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DEVELOPMENT AND VALIDATION OF A REVERSE PHASE HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CARBIDOPA AND LEVODOPA IN PHARMACEUTICAL DOSAGE FORMS

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Abstract:

A simple, rapid, and efficient reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous determination of Carbidopa and Levodopa in pharmaceutical dosage forms. The analysis was performed using a Waters Alliance 2695 HPLC system equipped with a PDA Detector 996 model. The separation was achieved using a Phenomenex Gemini C18 column (4.6 × 250 mm, 5 µm), with an isocratic mobile phase consisting of acetonitrile and water (75:25%, v/v). The flow rate was set at 1 mL/min, with a column temperature of 40ºC, and detection was carried out at a wavelength of 240 nm. The injection volume was 10 µL, and the total run time was 6 minutes. The method was validated according to ICH guidelines for parameters such as specificity, linearity, precision, accuracy, and robustness. The method demonstrated good resolution and reliable quantification of both drugs, making it suitable for routine quality control analysis of Carbidopa and Levodopa in pharmaceutical formulations.

KEY WORDS: *Carbidopa, Levodopa, HPLC, PDA Detector, quality control.*

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INTRODUCTION:

Analytical chemistry is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.

Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography

Advantages of instrumental methods

- Small samples can be used
- High sensitivity is obtained
- Measurements obtained are reliable
- Determination is very fast
- Even complex samples can be handled easily

Limitations of instrumental methods

- An initial or continuous calibration is required
- Sensitivity and accuracy depends on the instrument
- Cost of equipment is large
- Concentration range is limited
- Specialized training is needed
- Sizable space is required

High Performance Liquid Chromatography

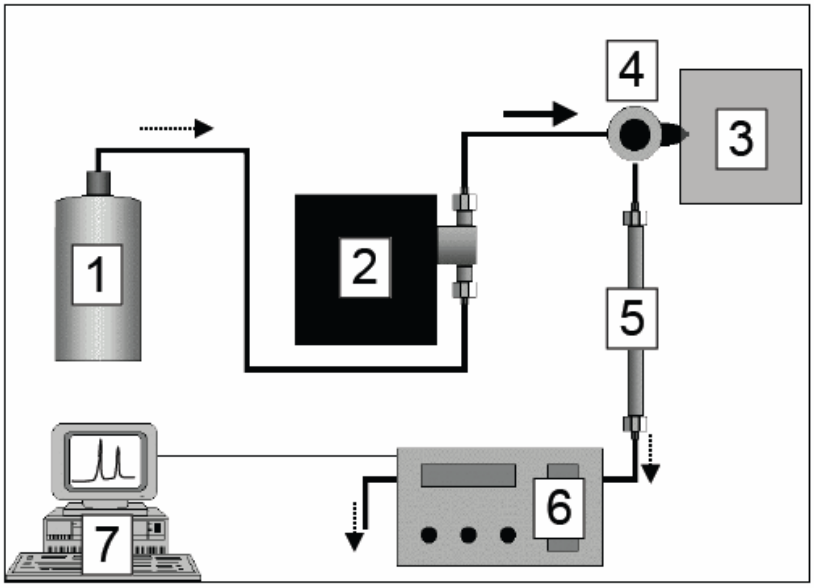
HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.

The rate of distribution of drugs between Stationary and mobile phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved .The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed,efficient ,accurate and highly resolved method of separation.

For the recent study metformin and Sitagliptin was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages

- Speed many analysis can be accomplished in 20min (or) less.
- Greater sensitivity(various detectors can be employed).
- Improved resolution(wide variety of stationary phases).
- Re usable columns(expensive columns but can be used for many analysis).
- Ideal for the substances of low viscosity.
- Easy sample recovery, handling and maintenance.
- Instrumentation leads itself to automation and quantification (less time and less labour).
- Precise and reproducible.
- Integrator itself does calculations.
- Suitable for preparative liquid chromatography on a much larger scale.

HPLC Basic Instrumentation:



1 – Reservoir

2 – Pump

3 – Auto Sampler

4 – Injection Valve

5 – Column

6 – Detector

HPLC components

The essential components[4] of a complete HPLC system are solvent delivery system (Pump), detector, fixed volume injector loop or autosampler, solvent reservoirs, packed column, data system and recorder. A schematic of a simplified HPLC system is shown in above Figure.

Column

The column is probably the heart of HPLC system. The development of this column technology leads to the evolution of the HPLC instrumentation systems used today. The conventionally used HPLC columns are particle packed columns. The key of column selection when previous separation is not available resides in knowing the chemistry of the sample. Columns should never be dry. A dry column will eventually have voids because the packing will shrink away from the wall, which would result in band broadening. Before running a sample in HPLC the column should be equilibrated. Usually column equilibrium is achieved after passage of 10 – 20 column volumes of the new mobile phase through the column. Insufficient column equilibrium usually leads to retention difference.

Pump

The solvent delivery system or as it is commonly called the pump includes two major types, constant volume or flow and constant pressure. Constant volume pumps are mechanically driven systems, most commonly using screw driven syringes or reciprocating pistons. On the other hand, constant pressure pumps are driven or controlled by gas pressure.

Injector or Auto sampler

Samples are usually introduced by syringe injection via a manual injector into the mobile phase stream or by the use of an auto sampler. The important aspects in sample introduction are precise and reproducible injections. This is especially important with quantitative analysis where the reproducibility of the peak response is dependent on the precision of the sample introduction. Direct syringe injection through a manual injector was the first popular method of sample introduction. As HPLC instrumentation evolved, many auto sampler techniques were applied so that sample introduction has become more precise and rapid.

Detector

HPLC detectors include ultraviolet-visible, fluorescence, electrochemical, refractometer, mass spectrometer and others. The UV visible absorption detector is the most widely used detector in liquid chromatography, since most organic compounds show some useful absorption in the UV region. This detector is fairly universal in application, although sensitivity depends on how strongly the sample absorbs light at a particular wavelength.

Solvent reservoir

Different containers are used as a solvent delivery system reservoir. The best material from which the containers are made is glass. Plastic containers are not recommended as it leads to plasticizer leaching. The container should be covered to prevent solvent evaporation. The tubing from the reservoir can be made of stainless steel or Teflon, and both are satisfactory.

Data handling and analysis

Data handling in HPLC is as important to the success of any experiment or analysis as any other

components in the system. It is part of good HPLC techniques to properly label and document the analytical results. The advanced computer softwares used now in data handling and analysis allow easy recording and storage of all chromatographic data.

Based on modes of chromatography:

- Normal phase chromatography
- Reverse phase chromatography

Normal phase chromatography

In normal phase mode the stationary base (eg; silica gel) is polar in nature and the mobile phase is non polar. In this technique, non polar compound travel faster and are eluted first. This is because less affinity between solute and stationary phase and take more time to elute.

Reverse phase chromatography

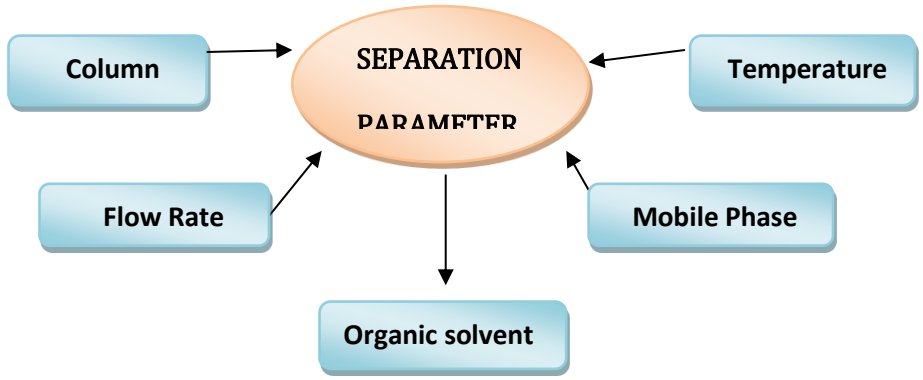
The popularity of reversed phase liquid chromatography is easily explained by its unmatched simplicity, versatility and scope. Neutral and ionic analytes can be separated simultaneously. Retention in RPLC is believed to occur through nonspecific hydrophobic interaction of the solute with the stationary phase. The near universal application of RPLC stems from the fact that almost all organic compounds have hydrophobic regions in their structure and are capable of interacting with the stationary phase.

A decrease in the polarity of the mobile phase leads to a decrease in retention. It is also generally observed in RPLC that branched chain compounds are retained to a lesser extent than their straight chain analogues and that unsaturated compounds are eluted before their fully saturated analogs. A wide variety of RP-HPLC columns are available. Most columns are silica based. Silica offers good mechanical stability. A typical stationary phase is formed by chemically bonding a long-chain hydrocarbon group to porous silica. Typical ligands are n-octadecyl (C18), n-octayl (C8), n-butyl (C4), diphenyl (C2),and cyano propyl.

Parameters affecting separation:

Separation in reversed phase chromatography is affected by stationary phase type and column length. It is also affected by organic solvent type and percentage in the mobile phase and by mobile phase pH. Flow rate could also affect separation in reversed phase chromatography; however it is usually limited by the developed backpressure. Moreover temperature of the column also has an effect on separation.

Parameters affecting separation in reversed phase chromatography are shown Schematically:



MATERIALS AND METHODS:

INSTRUMENTS USED

HPLC from WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.

CHEMICALS USED:

carbidopa and levodopa from Sura Pharma Labs, Water and Methanol for HPLC from LICHROSOLV (MERCK) and Acetonitrile for HPLC from Merck

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of carbidopa and levodopa working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3ml of carbidopa and 1.98ml of levodopa from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

RESULTS AND DISCUSSION

Optimised Chromatographic boundaries:

Mobile phase : Potassium dihydrogen phosphate (0.03M) (pH-2.8): Methanol (75:25)
Auto sample temperature : Ambient
Injection volume : 20µL
Column : Hypersil C18 (250 mm×4.6 mm, 5µm) particle size

Detector wavelength : 226 nm
Flow rate : 1.0ml/min
Run time : 6 minutes

Procedure:

Twenty microliters of the normal solution should be added to the chromatographic framework. Then, using the provided recipe, determine the areas to be tested for Levodopa and Carbidopa.

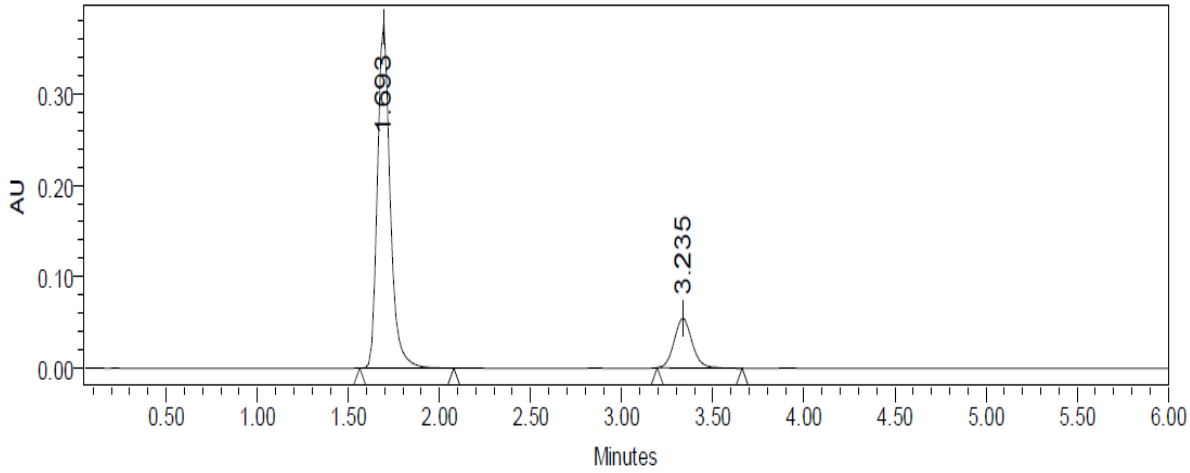


Fig: Ordinary Chromatogram for streamlined strategy

Table-: Consequences of streamlined strategy Chroma togram

S. No.	Name	R Time	A	US.P Resolution	US.P Tailing	US.P Plate Count
1	Levodopa	1.693	3658986		1.59	5699
2	Carbidopa	3.235	6528	7.29	1.64	7528

Observation: It appears that the cut off points are well-separated and located inside the endpoints.

PREPARATION OF MOVABLE PHASE:

Add 1.36086 grams of KH_2PO_4 to 1000 milliliters of measuring glass and adjust the pH to 2.80 using orthophosphoric acid (OPA).

Following the aforementioned procedure, 750 ml and 250 ml of methanol are used as a flexible stage. After 20 minutes of blending, they are subjected to sonication.

PLANNING OF THE LEVODOPA AND CARBIDOPA STANDARD AND TEST ARRANGEMENT:

PLANNING OF STANDARD ARRANGEMENT:

Add 10 milliliters of water to 50 milliliters of vol. cup with 50 milligrams of levodopa and 50 milligrams of carbidopa; sonicatorr for 10 min; agitation for 5 min; and then make with H_2O.

Additional 0.8 ml of Levodopa and 0.9 ml of Carbidopa should be transferred into a 10 ml vol. cup using a pipette from the aforementioned stock solutions. Then, dilute the mixture to your satisfaction.

PREPARATION OF SAMPLE STOCK SOLUTION:

After carefully opening and grinding six tablets, the amount of active ingredients (585.58 mg of Levodopa and Carbidopa) was transfer to a 50 ml vol. carafe. To this, 10 ml of methanol was extra, and the mixture was sonicater for 20 minutes (or stirred for 10 minutes) before being diluted with water. Using a pipette, transfer 0.8 milliliters of levodopa and 0.9 milliliters of carbidopa from the aforementioned stock solutions into a 10 milliliter vol. cup. Dilute to taste.

METHOD VALIDATION

1. SYSTEM SUITABILITY:

There should not be more than 2.0 in the next section for the tops due to Levodopa and Carbidopa in typical configuration. Under no circumstances could the typical arrangement's speculation plates for the Levodopa and Carbidopa tops be less than 2500.

Table: System fittingness data of Levodopa and Carbidopa

parameter	Levodopa	Carbidopa	A.criteria
Retntion tim	1.692	3.298	_
Theoretical p.	5699	7528	>2500
Tailling factor	1.59	1.64	<2.00
% RSD	0.03	0.04	<2.00

Table: Standard Effects of Levodopa

S. no	s. name	R.T	A	US.P p.c	USP tailing
1	Inj-1	1.695	3658987	5699	1.59
2	Inj-2	1.688	3659845	5656	1.58
3	Inj-3	1.691	3659863	5683	1.59
4	Inj-4	1.687	3654876	5675	1.59
5	Inj-5	1.689	3654513	5629	1.58
Avg.			3657618		
SD			2694.128		
% RSD			0.073658		

Table : Standard Effects of Carbidopa

S. no	S.name	R.T	A	US.P p.count	US.P tailing
1.	Inj-1	3.245	6599	7599	1.62
2.	Inj-2	3.239	6575	7548	1.63
3.	Inj-3	3.245	6524	7562	1.64
4.	Inj-4	3.264	6538	7593	1.62
5.	Inj-5	3.266	6577	7568	1.63
Avg.			6562.6		
SD			30.74573		
% RSD			0.468499		

RESULT:

The following table summarizes the outcomes of the construction appropriateness study. Both drugs demonstrated a respectable assessment framework with six consecutive standard strategy combinations showing consistent maintenance time, hypothetical plate count, following part, and objective.

2. SPECIFICITY:

The test reasoning team prepared the plan for the standard model and fake treatment, and then they soaked it into the HPLC framework.

Acceptance criteria:

Near maintenance time, the chromatogram of the standard and test should be hazy.

Blank interference:

Research into dispersing the blockage of clear was made possible. In accordance with the test plan, solvable was included into the HPLC framework.

Acceptance criteria:

The peak of the analyte shouldn't be visible on a fresh chromatogram. During the analyte maintenance season, there is no apparent impediment. Therefore, the approach is very clear.

Table: Specificity data for Levodopa and Carbidopa

S. no	Sample name	Levodopa		Carbidopa	
		A	R.t	A	R.t
1.	Standrad	3658986	1.692	6528	3.298
2.	Sam.	3785985	1695	6696	3.235
3.	Blank	-	-	-	-
4.	Placebo	-	-	-	-

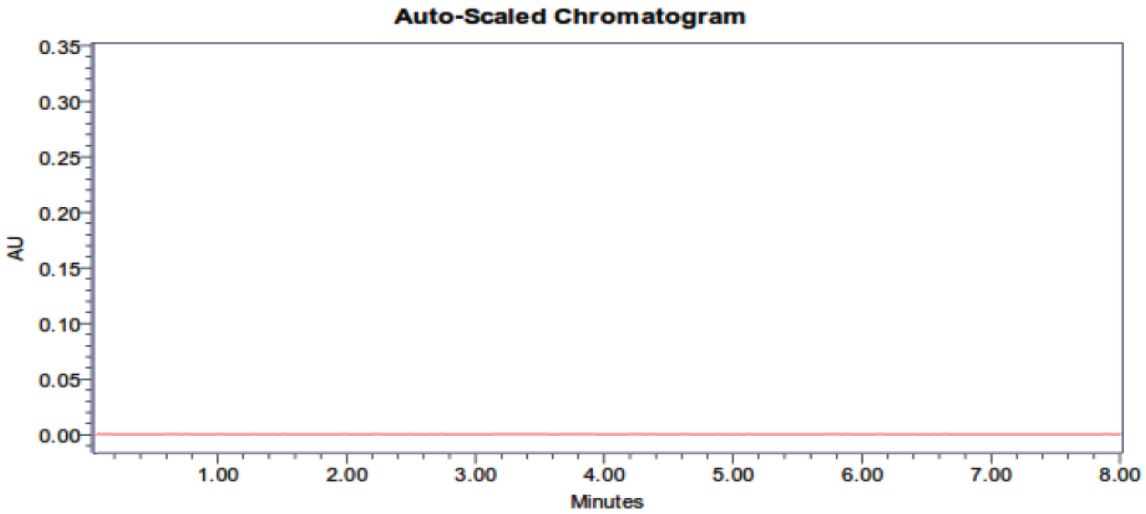


Fig: Normal chromatogram of the Clear Arrangement

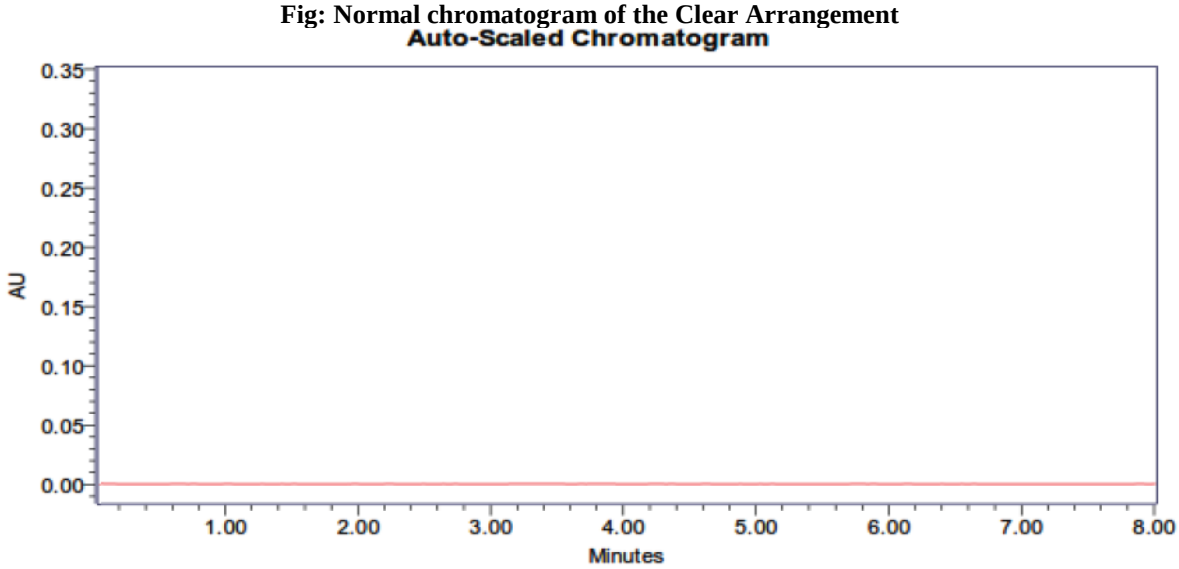


Fig : Common chromatograms of the Fake treatment

RESULT

Standard, model, and business delayed consequences of Levodopa and Carbidopa have the same maintenance time, according to chromatograms. This demonstrates that, additives have an effect on the constant philosophy. A transparent pinnacle obviously couldn't hide a drug top. This means that the framework is clear at its core.

3. ACCURACY/RECOVERY:

Between fifty percent and one hundred fifty percent of the test's objective social event might be used for recovery evaluation. We recommend a minimum of three fixations.

Acceptance criteria:

Recovery rates typically ranged from 97% to 103%, with a relative standard deviation of less than 2% among these recovery sites.

Table: Accuracy data for Levodopa

S. no	A.level	Injection	S.A	R.t
1	50%	1	1928493	1.687
		2	1935675	1.691
		3	1927547	1.688
2	100%	1	3859866	1.688
		2	3865142	1.688
		3	3858749	1.688
3	150%	1	5785848	1.686
		2	5786422	1.685
		3	5789659	1.684

Table : Accuracy (%recovery) outcomes of Levodopa

S. no	Acc. Level	Samp name	µg/ml added	µg/ml fond	% Rec	% M
1	50%	1	40	39.948	99.873	99.979%
		2	40	40.099	100.246	
		3	40	39.927	99.823	
2	100%	1	80	80.072	100.089	100.124%
		2	80	80.181	100.226	
		3	80	80.049	100.061	
3	150%	1	120	120.081	100.067	100.091%
		2	120	120.093	100.077	
		3	120	120.158	100.133	

Table : Accuracy data for Carbidopa

S. no	A.level	Sample name	S.area	R.t
1	50%	1	3288	3.277
		2	3279	3.275
		3	3283	3.266
2	100%	1	6517	3.265
		2	6519	3.265
		3	6528	3.265
3	150%	1	9748	3.268
		2	9759	3.268
		3	9747	3.266

Table: Accuracy (%recovery) effects of Carbidopa

S. no	Accuracy Level	Sample name	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	45	45.123	100.276	100.131%
		2	45	44.998	99.998	
		3	45	45.056	100.123	
2	100%	1	90	90.029	100.032	100.108%
		2	90	90.057	100.063	
		3	90	90.208	100.233	
3	150%	1	135	134.988	99.991	100.010%
		2	135	135.113	100.083	
		3	135	134.946	99.958	

RESULT

The accompanying table introduces the delayed effects of the exactness research. The recovery test yielded the intentional value. The recovery period was weighed against the spiked dosage of both medications.

% Levodopa and carbidopa both had recovery rates of 100%. According to the effects, the strategy is all about being careful.

4. PRECISION:

Preparation of sample:

Combine 802.04 milligrams of test with 100 milliliters of water, 10 milliliters of methanol, and 20 minutes of sonication. Rinse with water and apply beauty care products. Scale up the previous strategy from 5 milliliters to 25 milliliters by diluting the volume with water.

By calculating the percentage RSD of look regions from six replicate injections, the method accuracy limitations were observed from the test chromatograms obtained.

Acceptance criteria: The blend reproducibility supplies are satisfied if the %RSD aimed at top areas and help time are less than 2.0.

Table: Accuracy scholarships for Levodopa and Carbidopa

S. no	Intraday precision for Levodopa			Intraday precision for Carbidopa		
	P.	Mean p.a	%RSD	P. area	Mean p.	%RSD
1	3658953	3665966	0.436	6599	6548	0.391
2	3659855			6528		
3	3659875			6536		
4	3658749			6539		
5	3698548			6547		
6	3659817			6535		

RESULT

The above table summarizes the effects of the fluctuation. It is not possible to permanently set the %RSD of peak for various runs. It was determined that the rate qualified typical nonconformity (%RSD) was less than 2%, indicating that the technique is accurate.

5. LINEARITY:

Put into place an HPLC framework and set up a development of standard plans. Choose the affiliation and legitimization coefficient underlying a 100% response, then plot the diagram of standard against credible fixation in µg/ml.

Acceptance criteria:

When plotting the normal summit district reaction of repeat blends against individual fixation, the linearity apostatize coefficient should not be less than 0.999. It is expected that the % y-block, found after the linearity data (without extrapolation from starting 0, 0), will fall within ±2.0.

Numerical Assessment:

The fixation was plotted against the usual region. There were spots where linearity could be seen. The connection coefficient, slant, and y are still hanging around there after a line of best fit was occupied using the concept of least squares.

Table : Linearity data for Levodopa

S. no	Concentration (µg/ml)	R.t	A
1.	40	1.689	1923836
2.	60	1.691	2899875
3.	80	1.692	3868986
4.	100	1.689	4835985
5.	120	1.688	5758748

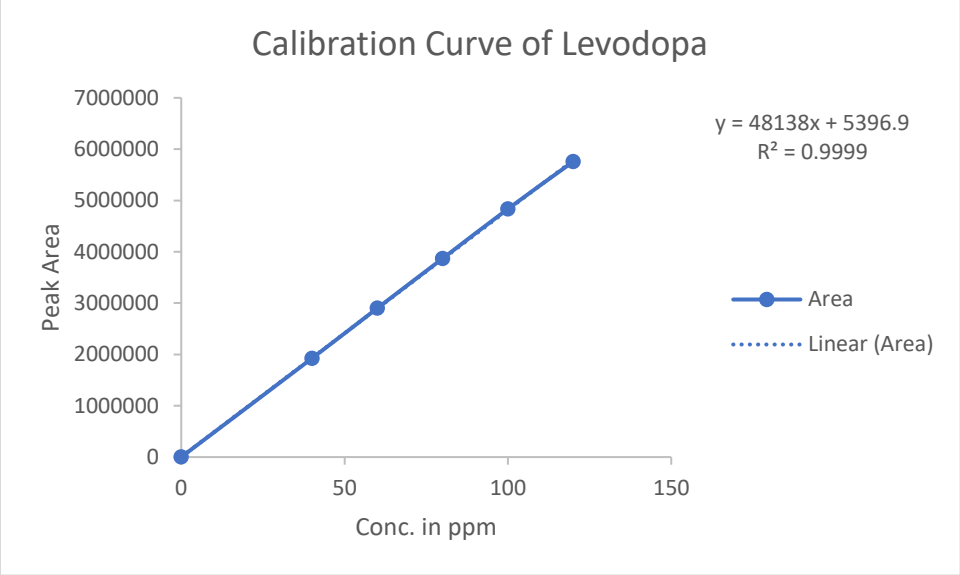


Fig: Linearity Curve of Levodopa

Table: Linearity data for Carbidopa

S. no	Concentration (µg/ml)	Rt	A
1.	50	3.203	3676
2.	70	3.299	5109
3.	90	3.294	6528
4.	110	3.290	7955
5.	130	3.288	9348

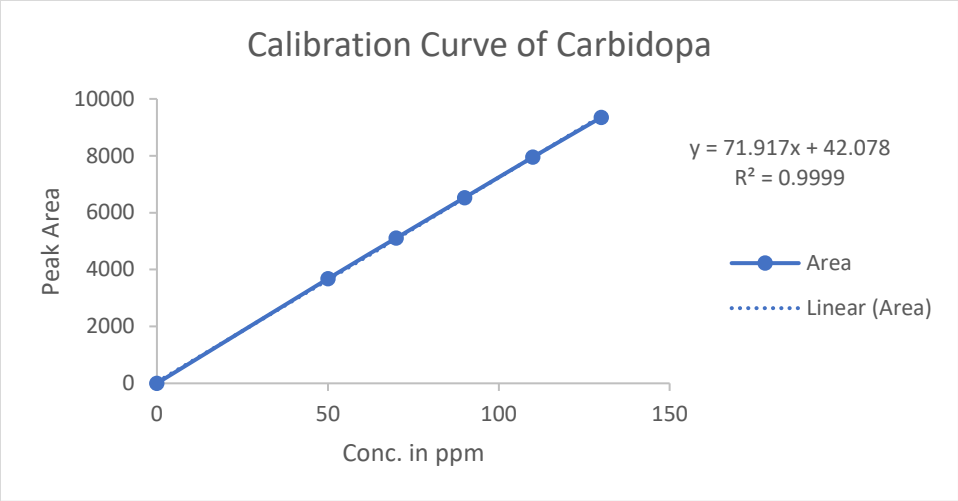


Fig: Linearity Curve of Carbidopa

RESULT

Between 50% and 150% of apparent fixation, a linear association between top regions and focuses was seen for Levodopa and Carbidopa. The system is linear from 50% to 150%, as shown by the relationship coefficients of 0.999 for levodopa and 1 for carbidopa.

6. ROBUSTNESS:

Effect of variation in flow rate:

Prepare the design fit strategy rendering to the exam technique and include it hooked on the HPLC scheme using about 0.2 ml of the process stream. For both flow rates, evaluate the construction fitness values precisely as the test protocol anticipates. We blended it into HPLC and evaluated the structural reasonableness after changing the certified stream rate from 1.0 ml/min to 0.8 ml/min and 1.2 ml/min.

Result of variation in Temperature:

Combine the framework reasonableness plan with the HPLC with ± 5º C of the procedure temperature, following the test methodology. Analyze the building reasonableness values in accordance with the test method's predictions for the two fevers.

Table: Robustness statistics for Levodopa

Parameter	R.t	Theoretical plate	Tailing facto
Decreased f.rat (0.8ml/min)	1.867	5855	1.56
Increased fl rate (1.2ml/min)	1.545	5366	1.57
Reduced temt (20^0c)	1.730	5419	1.53
Increased tempt (30^0c)	1.674	5497	1.54

Table: Robustness data for Carbidopa

Parameter	R.t	Theoretical plates	Tailing factor
Decreased rate (0.8ml/min)	3.621	7599	1.62
Increased rate(1.2ml/min)	2.998	7613	1.61
Reduced temp (20^0c)	6.242	7252	1.64
Increased temp (30^0c)	2.302	7196	1.61

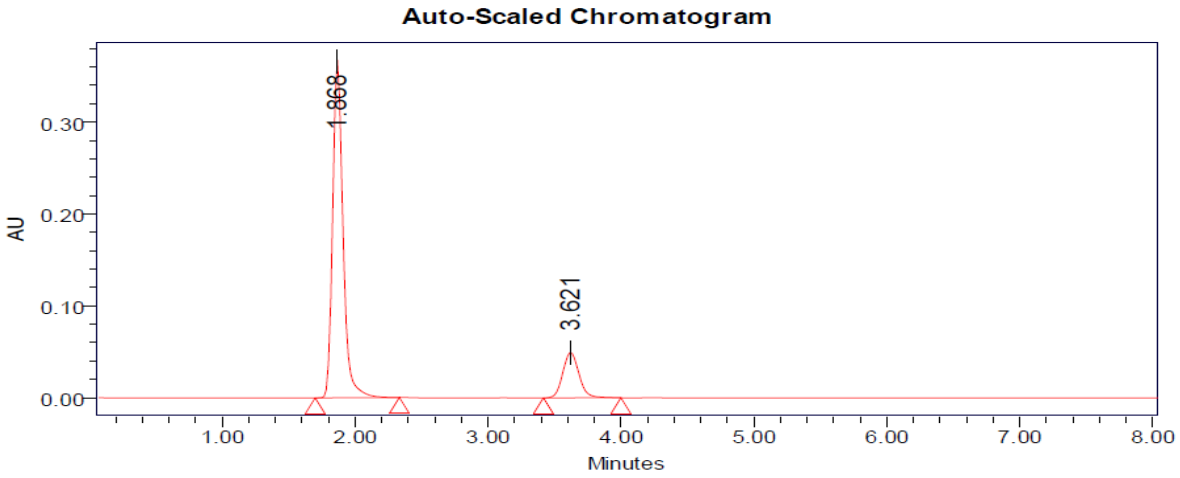


Fig: Chromatogram for diminished stream rate

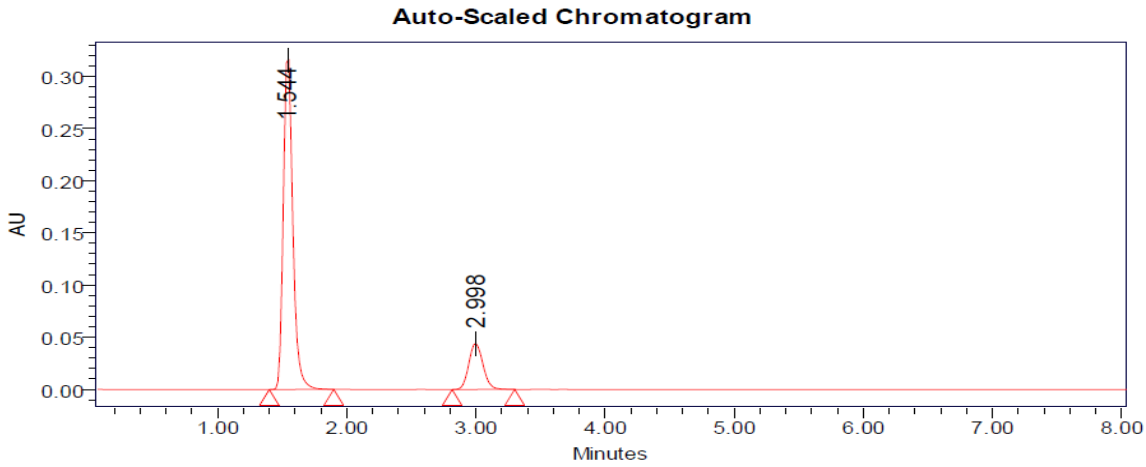


Fig: Chromatogram for lessened stream rate

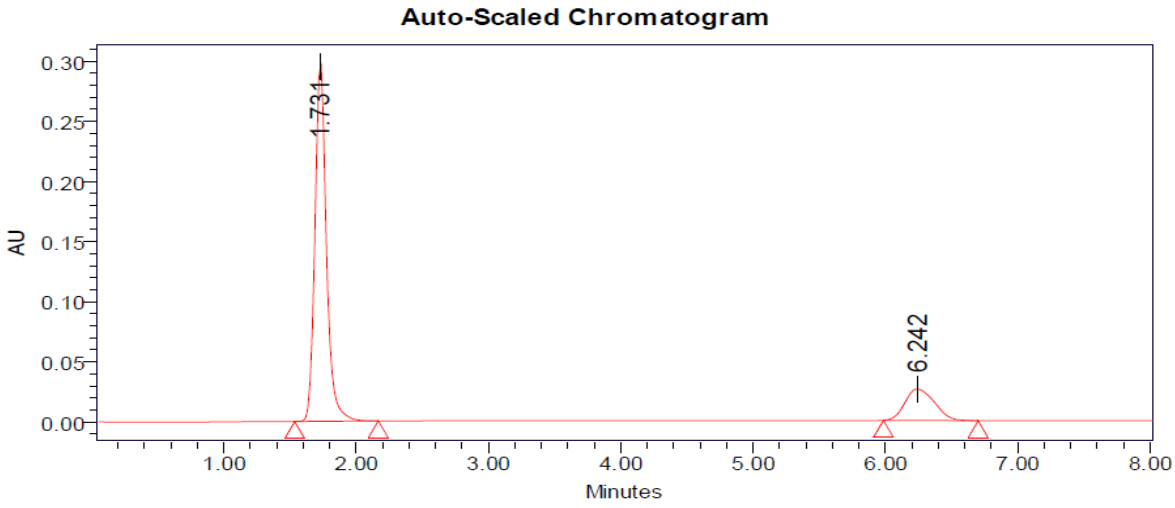


Fig: Chromatogram for diminished temperature

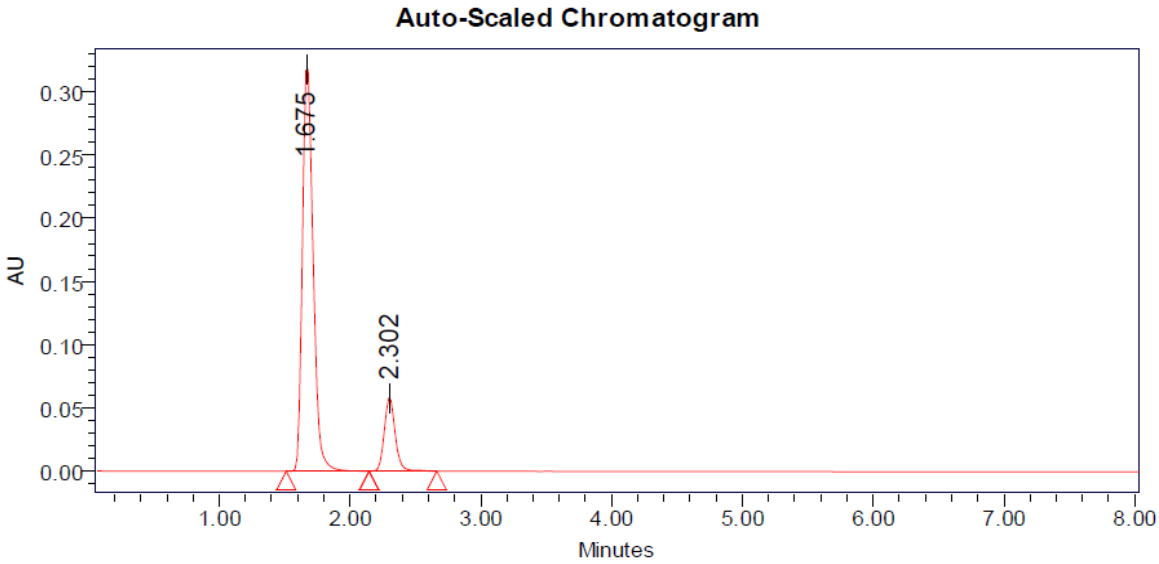


Fig: Chromatogram for expanded temperature

RESULT

Modifications to the stream and temperature did not provide significantly different plausible effects, as indicated in the previous table, according to the energy consequences of the continuous method. We might state the method is energetic since the advancements aren't crucial.

7. LIMIT OF DETCTION:

Concerning the limitation of territory (LOD), the suggested method was used to study the awareness of evaluation of carbidopa and levodopa. The use of sign to upset level does not fully resolve the. The LOD respect was verified by blending the reasonable model on several occasions, with the peak region of this model not being permanently set up as the noise level. The amount of disturbing impact is not always constant, and the setup is not permanent either.

LOD= 3.3 σ / S

Where,

σ = standad eccentricity of captures of alignment bends.

S = unpleasant of inclines of the alignment bends.

The slant S might be assessed from the adjustment bend of the analyse.

Least grouping of standa part in which the pinnacle of the standard gets converged with commotion called the LOD

LOD = 3.3* σ/S

Where;

σ = standard devi of reaction.

S = slant of alignment bend.

LOD for Levodopa = 1.27

LOD for Carbidopa = 1.16

8. LIMIT OF QUANTIFICATION:

In terms of LOQ, the suggested approach was used to examine the responsiveness of Levodopa and Carbidopa evaluations. Using sign to commotion level does not really permanently establish. In order to determine the LOQ respect, the reasonable model was applied to several occurrences, with the peak region remaining at the disturbance level. Despite several instances of the annoyance respecting the LOQ, the setup is not permanent.

LOQ = 10 σ / S

Where,

σ = standard deviation of captures of adjustment bends.

S = mean of inclines of the adjustment bends.

The incline S might be assessed from the alignment bend of the analyse.

Least convergence of normal part in which the pinnacle of the normal becomes identified and evaluation

LOQ = 10*σ/S

Where;

σ = standard deviation of the reaction.

S = incline of the alignment bend.

LOQ for Levodopa = 3.81

LOQ for Carbidopa = 3.48

CONCLUSION:

The developed RP-HPLC method provides a simple, rapid, and reliable approach for the simultaneous quantification of Carbidopa and Levodopa in pharmaceutical dosage forms. It offers several advantages, including short run time (6 minutes), high resolution, and reproducibility, making it ideal for routine quality control applications. The method meets the required validation criteria, demonstrating accuracy, precision, and robustness. Therefore, this method can be confidently used for the determination of Carbidopa and Levodopa in both formulation development and quality control of pharmaceutical products.

BIBLIOGRAPHY:

1. Shetti, P.D. High Performance Liquid Chromatography. 2001; 116.
2. The Merck Index, an encyclopedia of chemicals, drugs and biological. Fourteenth Edn. USA; 2006.
3. Beckett, A.H.; Stenlake, J.B. Practical Pharmaceutical Chemistry, 4th Edn., C.B.S. Publications, 1.
4. Malik, V. Drugs and Cosmetics Act 1940, 16th Edn., Eastern Book Company, Lucknow, 5.
5. Willard, H.H.; Merit, L.L.; Dean, F.A.; Settle, F.A. Instrumental methods of analysis, 7th Edn., C.B.S. Publishers, New Delhi, 2002.
6. Mendham, J.; Denney, R.C.; Barnes, V; Thomas, M.J.K. Vogel's Text book of Qualitative Chemical Analysis, 6th Edn., 261-287.
7. Skoog, D.A.; West, D.M. Fundamentals of Analytical Chemistry, 7th Edn.
8. Sharma, B.K., Instrumental methods of Chemical analysis, 19th Edn., 2000.
9. Willard, H.H.; Merrit, L.L.; Dean. J.A., Instrumental methods of analysis, 7th Edn., CBS Publishers, New Delhi.
10. Khopkar, S.M. Basic concepts of analytical chemistry, 2nd edition, 2005.
11. Tips on Liquid Chromatography, Waters www.waters.com.
12. Validationof Analytical Procedures: Methodology, ICH Tripartite Guidelines 1996.
13. Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines 1994.
14. ICH, Q2A Text on validation of analytical procedures, Oct, 1994www.ich.org.
15. ICH, Q3B Validation of analytical procedures: methodology, Nov, 1996.
16. Osman A. O. Mohammed. Development of an HPLC method for the Simultaneous Determination of Levodopa and Carbidopa in Pharmaceutical Dosage Forms. Hacettepe University Journal of the Faculty of Pharmacy Volume 41 / Number 4 / December 2021 / pp. 221-227.
17. Mithun Rudrapal, Shubham J. Khairnar, Devid Chutia, Soumya Bhattacharya, Khomendra Kumar Sarwa, Nazim Hussain. Development and Validation of Reverse-phase High-performance Liquid Chromatography Method for Simultaneous Estimation of Riluzole and Levodopa in Tablet Dosage Form. Asian Journal of Pharmaceutics • Jan-Mar 2021 • 15 (1) | 172.