International Journal of
Science Inventions Today
www.ijsit.com
ISSN 2319-5436
Research Article

DEVELOPMENT OF NEW ROBUST RP-HPLC METHOD FOR ANALYSIS OF LEVO DOPA IN FORMULATIONS

N. Bujji babu, P. Srinivasa rao and R. Ramesh raju

Department of Chemistry, Acharya Nagarjuna University, Guntur, A. P., India

ABSTRACT

HPLC method was developed for the analysis of Levo Dopa in formulations. In this method we focused on easy and more accuracy. A mixture of Acetonitrile: Methanol: orthophosphoric acid in the ratio of 30:50:20, v/v/v was prepared and used as mobile phase. The mobile phase is 6.8. The wave length of U.V detector 280 nm. We obtained a good resolution peak 2.4 min. The linearity of method is 10-25 ppm. The recovery range of method is 98.6-99.85.

Keywords: Levo Dopa, HPLC Mehtod, accuracy, Mobile phase, Recovery**,** Column.

INTRODUCTION

L-DOPA (L-3,4-dihydroxyphenylalanine)[1] is a chemical that is made and used as part of the normal biology of some animals and plants. Some animals including humans make it via biosynthesis from the amino acid L-tyrosine. L-DOPA is the precursor to the neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline) collectively known as catecholamines. L-DOPA can be manufactured and in its pure form is sold as a psychoactive drug with the INN levodopa; trade names include Sinemet, Parcopa, Atamet, Stalevo, Madopar, Prolopa, etc.). As a drug it is used in the clinical treatment of Parkinson's disease and dopamine-responsive dystonia[2]

L-DOPA crosses the protective blood-brain barrier, whereas dopamine itself cannot. Thus, L-DOPA is used to increase dopamine concentrations in the treatment of Parkinson's disease and dopamine-responsive dystonia. This treatment was originally developed by George Cotzias and his coworkers. Once L-DOPA has entered the central nervous system, it is converted into dopamine by the enzyme aromatic L-amino acid decarboxylase, also known as DOPA decarboxylase (DDC)[3]. Pyridoxal phosphate (vitamin B6) is a required cofactor in this reaction, and may occasionally be administered along with L-DOPA, usually in the form of pyridoxine[4].

Besides the CNS, L-DOPA is also converted into dopamine from within the peripheral nervous system. The resulting hyperdopaminergia causes many of the adverse side effects seen with sole L-DOPA administration[5]. In order to bypass these effects, it is standard clinical practice to co-administer (with L-DOPA) a peripheral DOPA decarboxylase inhibitor (DDCI) such as carbidopa (medicines combining L-DOPA and carbidopa are branded as Lodosyn, Sinemet, Parcopa, Atamet, Stalevo) or with a benserazide (combination medicines are branded Madopar, Prolopa), to prevent the peripheral synthesis of dopamine from L-DOPA[6]. Co-administration of pyridoxine without a DDCI accelerates the peripheral decarboxylation of L-DOPA to such an extent that it negates the effects of L-DOPA administration, a phenomenon that historically caused great confusion[7]

Side effects:

The side effects of L-DOPA may include:

- Hypotension, especially if the dosage is too high
- Arrhythmias, although these are uncommon
- Nausea, which is often reduced by taking the drug with food, although protein interferes with drug absorption
- Gastrointestinal bleeding
- Disturbed respiration, which is not always harmful, and can actually benefit patients with upper airway obstruction
- Hair loss
- Disorientation and confusion

- Extreme emotional states, particularly anxiety, but also excessive libido
- Vivid dreams and/or insomnia
- Auditory and/or visual hallucinations
- Effects on learning; there is some evidence that it improves working memory, while impairing other complex functions
- Somnolence and narcolepsy
- A condition similar to stimulant psychosis

Although there are many adverse effects associated with L-DOPA[8], in particular psychiatric ones, it has fewer than other antiparkinsonian agents, such as anticholinergics and dopamine receptor agonists

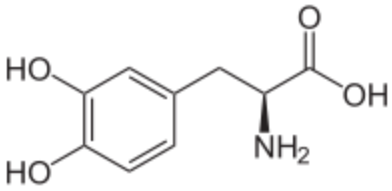

Figure 1

Structure of levo dopa:

IUPAC Name : (*S*)-2-amino-3-(3,4-dihydroxyphenyl) propanoic acid

Formula : $C_9H_{11}NO_4$

Molecular Weight : 197.19 g/mol

Routes : Oral

List of brand names of Levo dopa:

S.No	Brand Name	Formulation	Available Strength (Mg)
1	Syndopa	Tablet	275
2	Gemdopa	Tablet	110
3	Tidomet ferte	Tablet	250

Table 1

EXPERIMENTAL

Instrumentation:

To develop a liquid chromatographic method for quantitative estimation of Levo dopa using an isocratic peak HPLC instrument on a Chromosil C18 column (250 mm x 4.6 mm, 5µ). The instrument is equipped with a LC 20AT pump and variable wavelength programmable UV-Visible detector, SPD-10AVP. A 20µL Hamilton syringe was used for injecting the samples. Data was analyzed by using peak software. Techcomp UV-2301 UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Denver SI234 balance was used for weighing the materials.

Chemicals and Solvents:

The reference sample of Levo dopa (API) was obtained from Dr. Reddy's Laboratory, Hyderabad. The Formulation was procured from the local market. Acetonitrile, Methanol used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India. is orthophosphoric acid AR grade purchased from local market.

The mobile phase:

A mixture of Acetonitrile: Methanol: orthophosphoric acid in the ratio of 30:50:20, v/v/v was prepared and used as mobile phase. Orthophosphoric acid used to adjust the pH to 6.8 and filtered through 0.45µ nylon filter.

Standard solution of the drug:

For analysis 100ppm standard solution was prepared, required concentrations were obtained from 100ppm solution by proper dilution.

Sample (tablet) solution:

The formulation tablets of Levo dopa (Tidomet ferte 250 mg) were crushed to give finely powdered material. With Powder we prepared 14ppm solution in mobile phase and then filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper.

METHOD DEVELOPMENT

For developing the method, [27-32] a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

Detection wavelength:

The spectrum of diluted solutions of the Levo dopa in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of the both Levo dopa

were showed that a wavelength was found to be 280 nm.

Choice of stationary phase:

Preliminary development trials have performed with octadecyl columns with different types, configurations and from different manufacturers. Finally the expected separation and shapes of peak was succeeded chromosil C18 column (250 mm x 4.6 mm, 5µm) column.

Selection of the mobile phase:

In order to get sharp peak and base line separation of the components, a number of experiments were carried out by varying the composition of various solvents and its flow rate. To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like Methanol, OPA and Acetonitrile with or without different buffers indifferent combinations were tested as mobile phases on a C18 stationary phase. A mixture of Methanol: Acetonitrile: ortho phosphoric acid 50:30:20 v/v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Flow rate:

Flow rates of the mobile phase were changed from 0.5 – 1.5 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.5 mL/min flow rate was ideal for the successful elution of the analyte.

Optimized chromatographic conditions:

Chromatographic conditions as optimized above were shown in Table 4.2 These optimized conditions were followed for the determination of Levo dopa in bulk samples and its combined tablet Formulations. The chromatograms of standard and sample were shown in Figure 2.

S.NO	Mobile phase	Methanol: Acetonitrile: OPA 50:30:20 (v/v/v)
1	Pump mode	Isocratic
2	Ph	6.8
3	Diluents	Mobile phase
4	Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
5	Column Temp	Ambient
6	Wavelength	280 nm
7	Injection Volume	20 µl
8	Flow rate	1.5 ml/min
9	Run time	10 minutes
10	Retention Time	2.49 minutes
11	Concentration	14

Table 2: Optimized chromatographic conditions for estimation Levo dopa

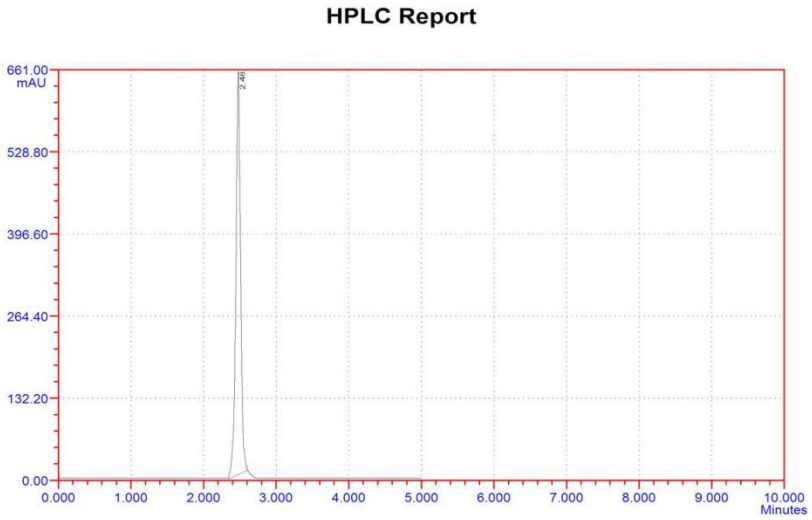

Figure 2: Chromatogram of standard solution

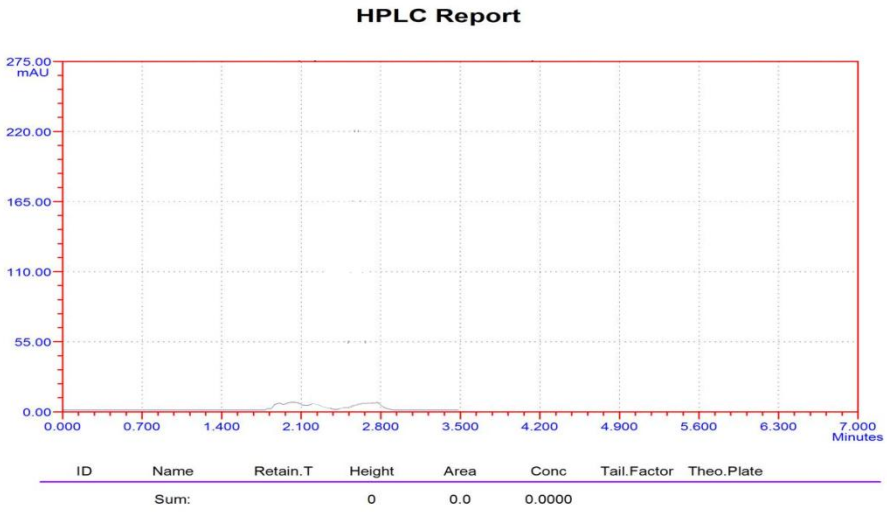

Figure: 3: Chromatogram of blank (No Peak)

Validation of the proposed method:

The proposed method was validated [32-50] as per ICH guidelines [32]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity:

The specificity of method was performed by comparing the chromatograms of blank, standard and sample. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation

between the retention times of standard and sample. The specificity results are shown in Table 3.

Name of the solution	Retention Time in Min
Blank	No peaks
Levo dopa	2.4

Table 3: Specificity study

Linearity:

Linearity was performed by preparing mixed standard solutions of Levo dopa at different concentration levels including working concentration mentioned in experimental condition i.e. 14ppm. Twenty micro liters of each concentration was injected in duplicate into the HPLC system. The response was read at 280 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. Linearity results were presented in Table 4.

LEVEL	CONCENTRATION OF LEVO DOPA IN PPM	PEAK AREA
Level 1	10	65124
Level 2	12	76841
Level 3	14	87986
Level 4	16	100561
Level 5	20	124395
Level 6	25	157319
Range 10 ppm to 25 ppm	SLOPE INTERCEPT CORREALATION COEFFICIENT	6239.191 1003.49 0.99975

Table 4

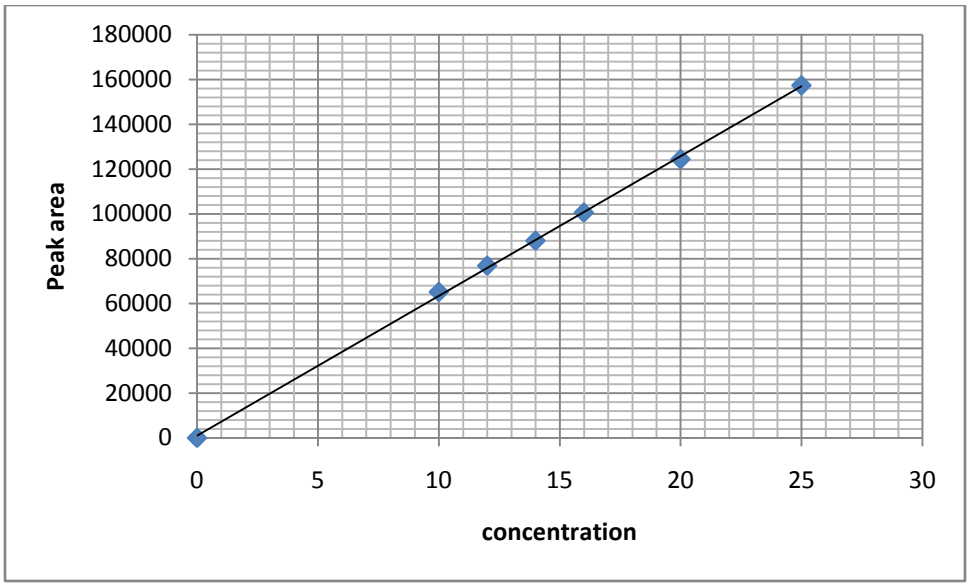

Figure 4

On X axis concentration of sample, On Y axis peak area response.

Precision:

Precision is the degree of repeatability of an analytical method under normal Operational conditions. Precision of the method was performed as intraday precision, Inter day precision.

Intraday precision:

To study the intraday precision, six replicate standard solution of Levo dopa was injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.56, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 5.

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTRA DAY RSD (Acceptance criteria ≤ 2.0%)
Levo dopa	14	1	87152	0.56
		2	87438	
		3	86979	
		4	88012	
		5	87325	
		6	86540	

Table 5

Inter Day precision:

To study the interday precision, six replicate standard solution of Levo dopa was injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.83, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 6.

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTER DAY RSD (Acceptance criteria ≤ 2.0%)
Levo dopa	14	1	86245	0.83
		2	86902	
		3	87330	
		4	88076	
		5	88147	
		6	87648	

Table 6

Accuracy:

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% level. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table 7 Satisfactory recoveries ranging from 98.6 to 99.85 were obtained by the proposed method. This indicates that the proposed method was accurate.

% Recovery	Target Conc., (ppm)	Spiked conc. (ppm)	Final Conc. (ppm)	Conc., Obtained	% of Recovery
50%	10	5	15	14.83	98.86
	10	5	15	14.79	98.6
	10	5	15	14.92	99.46
100%	10	10	20	19.97	99.85
	10	10	20	19.85	99.25
	10	10	20	19.91	99.55
150%	10	15	25	24.87	99.48
	10	15	25	24.77	99.08
	10	15	25	24.94	99.76

Table 7

Robustness:

The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. Levo dopa at 14 ppm concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in Table 8.

S.NO	Parameter	Change	Area	% of Change
1	Standard	.....	87986	.....
2	MP	MeOH :ACN:OPA 50:30:20	87106 86991	1.0 1.13
3	PH	6.6 7.0	87580 88103	0.46 0.13
4	WL	282nm 278nm	86875 87294	1.26 0.78

Table 8

Stability test:

To perform the Stability test the standard solution of 14ppm was stored at ambient temperature (±10^{0}C) for two days. After this these storage solutions and freshly prepared solution were tested with proposed method. It is noticed that assay of these results were did not decreased below 98%. The results of stability test were shown in Table 9.

S. No	Concentration Ppm	Standard solution	Area	%of Assay
1	14	Fresh	87986	100.0
2	14	Stored at +10°c 1 2 3	87576 87741 87062	99.53% 99.72% 98.94%

Table 9

System suitability:

System suitability was studied under each validation parameters by injecting six replicates of the standard solution. The system suitability parameters are given in Table 10.

Parameter	Tailing factor	Theoretical plates
Specificity study	1.39	4222
Linearity study	1.25	4309
Precision study	1.43	4186

Table 10

Limit of detection and Limit of quantification:

To determine the Limit of detection (LOD) sample was dissolved by using Mobile Phase and injected until peak was diapered. After 1ppm dilution, Peak was not clearly observed. So it confirms that 1ppm is limit of Detection and limit of Quantification is 3ppm. For this study six replicates of the analyte at lowest concentration were Measured and quantified. The LOD and LOQ of Levo dopa are given in Table 11.

Parameter	Measured Value
Limit of Quantification	3ppm
Limit of Detection	1ppm

Table 11

Formulation:

For assay Levo dopa (Tidomet ferte - 250 Mg) 20 tablets were weigh and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of Levo dopa in to a 10ml volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents and finally 14ppm were prepared. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Levo dopa was measured for the determination.

S.NO	Brand Name	Dosage	Sample Concentration	Drug estimated	% of Drug Estimated
1	Tidomet ferte	250 mg	20 ppm	19.89 ppm	99.45

Table 12

DISCUSSION ON THE RESULTS

To develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of Levo dopa different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. Proper selection of the stationary phase depends up on the nature of the sample, and molecule Physico- chemical properties. Mixture of Acetonitrile : Methanol: orthophosphoric acid (30:50:20 v/v/v) was selected as mobile phase and the effect of composition of mobile phase on the retention time of Levo dopa was thoroughly investigated. Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug Levo dopa in non - polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. The system suitability results obtained for proposed method were within acceptable limits (capacity factor >2.0, tailing factor =2.0 and theoretical plates >2000) and are represented in Table 4.10 Thus, the system meets suitable criteria.

The calibration curve for Levo dopa was obtained by plotting the peak area of Levo dopa versus concentration of Levo dopa over the range of 10-25ppm, and it was found to be linear with r = 0.99975. The regression equation for LP and AT were found to be y =6239.191 x+1003.49. The data of regression analysis of the calibration curves are shown in Table -4.4 Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day (Intra-0.56) and day to day (Inter-0.83) variations, which proves that method is precise.

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Results of recovery studies are shown range 98.6-99.85%. The mean recovery data obtained for each level as well as for all levels combined (Table 4.7) were within 2.0% of the label claim for the active substance with an R.S.D. < 2.0%, which satisfied the acceptance criteria set for the study.

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, pH and mobile phase ratio on the Area were studied. These parameters were found to proportionally changes which are given in Table 4.8. Also as part of evaluation of robustness, solution stability was evaluated by monitoring the peak area response. Standard solutions were analyzed three successive days right after its preparation. The change in Levo dopa peak area response did not decrease below 98% and there was no change in chromatographic parameters of drug peak. Also the method has been applied for the estimation of drug in pharmaceutical dosage forms. Levo dopa tablets (Tidomet - 250 Mg) were evaluated for the amount of Levo dopa present in the formulation. Sample was analyzed in triplicate and the assay of Levo dopa in 'tidomet' is 99.45%.

The sensitivity test results of Levo dopa indicated that the method was sensitive enough to detect a concentration of 1ppm and able to quantify at a concentration of above 3ppm.

The proposed method uses a simple mobile phase composition, easy to prepare with little or no variation. The rapid run time of 10 min and the relatively low flow rate allows the analysis of large number of samples with less mobile phase that proves to be cost-effective. Efficient UV detection at 280nm was found to be suitable without any interference from injectable solution excipients or solvents. The proposed HPLC method is fast, precise, accurate, sensitive, and efficient and can be used in routine analysis in quality control laboratories.

REFERENCES

1. "L-dopa for RLS". Bandolier. 1 April 2007. Retrieved 2008-10-16.
2. http://students.cis.uab.edu/porce/page4.html
3. Hyland K, Clayton PT (December 1992). "Aromatic L-amino acid decarboxylase deficiency: diagnostic methodology" (PDF). Clinical chemistry 38 (12): 2405–10.
4. Merims D, Giladi N (2008). "Dopamine dysregulation syndrome, addiction and behavioral changes in Parkinson's disease". Parkinsonism Relat Disord 14 (4): 273–280.
5. Cheng N, Maeda T, Kume T, et al. (December 1996). "Differential neurotoxicity induced by L-DOPA and dopamine in cultured striatal neurons". Brain research 743 (1-2): 278–83.
6. Basma AN, Morris EJ, Nicklas WJ, Geller HM (February 1995). "L-dopa cytotoxicity to PC12 cells in culture is via its autoxidation". Journal of neurochemistry 64 (2): 825–32..
7. Pardo B, Mena MA, Casarejos MJ, Paíno CL, De Yébenes JG (June 1995). "Toxic effects of L-DOPA on mesencephalic cell cultures: protection with antioxidants". Brain research 682 (1-2): 133–43.
8. Mytilineou C, Han SK, Cohen G (October 1993). "Toxic and protective effects of L-dopa on mesencephalic cell cultures". Journal of neurochemistry 61 (4): 1470–8.
9. J Coello, S Maspoch , N Villegas, Simultaneous kinetic-spectrophotometric determination of levodopa and benserazide by bi- and three-way partial least squares calibration, Talanta, Volume 53, Issue 3, 4 December 2000, Pages 627-637
10. Min Zhu, Xuemei Huang, Jie Li, Hanxi Shen , peroxidase-based spectrophotometric methods for the determination of ascorbic acid, norepinephrine, epinephrine, dopamine and levodopa, Analytica Chimica Acta Volume 357, Issue 3, 31 December 1997, Pages 261-267
11. P. Nagaraja, K.C.Srinivasa Murthy, K.S. Rangappa, N.M.Made Gowda, Spectrophotometric methods for the determination of certain catecholamine derivatives in pharmaceutical preparations, Talanta, Volume 46, Issue 1, May 1998, Pages 39-44.
12. Tricia C. Damiani, Andrea C. Moschetti, Adrián J. Rovetto, Fernando Benavente, Alejandro C. Olivieri, Design and optimization of a chemometrics-assisted spectrophotometric method for the simultaneous determination

of levodopa and carbidopa in pharmaceutical products, Analytica Chimica Acta, Volume 543, Issues 1-2, 6 July 2005, Pages 192-198.

13. P. Nagaraja , R.A. Vasantha, K.R. Sunitha, A new sensitive and selective spectrophotometric method for the determination of catechol derivatives and its pharmaceutical preparations, Journal of Pharmaceutical and Biomedical Analysis, Volume 25, Issues 3-4, June 2001, Pages 417-424.
14. Maspoch, determination of binary mixtures of levodopa and benserazide in pharmaceuticals by ratio-spectra derivative spectrophotometry, volume 35, issue 2, 2002
15. HELALEH M. I. H. RAHMAN N. ABU-NAMEH E. S. M, Analytical Research Division, Department of Chemistry, Aligarh Muslim University, Aligarh-202002, INDE, Analytical sciences ISSN 0910-6340
16. Massoumeh mohammadnejad, tayyebeh madrakian, simultaneous spectrophotometric determination of levodopa and carbidopa in pharmaceutical formulations and water samples by using mean centering of ratio spectra and h-point standard addition methods, chemical & pharmaceutical bulletin, vol. 55 (2007) , no. 6 865
17. Erdal Dinç, Süha Kaya, Tanver Doganay, Dumitru Baleanu, Continuous wavelet and derivative transforms for the simultaneous quantitative analysis and dissolution test of levodopa–benserazide tablets, Journal of Pharmaceutical and Biomedical Analysis Volume 44, Issue 4, 15 August 2007, Pages 991-995.
18. Babu g. gowda, mahaveer b. melwanki, jaldappa seetharamappa, spectrophotometric determination of certain vicinal dihydroxybenzene derivatives in pharmaceutical preparations, analytical sciences, the japan society for analytical chemistry.
19. Sophie dethy , marie aline laute, nadège van blercom, philippe damhaut, serge goldman and jerzy hildebrand**,** microdialysis-hplc for plasma levodopa and metabolites monitoring in parkinsonian patients, clinical chemistry 43: 740-744, 1997.
20. Guang Wu, The determination of levodopa in plasma by HPLC: A cautionary note , chromatographia , Volume 52, Numbers 5-6, 371-372, DOI: 10.1007/BF02491035.
21. Loren R. Gelber, John L. Neumeyer, Determination of the enantiomeric purity of levodopa, methyldopa, carbidopa and tryptophan by use of chiral mobile phase high-performance liquid chromatography, Journal of Chromatography A, Volume 257, 1983, Pages 317-326.
22. Li sf, wu hl, yu yj, li yn, nie jf, fu hy, yu rq, 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, State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, China
23. Dethy S, Laute MA, Van Blercom N, Damhaut P, Goldman S, Hildebrand J, Microdialysis-HPLC for plasma levodopa and metabolites monitoring in parkinsonian patients, Clin Chem. 1997 May;43(5):740-4.
24. Ashwah gadallah mohamed, mohamed sultan mohamed, determination of antiparkinsonism drug entacapone, journal of the chilean chemical society, versión on-line issn 0717-9707.
25. Ankara Ecz. Fak. Der. J. Fac. Pharm. Ankara, Simultaneous Determination of Levodopa and Benserazide, Using

Derivative Spectrophotometry, 23, 1-2 (1994) 23 1-2 (1994).

26. T. Madrakian,* A. Afkhami, M. Borazjani, and M. Bahram, Simultaneous Derivative Spectrophotometric Determination of Levodopa and Carbidopa in Pharmaceutical Preparations, 1764 Bull. Korean Chem. Soc. 2004, Vol. 25, No. 12
27. http://www.drugbank.ca/drugs/DB01029
28. http://www.drugs.com/mtm/levo dopa.html