ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2015, *60*(5-6), 555-561

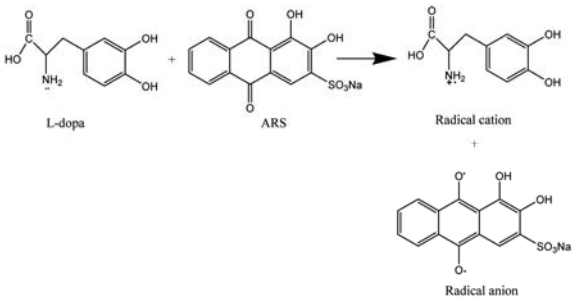
DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF LEVODOPA (L-dopa) IN PHARMACEUTICAL FORMULATIONS

Banan Elshiekh Alsied BASHEIR ,[a] Abdalla A. ELBASHIR[a,*] and Hassan Y. ABOUL-ENEIN[b,*]

[a]University of Khartoum, Faculty of Science, Chemistry Department, Khartoum, Sudan
[b] Pharmaceutical and Medicinal Chemistry Department, Pharmaceuticals and Drug Industries Research Division, National Research Centre, Dokki, Giza 12622, Egypt

Received October 15, 2014

An accurate, simple and fast spectrophotometric method has been developed for the determination of L-dopa in bulk drugs. This method is based on the reaction of Alizarin red with primary amino group present in the L-dopa in basic medium. This reaction produces a purple color product which absorbs maximally at about 588 nm. Beer's law was obeyed in the range of 10-60 µg/mL with good regression value 0.9985. The effects of variables such as temperature, heating time, concentration of color producing reagent and pH was investigated to optimize the procedure. The results are validated statistically. The value of LOD and LOQ was found to be 2.4 µg/mL and 7.3µg/mL for L-dopa that indicates good sensitivity of proposed method.



INTRODUCTION

L-dopa (L-3, 4-dihydroxyphenylalanine) is a chemical that is biosynthesized by humans, and some animal from the amino acid L-tyrosine. L-Dopa is used in the treatment of Parkinson's disease and dopamine-responsive dystonia.[1]

Parkinson's disease is one of the most difficult medical condition. The cause of this disease is a significant depletion of dopamine due to the death of neurons which can produce dopamine in brain. It leads to tremor, muscle stiffness, bradykinesia. Levodopa is a precursor of dopamine which is an important neurotransmitter which is used for the medication of neural disorders such as Parkinson's disease. After administration, levodopa is converted into dopamine through enzymatic reaction catalyzed by dopadecarboxylase.[2,3]

A number of analytical methods have been reported for the analysis of L-dopa in pharmaceutical formulation including LC-MS-MS,[4] chemiluminescence,[5] voltammetery,[6] HPTLC[7] using a modified carbon nanotube paste[8] and spectrophotometry.[9-12]

Spectrophotometry is probably the most convenient analytical technique for routine analysis because of its inherent simplicity, low cost and wide availability in quality control laboratories.

Alizarin Red sulphonate (ARS) has been used as a color-developing reagent in the spectrophotometric determination of metal ions[13-16] and pharmaceutical amines.[17-25] The reaction between ARS and L-dopa has not been investigated yet. Therefore, the present study is devoted to investigate the reaction between ARS

* Corresponding authors: haboulenein@yahoo.com; tel.:+00201003678948; fax: +0020233370931 and hajaae@yahoo.com, aaelbashir@uofk.edu

and L-dopa, and use this color reaction in the development of simple rapid spectrophotometric method for determination of L-dopa in its dosage form.

EXPERIMENTAL

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 (Tokyo, Japan) with 1 cm matched quartz cell was used for the absorbance measurements. Other instruments used were: Water bath (Lab. Companion, BS – 11, Dorval, Canada); Electronic balance (Sartorius AG GÖTTINGEN B2 2105 Gerrmany); pH – Metter (PW- 9421, Giessen, Germany) .

Reagents

Alizarin Red Sulphonate (ARS) was supplied by Hopkin & Williams LTD (Essex, England).

Preparation of reagents and solutions

Stock standard solution of L-dopa

An accurately weighed amount 0.01 g of L-dopa was dissolved in 15 mL water then transferred into a 50 mL volumetric flask, completed to the mark with ethanol (ratio of water to ethanol was 30:70 v/v) concentration of 0.2 mg/mL was obtained.

Standard solution of Alizarin Red Sulphonate (ARS)

An accurately weighed amount (0.2401 g) of ARS was dissolved in 30:70 v/v water and ethanol, transferred into a 100 mL standard flask, completed to the mark with the same solvent to obtain a solution of 10^{-3} mol/L. Ten mL of this solution was transferred into 25 mL volumetric flask and it was completed to the mark with the solvent (water-ethanol 30:70v/v). The solution was freshly prepared and protected from light during use.

Sample solution

Ten tablets were weighed accurately and pulverized. Then an accurately weighed amount equivalent to 0.01 g was dissolved in 7.5 mL water then transferred into a 25 mL volumetric flask, completed to the mark with aqueous ethanol (ratio of water to ethanol was 30:70v/v). The prepared solution was diluted quantitatively to obtain a suitable concentration for the analysis, and then the general procedure was followed as described below.

Assay procedures

Aliquots of solution were added to 10 mL volumetric flasks to give final concentrations of 10-60 µg/mL. Buffer solution (pH 9.0, 2.0 mL) was added followed by 2mL ARS solution ($4x10^{-4}$). The reaction was allowed to proceed at 70 ^{o}C for 20 min after which the reaction mixture was made up to the mark with water and the absorbance measured at 588 nm against a blank similarly prepared but without the analyte.

Job's method

The Job's method of continuous variation[26] was employed. Master equimolar (4×10^{-4} M) aqueous solution of L-dopa and ARS were prepared in the same manner of the standard solution. Series of 15ml portions of the master solution of L-dopa and ARS were made up comprising different complementary proportions (1:9,…9:1, inclusive) in 10 mL volumetric flask and 2.0 mL of buffer solution (pH 9.0) were added. The solution was further manipulated as described under the general recommended procedure.

RESULTS AND DISCUSSION

Alizarin Red S (ARS) has been used as a color-developing reagent in the spectrophotometric determination of pharmaceutical amines.[17-25] The reaction of L-dopa with ARS results in the formation of a charge transfer complex of the *n*–π - type. This compound is considered to be an intermediate molecular association complex which dissociates in the corresponding radical anions in ethanolic solvent. The radical anion (absorbing species) absorbs at 588 nm as shown in Fig. 1.

Optimization of the experimental conditions

Effect of pH

The effect of pH was studied by forming the colored product in the presence of different pH. The absorbance of the proton transfer product was measured. Fig. 2 shows poor absorbance at pH 7.0 and 8.0 then a high absorbance occurs at pH 9.0 and then decrease at a higher pH. Thus pH 9 was chosen as the suitable pH to attain maximum absorbance.

Effect of ARS concentration

The reaction depends on the ARS concentration. The optimum concentration that gives maximum color formation was 2.0 mL of solution (4.0×10^{-4} M) ARS solution was found to be sufficient for the production of maximum and reproducible color intensity. At higher concentrations of the reagent, the absorbance of the product was observed to decrease as shown in Fig. 3.

Effect of reaction time

By following the reaction for various lengths of time, it was found that the reaction went to completion after 20 min and a longer reaction time was not necessary (Fig. 4).

Effect of temperature

The effect of temperature on the reaction was studied by varying the temperature from 30°C to 90°C. The highest absorbance is obtained at 70°C (Fig. 5).

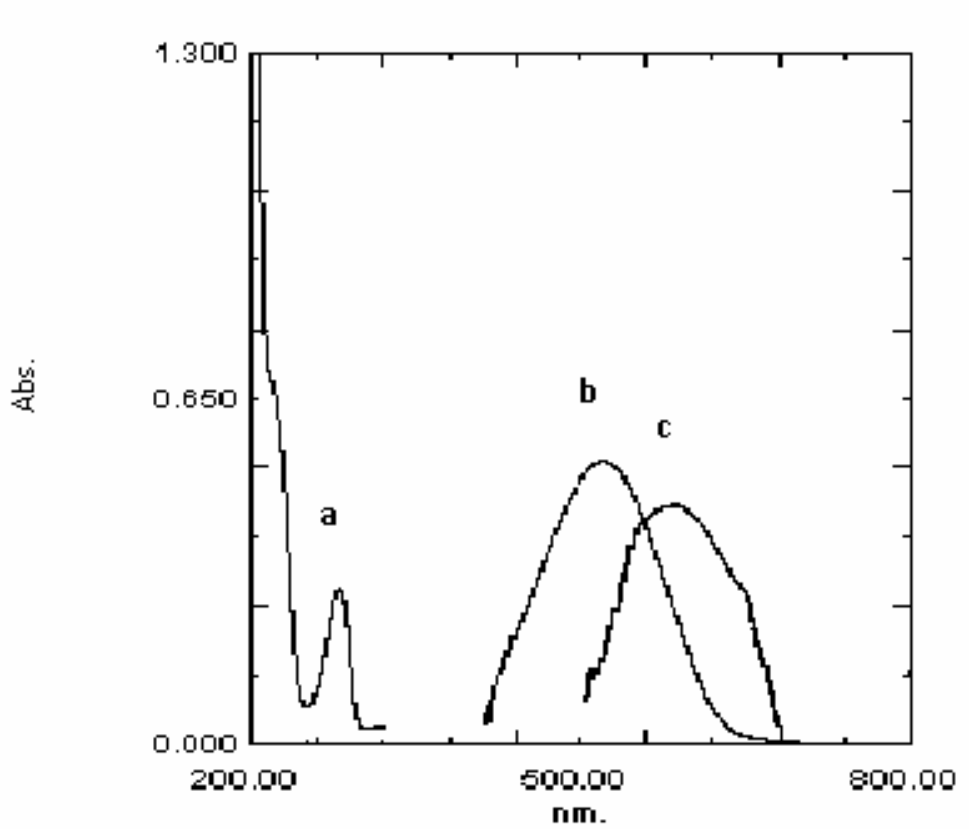


Fig. 1 – (a) Absorption spectrum of L-dopa (20μg/mL) in water. (b) Absorption spectrum of alizarin red ARS (8μM) in water. (c) Absorption spectrum of reaction of L-dopa (30 μg/mL) with alizarin red.

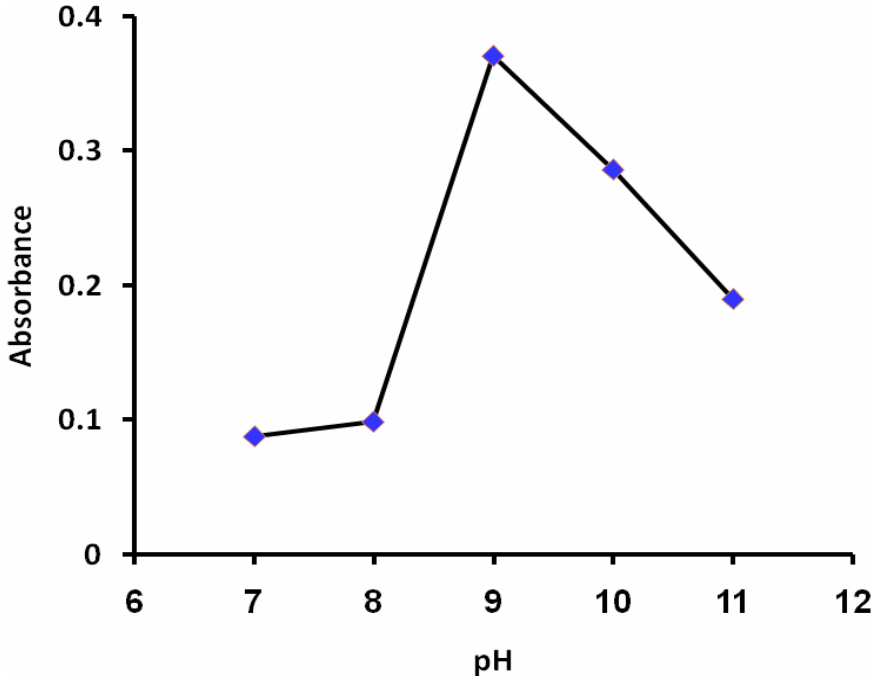


Fig. 2 – Effect of pH on the reaction of l-dopa with alizarin red. L-dopa (20 μg/mL): 1.0 mL;
buffer solution of different pH values: 2 mL; alizarin red (0.0004M): 2.0 mL; temperature: 50 °C; reaction time: 30 min.

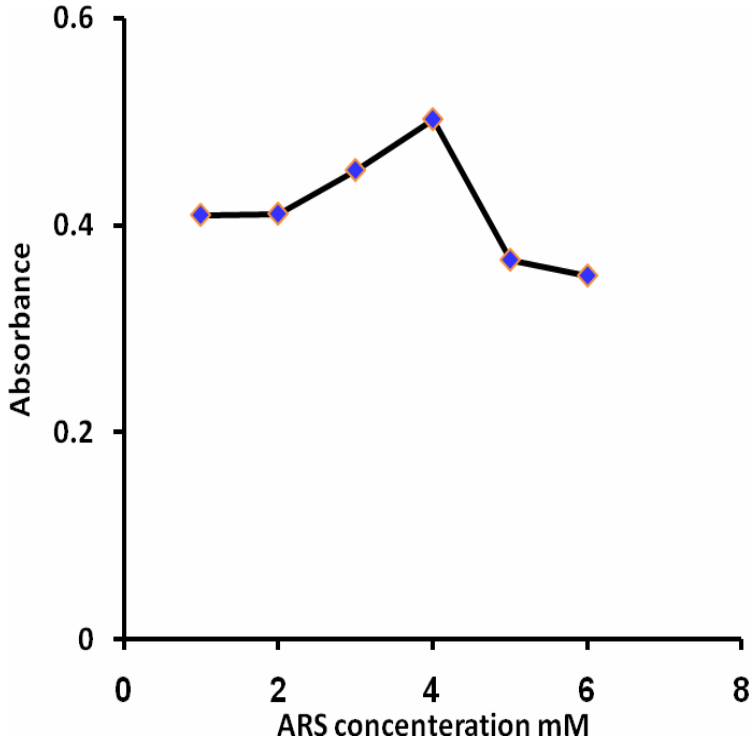


Fig. 3 – Effect of alizarin red concentrations on the reaction of L-dopa with alizarin red, L-dopa (20 µg/mL): 1.0 mL; alizarin red: 2.0 mL; buffer solution (pH 9): 2.0 mL; temperature: 50 °C; reaction time: 30 min.

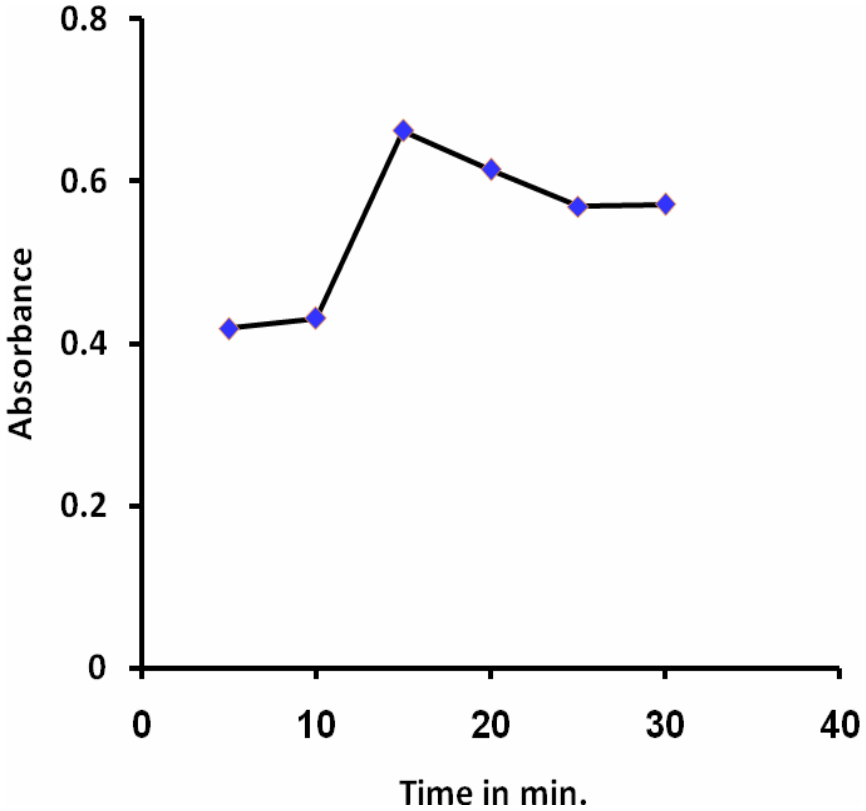


Fig. 4 – Effect of standing time on the reaction of L-dopa with alizarin red. L-dopa (20 µg/mL); alizarin red (0.0004M) 2 mL; buffer (pH 9.0): 2 mL; temperature 50 °C.

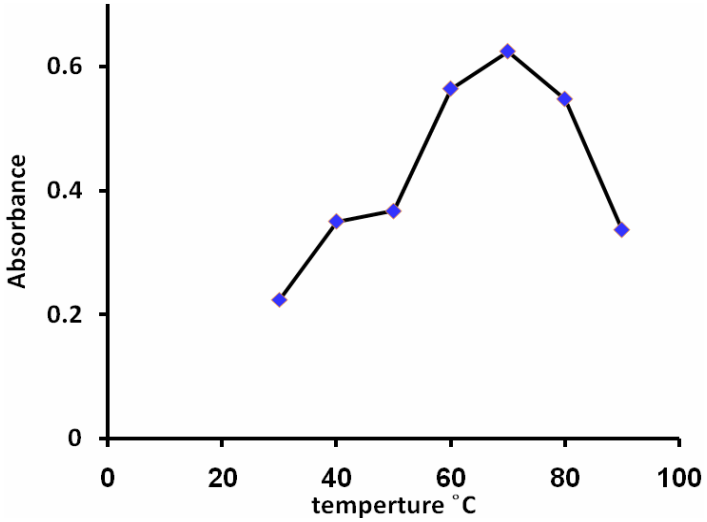
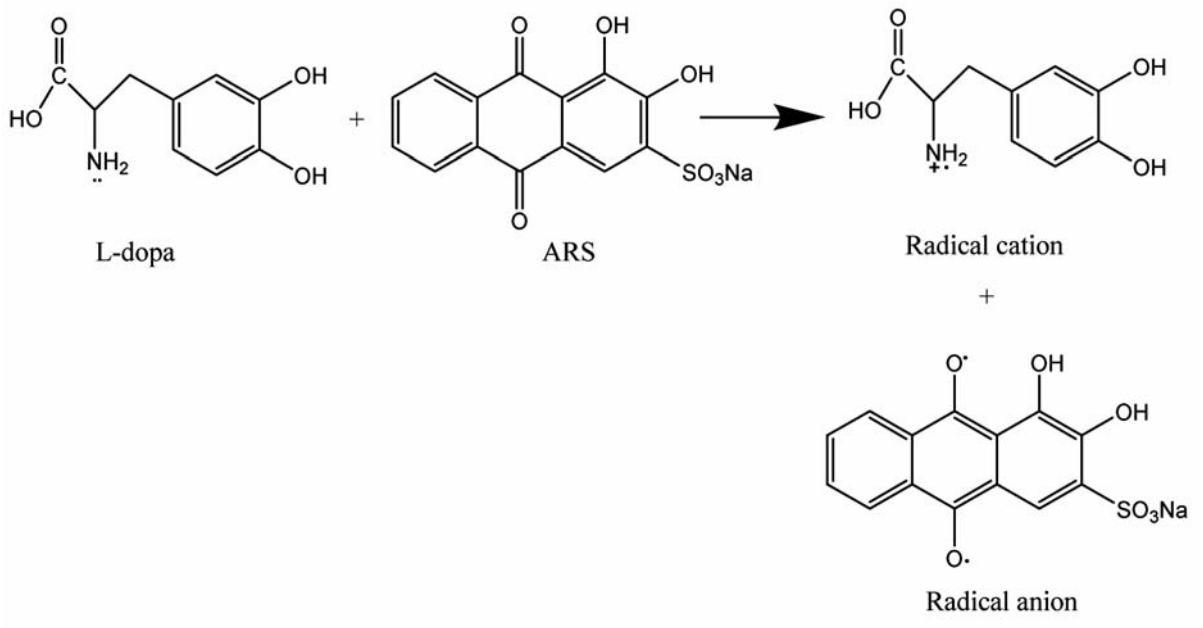


Fig. 5 – Effect of temperature on the reaction of L-dopa with alizarin red. L-dopa (20 µg/mL): 1.0 mL; alizarin red (0 .0004 M): 2.0 mL; buffer solution (pH 9.0): 2.0 mL; reaction time: 20 min.



Scheme 1– Proposed reaction pathway between L-dopa and ARS.

Stoichiometry of the reaction (Job's method)

Under the optimum conditions the stoichiometric ratio between L-dopa and the investigated reagent ARS was found to be 1:1. Based on this ratio, the reaction pathway was postulated to be proceeded as shown in Scheme 1.

Validation of the proposed method

Linearity, detection, and quantitation limits

Following the proposed experimental conditions, the relationship between the absorbance and concentration was quite linear in the concentration ranges given in (Table 1). The intercept (a), slope (b), correlation coefficient (r), and molar absorptivity (ε), values are summarized in Table 1. The limit of detection (LOD) is defined as the minimum level at which the analyte can be reliably detected.[27,28] The LOD was 2.4 µg/mL as shown in Table 1.

The limit of quantitation (LOQ), is defined as the lowest concentration that can be measured with acceptable accuracy and precision.[27, 28] The limit of quantitation was 7.3µg /ml as shown in Table 1.

Table 1

Parameters for the performance of the proposed method

Parameter	Results
λ_{max}, nm (Drug)	280
λ_{max}, nm(product)	588
Beer's law limits, µg/mL	10-60
Molar absorptivity, l/mol cm	$3.187*10^3$
Limit of detection, µg/mL	2.4
Limit of quantification, µg/mL	7.3
Regression equation, Y*:	
Intercept (a)	0.1198
Standard deviation of intercept	0.008157
Slope (b)	0.011163
Standard deviation of slope	0.000209
Correlation coefficient (r^2)	0.998594
Standard deviation	0.008762

Table 2

Precision results for the proposed method

Concentration added µg/mL	Concentration found µg/mL	%±RSD
20	18.5	92.5±0.3
30	29.45	98.16±0.84
40	40.38	100.95±1.08

Table 3

Recovery of the proposed method

Sample No.	Sample contact µg/mL	Added µg/mL	Found µg/mL	%±SD
1	10	10	19.3	96.5±0.016
2	10	15	25.3	101.2±.041
3	10	20	27.63	92.1±0.0136

Accuracy and precision

The accuracy and precision of the methods were evaluated by performing six replicate analyses on pure drug solution at four different concentration levels (within the working range). Relative standard deviation (RSD %) as accuracy of the proposed spectrophotometric method was calculated. The relative standard deviation (RSD) values were less than 2% in all cases, indicating good repeatability of the proposed method (Table 2).

Recovery studies

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure L-dopa at different levels and the total was determined by the proposed methods using standard addition technique. The percent recovery of pure L-dopa added was in the range 92.10–101.2% with standard deviation of 0.041-0.0136, indicating good recoveries, and that the co-formulated substances and common excipients did not interfere in the determination Table 3.

Robustness

Robustness of the procedures was assessed by evaluating the influence of small variation of experimental variables, i.e., concentrations of reagent and reaction time, on the analytical performance of the methods. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results. This provided an indication of the reliability of the proposed method during routine work Table 4.

Applications of the method

The proposed method was applied for the analysis of L-dopa in pharmaceutical formulations. The results indicate the high accuracy of the proposed method and the recovery was 99.7±0.4 (value is means of five determinations). The proposed method has the advantage of being virtually free from interferences by excipients.

Table 4

Robustness of the proposed method

Parameter	Recovery% ± RSD*
pH	
8.8	100.6±0.01
9.2	100.1±0.01
Temperature (˚C)	
68	95.5±0.40
72	99.2±0.03
Time (min)	
18	95.9± 3.1
22	100.9± 0.21
Alizarin red S concentration M	
0.0038	96.69±0.57
0.0042	100.4±0.05

CONCLUSIONS

The described spectrometric method for the determination of L-dopa in pharmaceutical formulation is simple, sensitive, rapid and accurate. The method is reliable and efficient for routine application in quality control laboratories for analysis of L-dopa.

REFERENCES

1. W. C. Bowman and M. J. Rand, "Text book of pharmacology", 2nd edition, Blackwell, Cambridge, UK, 1980.
2. M. Karimi, J. L. Carl, S. Loftin and J. S. Perlmutter, *Chromatogr., B: Anal. Technol. Biomed. Life Sci.,* 2006, *836*, 120.
3. T. H. Kim, K. H. Cho, W.S. Jung and M. S. Lee, *PLoS ONE* , **2012**, *7*, 35695.
4. L. Lv, W. Jiang, S. Zhou, S. Huang, X. Shi, X. Lv, L. Wu and C. Xu, *Chromatographia*, **2010**, *72*, 239.
5. S. M. Alam, M. M. Karim, S. H. Lee, S. M. Wabaidur, H. Y. Chung, J. H. Choi and M. Kang**,** *Luminescence*, **2008**, *23*, 327.
6. M. F. S. Teixeira, M. F. Bergamini, C. M. P. Marques and N. Bocchi, *Talanta,* **2004**, *63*, 1083.
7. K. P. Modi, N. M. Patel and R. K. Goyal, *Chem Pharm Bull.*, **2008**, *56*, 357.
8. H. M. Moghaddam, *Int. Electrochem Sci.,* **2011,** *6* , 6557.
9. D. Pecanac, K. Karljikovic-Rajic and D. Radulovic, *Anal. Lett*, **1997**, *30*, 1833.
10. E. S. Abu-Nameh, M. I. Helaleh and S. A. Nabi*, Acta Pol Pharm.*, **1997**, *54*, 347.
11. A. Afkhami, D. Nematollahi and H. A. Khatami, *Asian J. Chem*., **2002**, *14*, 333.
12. A. Safavi and M. Tohidi, *J. Pharm. Biomed. Anal*., **2007,** *44*, 313.
13. M. Alkan, M. Karun and F. Chmilenko, *Talanta*, **2003**, *59*, 605.
14. J. Hernandez-Mendez, R. Carabias-Martinez, B. Moreno-Cordero and L. Gutierrez-Davila, *Anal. Chim. Acta*, **2008**, *149*, 379.
15. H. A. Panahi, M. Karimi, E. Moniri and H. Soudi, *Afric. J. Pure Appl. Chem*., **2008**, *2,* 96.
16. A. Abbaspour and L. Baramakeh, *Talanta*, **2002**, *57*, 807.
17. K. Basavaiah, S. Latha and J. M. Swamy, *Talanta*, **1999**, *50*, 887.
18. K. Srikanth, K. A. Emmanuel and R. K. Raju, *Rasayan J. Chem.*, **2010**, *3*, 179.
19. M. Kishore, Y. H. Rao and M. Janardhan, *Int. J. Pharm. Sci. Res*., **2010,** *1*, 438.
20. K. Farhadi, A. K. Savojbolaghi and R. Maleki, *J. Chinese. Chem. Soc*., **2003**, *50*, 153.
21. T. S. Al-Ghabsha and A. M. S. Al-Delymi, *J. Edu. & Sci.,* **2008**, *21*, 62.
22. A. A. Gouda, R. El Sheikh and R.M. El-Azzazy, *Anal. Bioanal. Tech.*, **2012**, *3*, 6.
23. A.A. Elbashir and F.A.A. Abdalla, *Amer. Acad. Scholarly Res.J.,* **2013**, *5*, 22-31.
24. W. S. Hassan, M. M. El-Henawee and A. A. Gouda, *Spectrochim Acta. Part A*., **2008**, *69*, 245.
25. A. A. Elbashir and F. A. A. Abdalla*, Med Chem.*, **2014**, *4*, 361.
26. P. Job, "Advance physicochemical experiment", 2nd edition, Edinburgh, UK, Oliner and Boyd, 1964, p.54, 27. Topic Q2 (R1). Validation of analytical procedures: Text and methodology. International Conference on Harmonization (ICH), **2005**.
28. ICH-Q2A guideline for industry March, Text on validation of analytical procedures, 1995.