corresponding author:
Shaza W. Shantier
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, Sudan, P.O.Box 1996
E-mail: sshantier@yahoo.com

Received date : 08.10.2021
Accepted date : 24.11.2021

DOI: 10.52794/hujpharm.1006726

ABSTRACT

Multicomponent analysis has presented a great challenge in pharmaceutical quality control analysis. Thus, in the present work a selective, accurate and precise liquid chromatographic method (HPLC) has been developed for the simultaneous determination of levodopa and carbidopa in bulk and tablet form. Best resolution was achieved on a C_{18} column (250 mm×4.6 mm, 5 µm particle size) using a mixture of phosphate buffer pH 2.8 and acetonitrile (95: 5 v/v) as the mobile phase pumped at a flow rate of 1.0 mL/min. The constructed calibration curves were linear in the concentration range (25-250 µg/mL and 2.5 – 25.0 µg/ml for levodopa and carbidopa, respectively) with correlation coefficients close to 1.0. The accuracy and the precision of the developed method were very good (RSD < 2%). The validation of the proposed method was confirmed through the statistical comparison of the obtained data with those of the official USP method. The developed method proved to be accurate and valid for the simultaneous analysis of levodopa and carbidopa.

Keywords: Chromatography; HPLC; Levodopa; Carbidopa; Official method, Validation

ÖZET

Çok bileşenli analiz, farmasötik kalite kontrol analizinde büyük bir zorluk teşkil etmiştir. Bu nedenle, mevcut çalışmada, levodopa ve karbidopa'nın toplu ve tablet biçiminde eşzamanlı tayini için seçici, doğru ve kesin bir sıvı kromatografik yöntem (HPLC) geliştirilmiştir. En iyi çözünürlük, mobil faz 1 akış hızında pompalanırken fosfat tamponu pH 2.8 ve asetonitril (95: 5 v/v) karışımı kullanılarak bir C18 kolonunda (5 um partikül boyutu, 250 mm x 4.6 mm ID) elde edildi. ml/dak. Oluşturulan kalibrasyon eğrileri, 1.0'a yakın korelasyon katsayıları ile konsantrasyon aralığında (sırasıyla levodopa ve karbidopa için 25-250 µg/ml ve 2.5 – 25.0 µg/mL) doğrusaldı. Geliştirilen yöntemin doğruluğu ve kesinliği çok iyiydi (RSD < %2). Önerilen yöntemin geçerliliği, elde edilen verilerin resmi USP yöntemininkilerle istatistiksel olarak karşılaştırılması yoluyla doğrulandı. Geliştirilen yöntemin levodopa ve karbidopanın eş zamanlı analizi için doğru ve geçerli olduğu kanıtlanmıştır.

Anahtar Kelimeler: Kromatografi; HPLC; Levodopa; Karbidopa; Resmi yöntem, Doğrulama

1. Introduction

Levodopa, a naturally occurring amino acid, is the direct precursor to the neurotransmitter dopamine (Figures 1a). Levodopa's effects are mostly those of dopamine. Unlike dopamine, levodopa quickly penetrates the CNS and is used to treat diseases linked with dopamine depletion in the brain, such as Parkinson's disease.

Carbidopa (Figures 1b), is a peripheral dopa-decarboxylase inhibitor with little or no pharmacological activity when given alone in usual doses. It inhibits the peripheral decarboxylation of levodopa to dopamine, unlike levodopa, it does not cross the blood-brain barrier, and effective brain concentrations of dopamine are produced with lower doses of levodopa. The combination of levodopa and carbidopa is used in the treatment of Parkinson's disease [1]. Levodopa and carbidopa tablets are available in dose ratio of 1:4 or 1:10 carbidopa to levodopa.

Both the British Pharmacoepia (BP) and United States Pharmacoepia (USP) [2, 3] use reversed phase high performance liquid chromatographic method for the determination of the two drugs combination. Literature search revealed that various spectrophotometric and chromatographic methods also have been used for the analysis of the two drugs in combination [4-13].

In this study, we are reporting a simple and accurate high performance method using a relatively cheap mobile phase constituents for the simultaneous determination of levodopa and carbidopa with optimum resolution between the two analytes within a reasonable analysis time.

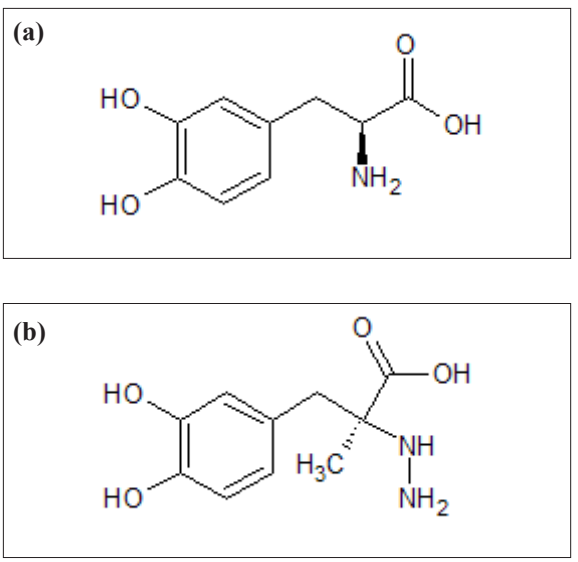

Figure 1. Chemical structure of (a) levodopa and (b) carbidopa

2. Materials and Methods

2.1. Instruments and apparatus

Experiments were performed on a Shimadzu Prominence HPLC system consisted of: degasser (Model DGU-20A5), pump (Model LC-20AD), Rheodyne manual injector fitted with 20 µl loop, variable wavelength UV–VIS detector (ModelSPD-20A). Chromatographic separations were carried out on an Intersustain® (250 mm×4.6 mm, 15 µm particle size) from GL Sciences Inc., Japan. A Shimadzu UV-1800 240V spectrophotometer.

2.2. Standard and samples

Levodopa (99.3%) and Carbidopa (100.2%) were kindly provided by National Medicines Quality Control Laboratory (NMQCL) - Sudan. Levocar® tablets (25/250 mg) and Credanil® tablets (25/250 mg) were purchased from the local market.

2.3. Chemicals and reagents

Chemicals

Sodium dihydrogen phosphate dehydrate, phosphoric acid (analytical grade), acetonitrile and sodium 1-decanesulfonate (SDS; HPLC grade) were purchased from Scharlau, Spain.

Diluent

Phosphoric acid (0.1M) was used as the diluent throughout the experimental work.

Phosphate buffer pH 2.8

Eleven grams of sodium dihydrogen phosphate were dissolved in 900 mLof distilled water, the pH was adjusted to 2.8 using phosphoric acid and the volume was made to one litre with distilled water.

2.4. Preparation of standards and solutions

Levodopa Standard stock solution

An accurately weighed about 125 mg of the working standard were transferred into a 100 mL volumetric

flask, 70 mL of the diluent were added and the solution was sonicated for 5 minutes. The volume was then completed to the mark with the same diluent (Solution A; 1.25 mg/mL).

Carbidopa standard stock solution

An accurately weighed about 125 mg carbidopa of the working standard were transferred into a 100 mL volumetric flask, 70 mL of the diluent was added and the solution was sonicated for 5 minutes. The volume was then completed to the mark with the same diluent (Solution B; 0.125 mg/mL).

Linearity standard solution

A five points calibration solution was prepared by transferring aliquot volumes (2-20 mL) from each stock standard solution (Solution A and B) into five separate 100 ml volumetric flasks; the volumes of the flasks were then made to mark with the diluent.

Assay standard solution

This solution was prepared by transferring 10 mL each of solution A and B into a 100 mL volumetric flask and making the volume to mark with the diluents.

Sample preparation

An amount of powdered tablets equivalent to 25 mg carbidopa and 250 mg levodopa was accurately weighed and transferred into 100 mL volumetric flask, 70 ml of the diluent were added and solution was sonicated for 15 minutes with occasional swirling. The volume was then completed to the mark with diluent and filtered through 0.45µ nylon filter (Solution C). 5 mL of solution C were diluted to 100 mL using the diluents.

2.5. Procedure

Determination of detection wavelength

Two milliliters of solution A and B were transferred to two separate 100 ml volumetric flasks and diluted to mark with the diluent. The solutions were then scanned in a UV spectrophotometer in the range 190-400 nm to determine the suitable detection wavelength of each analyte.

Method development and optimization

Mobile phases containing different proportions of acetonitrile: phosphate buffer pH 2.8 pumped at 1 mL/minute flow rate were tried. Standard mixture containing 0.25 mg/ml levodopa and 0.025 mg/ml carbidopa was injected every time.

2.6. Method validation

Linearity, precision, accuracy and robustness were investigated as method validation parameters. The compound peaks were identified by their retention times and compared with the standards. Quantification was performed with five point external calibration curves. Precision was determined as repeatability (three samples) and intermediate precision (three samples on a different day). Accuracy was determined by sample spiking and robustness was determined by intentionally varying the method parameters within small range.

Linearity

Triplicate 20 µL injections were made from each linearity standard solution were made. The calibration curve of each analyte was obtained by plotting its average peak area against its corresponding concentration. The regression analysis data (slope, intercept and correlation coefficient) were calculated, further the limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the regression analysis data according to the following formulae [14]:

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ = the standard deviation of the response, S = the slope of the calibration curve

Accuracy

To assess the accuracy of the method, recovery studies were carried out by spiking the sample with standard equivalent to 20%, 100%, and 200% of the nominal sample concentration ($n = 3$). The recovery% was then calculated using the following equation [15]:

$$\%\ recovery = (C_s - C_u)/C_A \times 100$$

Where

C_s =concentration of spiked samples.

C_u = concentration of unspiked samples.

C_A = concentration of analyte added to the test sample

Precision

The method's within the day precision was determined by analyzing three sample solutions containing different concentrations of the analytes in the range of (20 -200%) of the nominal sample concentration; prepared by proper dilution from Solution

ISSN: 2458 - 8806

C. Each sample was injected three times; the mean, standard deviation (SD) and the relative standard deviation (RSD) were calculated. To determine the between the days precision the procedure was repeated by a different analyst on a different day using a different instrument.

Robustness

Robustness was investigated by varying the following method critical parameters: pH (± 0.1), organic solvent ratio (± 1.0%), flow rate (± 0.1 mL/min), column supplier (different brand) and detection wavelength (± 5 nm).

3. Results and Discussion

Due to their structural similarity the two analytes showed very similar absorption features in the ultraviolet region (Figures 2a and 2b). The wavelength away from the stray light region and giving reasonable response for both analytes (280 nm) was selected as the chromatographic detection wavelength.

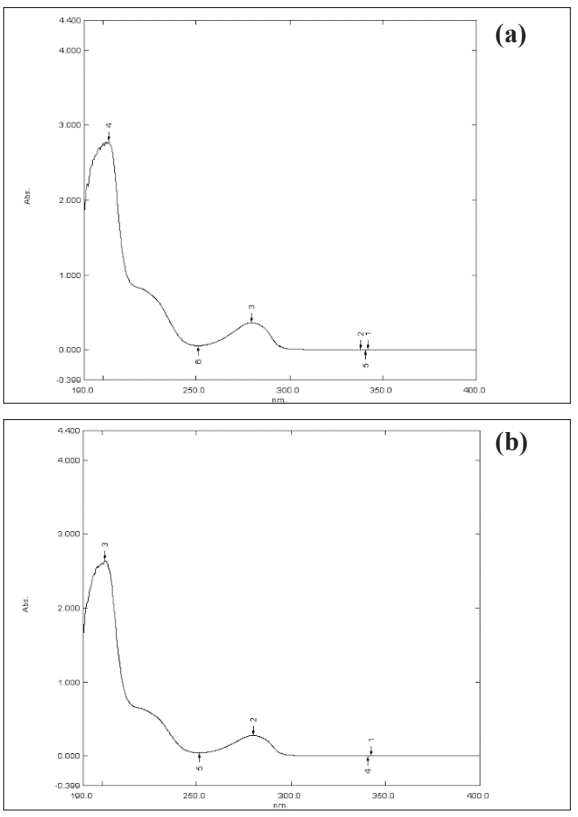

Figure 2. UV spectrum of (a) levodopa and (b) Carbidopa

3.1. Method development and optimization

The official USP method for the determination of levodopa and carbidopa combination in tablets uses a mobile phase consisting of pH 2.8 buffer and a small amount of sodium-1- decane sulfonate; (polarity index = 10.20), although the mobile phase flow rate was 2 mL/min still the elution of carbidopa was very much delayed (11.7 minutes for levodopa) leading to unnecessarily long analysis time. Additionally, various methods have been reported for the simultaneous determination of levodopa and carbidopa in pharmaceutical dosage forms. However, the present method was found to be superior on these methods in terms of simplicity, environmental friendly and cost-effectiveness. Therefore, a simple comparison has been conducted and summarized in Table 1 in order to support our findings and discuss the drawbacks of some reported methods.

Thus, the main objective of this study was to develop a simpler and cost-effective method in order to overcome the disadvantages of the previous methods, and to avoid using the ion-pairing reagent for its cost and deleterious effect on the column. Since the two molecules are zwitterionic and possess very similar chemical structure it is possible affect separation by suppressing the ionization of one functional group; mainly the carboxylic function by using mobile phase of acidic pH namely 2.8 and exploiting the ionization of the amino functions to derive the separation of the two analytes. Different volume ratios of acetonitrile and the phosphate buffer pH 2.8 were tried at a flow rate of 1 ml/min. The use of 30% acetonitrile in the mobile phase (polarity index = 8.88) resulted in completely overlapping peaks, with the reduction of acetontrile to 10% (polarity index = 9.76) the two peaks were almost separated; however carbidopa peak was broad and not symmetrical. Table 2 summarizes the results of the preliminary trials carried out to optimize the chromatographic conditions which gave satisfactory resolution of the two drugs when the proportion of acetonitrile in the mobile phase was 5% (polarity index = 9.98) as shown in Fig. 3.

3.2. Method validation

Linearity

The constructed calibration curves were linear over the concentration range 25-250 µg/mL and 2.5-25.0 µg/mL for levodopa and carbidopa, respectively with

Table 1. Comparison to the reported methods

Parameter Method	Column	Detector	Linear range (µg/mL)	Mobile phase	Retention time	Drawbacks
Kumar et al [4]	C18	UV detector at 282 nm	Levodopa (60-140), Carbidopa (6-14)	Phosphate buffer (pH 2.8), and methanol (20:80 v/v)	Levodopa 3.2, Carbidopa 4.3	not environmental friendly mobile phase
Sravanthi et al [5]	C18	UV detector at 220 nm	Levodopa (20-100), Carbidopa (10-50)	0.05%(v/v) o-phosphoric acid: acetonitrile (96:4%v/v)	Levodopa 4.2, Carbidopa 7.4	Mobile phase is an acidic solution, which is harmful to the column.
Raut et al [7]	C18	fluorescence detector at an excitation wavelength of 280 nm and an emission wavelength of 310 nm	0.055 – 0.5 for both	10mM potassium dihydrogen phosphate buffer, (pH 4.0) and methanol (90:10 v/v)	-	Complicated with a sophisticated detector
Nadendla and Abhinandana [8]	Phenomenex ODS	PDA detector	5-160 µg/ml	Acetonitrile and 0.1% ortho phosphoric acid (50:50 v/v)	-	High acid percentage in the mobile phase and complicated
Proposed method	C18	UV detector at 280 nm	Levodopa (25-250), Carbidopa (2.5-25)	Phosphate buffer pH 2.8 and acetonitrile (95: 5 v/v)	Levodopa 4.0, Carbidopa 6.2	-

Table 2. Results of optimization trials of the system

System	T_0	Levodopa				Carbidopa				Resolution
		RT	T. plate	T.F.	K_1	RT	T.plate	T.F.	K_2	
ACN: Buffer (70:30%)						Overlapped				
ACN: Buffer (30:70)						Overlapped				
ACN: Buffer (5:95%)	2.4	3.8	2966.8	1.01	0.58	6.3	10645.6	1.15	1.6	4.4
0.24g/l SDS in ACN: Buffer (5:95%)	2.4	3.8	3021.4	1.03	0.58	6.2	10622.4	1.15	1.6	4.3

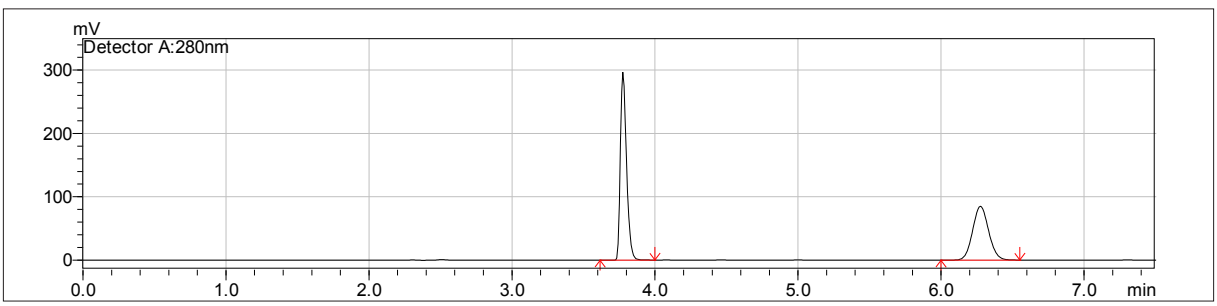

Figure 3. Typical chromatogram for drugs mixture using 5: 95 v/v acetonitrile: buffer solution (25:2.5µg/ml levodopa: carbidopa)

ISSN: 2458 - 8806

correlation coefficients close to 1.0 for both analytes, indicating the proportionality of their responses with the concentration. Table 3 summarizes the regression analysis data and the analytes LOD and LOQ.

Accuracy

The obtained high percentage recoveries (minimum 97.5% and maximum 100.4%) indicate the accuracy of the developed method and freedom from interference. The recovery data at three levels (20, 100 & 200 %) is presented in Table 4.

Precision

The method repeatability precision (within the day) was proved by the low RSD% values; 0.12 - 1.14% and 0.12- 0.44% for levodopa and carbidopa, respectively. Further the intermediate precision (between the days) performed with a different analyst on a different day using a different instrument resulted in RSD% values of 0.14 and 0.71 % for levodopa and carbidopa, respectively.

Robustness

Robustness was assessed by intentionally varying the studied parameters within small range. The obtained results showed that the developed method can withstand small deliberate changes without affecting the method's system suitability parameters.

3.3. Method application

The developed method was applied for the determination of content uniformity. Good assay results were obtained for levodopa and carbidopa (99.7 ± 0.2 and 99.9 ± 0.2; n=3, respectively).

Table 3. Regression analysis data for levodopa and carbidopa

Parameter	Levodopa	Carbidopa
Retention time (minutes)	3.9	6.2
Concentration range (µg/ml)	25-250	2.5-25.0
Slope± SE (*106)	15.3 ± 0.1	11.50±0.3
Intercept± SE (*103)	51.4±15.3	-3.2 ±1.9
Correlation coefficient (r2)	0.9998	0.9994
Limit of detection (µg/ml)	3.8	0.9
Limit of quantitation (µg/ml)	11.4	2.9

Table 4. Percentage recovery at three levels

Analyte	Percentage recovery mean ± SD; n = 3		
	20 %	100 %	200 %
Levodopa	100.4 ± 0.4	99.1 ± 0.1	98.6 ± 0.4
Carbidopa	97.5 ± 0.2	97.9 ± 0.1	99.8 ± 0.9

The validity of the method was also assessed by comparing the statistical results obtained with those of the official USP liquid chromatography method. As the calculated t- values were less than tabulated ones (n =4, P=0.05), the result of the developed method can be considered as accurate and precise as the official liquid chromatographic method (Table 5).

4. Conclusions

The developed method proved to be selective, accurate and precise for the analysis of levodopa and carbidopa in bulk and tablets form. The wide linearity range of the developed method allows the analysis of the marketed products of the two drugs having different dose ratios using one standard preparation. The method can also be used for the routine analysis of the drugs either in single or combination forms.

List of Abbreviations

HPLC: High performance liquid chromatography

BP: British Pharmacopeia

USP: United States Pharmacopeia

SDS: Sodium 1-decanesulfonate

ICH: International Conference of on Harmonization

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All dataset supporting this article is available within the article.

Competing Interests

The authors declare no conflict of interest.

Table 5. Results of the proposed method compared to the official method

		% content ± SD	t - calculated (t - tabulated)
Developed method	Carbidopa	99.90± 0.19	0.85 (2.78)
	Levodopa	99.70± 0.15	
USP method	Carbidopa	98.00±0.17	
	Levodopa	99.10±0.10	

Funding

The authors received no financial support and all the research was self-funded.

Authors' contributions

O. M. designed the methods, analyzed and interpret the data, wrote the manuscript draft; S.S supervising the research work, critically reviewed the manuscript and approved the final draft; I.A. reviewed the manuscript and approved the final draft; E.G. conceptualization, supervising the research work, critically reviewed the manuscript and approved the final draft. All the authors have read and approved the manuscript.

Acknowledgments

The authors acknowledge Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum and National Drug Quality Control Laboratory for the technical support.

References

1. Wisher D. (2012) Martindale: The complete drug reference. Journal of the Medical Library Association. 100 (1):75-7.
2. British Pharmacopeia (2017), volume III. London: stationary office. pp. 393-393.
3. The United States pharmacopeia convention, (2016) National formula (34th ed.), pp. 2925
4. Kumar SS, Natraj K, Khan A, Kumar BK, Rao J. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Levodopa and Carbidopa in Pharmaceutical Dosage Forms. Journal of Pharmacy Research Vol. 2011;4(11):4060-2.
5. Sravanthi D, Anusha M, Madhavi S, Firdose S, Nalluri BN. Simultaneous Estimation of Levodopa and Carbidopa in Bulk, Pharmaceutical Dosage Forms and Dissolution Sample Analysis by RP-HPLC-PDA Method. Journal o f Chemical and Pharmaceutical Research. 2013;5:422-8.
6. Elbashir AA, Basheir BEA, Aboul-Enein HY. Development and Validation of Spectrophotometric Method for the Determination of Levodopa (L-Dopa) In Pharmaceutical Formulations. 2016.
7. Raut PP, Charde SY. Simultaneous estimation of levodopa and carbidopa by RPHPLC using a fluorescence detector: its application to a pharmaceutical dosage form. Luminescence. 2014;29(7):762-71.
8. Nadendla R, Abhinandana P. Anti- Parkinsonian Drug Estimation by RP-HPLC, Journal of Pharmaceutical Research International, 2021, 33 (2): 14-25
9. Patel M. Development and Validation of Simultaneous Estimation of Two Catecholamines in Combined Dosage Form by HPTLC Method. Asian Journal of Pharmaceutical Analysis. 2014;4(2):57-77.
10. Khanmohammadi M, Mobedi E, Garmarudi AB, Mobedi H, Kargosha K. Simultaneous determination of levodopa and carbidopa in levodopa-carbidopa tablets by ATR-FTIR spectrometry. Pharmaceutical development and technology. 2007;12(6):573-80.
11. Sima IA, Casoni D, Sârbu C. Simultaneous determination of carbidopa and levodopa using a new TLC method and a free radical as detection reagent. Journal of Liquid Chromatography & Related Technologies. 2013;36(17):2395-404.
12. Miller R, Dehelean L, Belanger L. Determination of carbidopa and levodopa in human plasma by high-performance liquid chromatography with electrochemical detection. Chromatographia. 1993;35(9):607-12.
13. Madrakian T, Afkhami A, Borazjani M, Bahram M. Simultaneous derivative spectrophotometric determination of levodopa and carbidopa in pharmaceutical preparations. Bulletin of the Korean Chemical Society. 2004;25(12):1764-8.
14. ICH G. Validation of analytical procedures: text and methodology. 2005, Q2 (R1).1.
15. Elimam MM, Shantier SW, GadkariemEA, Mohamed MA. Derivative Spectrophotometric Methods for the Analysis and Stability Studies of Colistin Sulphate Journal of Chemistry, 2015; Article ID 624316, 5

ISSN: 2458 - 8806