Original Research Article

DETERMINATION OF LANTHANUM IN LANTHANUM DIOXYCARBONATE DRUG SUBSTANCE BY POTENTIOMETRIC TITRATION

ABSTRACT

This research focused on the determination of lanthanum in lanthanum dioxycarbonate drug substance through potentiometric titration, a vital aspect in ensuring pharmaceutical formulation quality and efficacy. Lanthanum dioxycarbonate, employed in treating hyperphosphatemia linked to chronic kidney disease, demands precise analytical methods for quality control. The analytical approach involves titrating lanthanum dioxycarbonate samples with a known concentration of ethylenediaminetetraacetic acid (EDTA) solution, with the endpoint detected potentiometrically. The study includes a rigorous analytical method validation encompassing various parameters. The method demonstrated specificity, confirmed by the absence of blank interference and the yellow color of the solution at the endpoint with the potentiometric curve peaks indicating the absence of lanthanum. Filter compatibility study results showed that the % lanthanum content for centrifuged and filtered samples met the acceptable limits, allowing the use of 0.45 µ PVDF or 0.45 µ nylon syringe filters for regular analysis. Precision was evident from consistent results, with a mean lanthanum content of 99.1% and %RSD of 0.6%. Accuracy was validated through recovery at 10%, 100%, and 150% levels, averaging 96.9% with a %RSD of 4.2%. Linearity was confirmed by a linear relationship between sample weight and titer value, with correlation coefficients exceeding 0.99 for all levels. The robustness study results indicated the methods' resistance under varied conditions. Thus, the validated potentiometric titration method proved effective for determining lanthanum content in lanthanum dioxycarbonate drug substance, exhibiting precision, accuracy, linearity, and robustness, supporting its suitability for pharmaceutical quality control. This research addresses critical gaps in analytical methodologies for pharmaceutical formulations containing lanthanum, contributing to the understanding of lanthanum determination.

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UNDER PEER REVIEW

1. INTRODUCTION

Lanthanum (LAN), a member of the lanthanide series, has a notable role in the pharmaceutical industry. In pharmaceuticals, lanthanum takes center stage as lanthanum dioxycarbonate ($La_2O_2CO_3$). This compound serves a crucial purpose in the medical field, particularly in the treatment of hyperphosphatemia, a condition frequently associated with chronic kidney disease [1]. Hyperphosphatemia poses risks of complications such as bone and cardiovascular issues due to elevated phosphate levels in the bloodstream [2]. Lanthanum dioxycarbonate emerges as an effective phosphate binder, mitigating phosphate absorption in the gastrointestinal tract and aiding in the management of phosphate levels [3].

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The determination of LAN in lanthanum dioxycarbonate holds significant importance in ensuring the quality and efficacy of pharmaceutical formulations [4]. Analytical methods, such as potentiometric titration, play a key role in accurately quantifying LAN content [5]. This involves titrating a sample of lanthanum dioxycarbonate with a known concentration of an EDTA solution. The potentiometric detection of the titration endpoint facilitates precise calculations of LAN content.

Despite the importance of these analytical methods, a comprehensive understanding of their validation, particularly in the context of LAN determination in pharmaceutical formulations, remains an area that demands focused exploration [6]. The current research aims to bridge this knowledge gap by undertaking an analytical method validation (AMV) for the potentiometric titration of LAN in lanthanum dioxycarbonate. This validation encompasses critical parameters like specificity, filter compatibility, precision, accuracy, linearity, range, and robustness. Through this endeavor, the study aims to enhance the reliability and robustness of analytical methods crucial for ensuring the pharmaceutical quality of Lanthanum-containing formulations.

2. DETAILS OF DRUG PRODUCT

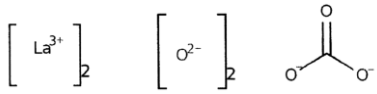

Figure 1. Structure of Lanthanum Dioxycarbonate

Chemical Name: Lanthanum Dioxycarbonate
Molecular Weight: 369.82 g/mol
Molecular Formula: $La_2O_2CO_3$

3. EQUIPMENTS AND INSTRUMENTS USED

The study involved the use of Borosil glass volumetric flasks and beakers, both of Class-A quality, along with Class-A glass measuring cylinders and pipettes, including both bulb and graduated types, from Borosil. An Auto titrator from Metrohm is employed for precise titration purposes, while a vacuum oven from Quesst International helps in sample preparation. The analytical precision and accuracy are ensured by Sartorius Analytical and Semi Micro balances. Other essential instruments include an Ultra sonicator from Samarth Electronics, a Dissolution apparatus from Electrolab, a pH meter from Horiba Scientific, and a Centrifuge from Eppendorf. The Optrode electrode used for photometric measurements has the serial number 0806322 and part number 6.1115.000, manufactured by Metrohm.

4. CHEMICALS, REAGENTS, STANDARD, AND SAMPLES USED

The study incorporated a variety of high-quality chemicals and reagents. The chemicals/reagents utilized in the process include Calcium Carbonate (Merck, SRM grade), EDTA (Merck, Emparta grade), Hexamethylenetetramine (Hexamine) from Sigma Aldrich (ACS grade), Hydrochloric Acid (Thomas Baker, LR grade), Hydroxy Naphthol Blue (Merck, Indicator), Sodium Hydroxide (Merck, Emparta grade), Xylenol Orange Tetra Sodium salt (Merck, AR grade), Sodium

UNDER PEER REVIEW

chloride (Merck, Emplura grade), Tween-80 (Merck, AR grade), and ultrapure Water (Evoqua). The standard/sample used for the analysis is Lanthanum Dioxycarbonate.

5. REFERENCE FOR METHOD VALIDATION

Established standards and protocols guide the analytical methodology. The references guiding the method validation process include the International Council for Harmonisation guidelines for Method Validation Q2 (R1). These guidelines provide a comprehensive framework for validating analytical procedures, ensuring accuracy, precision, specificity, and other critical parameters. Additionally, the validation of compendia procedures follows the current United States Pharmacopeia guidelines, specifically USP <1225>. Adhering to these references ensures that the analytical method used for LAN determination aligns with globally recognized standards, enhancing the reliability and acceptance of the obtained results in the pharmaceutical industry.

method

6. MATERIALS AND METHODS

The $La_2O_2CO_3$ was synthesized by a conventional precipitation method via $La(OH)CO_3$ with the precipitation time was longer than 12 h [7]. The assay of LAN content involved potentiometric titration of lanthanum dioxycarbonate drug substance. The critical control points had been identified to ensure the accuracy and reliability of the analysis. First, a flat-bottomed 100 mL glass beaker was used for analysis to minimize errors and enhance result precision. The cleanliness of the Optrode electrode is crucial; it should be thoroughly cleaned with water before the analysis to prevent any potential contamination. Care must be taken to avoid the generation of air bubbles throughout the analysis process, as they can introduce inaccuracies. Additionally, during complete titration, it is essential to ensure that the Optrode electrodes' slit portion is fully immersed inside the solution. Following these, critical control points are essential for maintaining the integrity of the potentiometric titration process and obtaining reliable results for the LAN content in the drug substance.

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6.1 Reagent And Apparatus Details

The reagents used to meet the specific quality standards for the study are as follows. These include Calcium Carbonate of SRM grade from Merck or equivalent, EDTA of Emparta grade from Merck or equivalent, Hexamethylenetetramine (Hexamine) of ACS grade from Sigma Aldrich or equivalent, Hydrochloric Acid of AR grade from Rankem or equivalent, Hydroxy Naphthol Blue of Indicator grade from Merck or equivalent, Sodium Hydroxide of Emparta grade from Merck or equivalent, Xylenol Orange Tetra Sodium Salt of AR grade from Merck or equivalent, Sodium Chloride of Emplura grade from Merck or equivalent, Tween-80 of AR grade from Merck or equivalent, and Milli-Q or equivalent quality Water. The use of specific reagent grades is crucial for the accuracy and reliability of the analytical results. The Standardization method parameters for 0.01M EDTA and Potentiometric Titrator details are given in Table 1. The Assay Method Parameters for LAN and the details of the Instrument Parameters for Autotitrator are detailed in Table 2.

Table 1. Standardization method Parameters for 0.01M EDTA

Sensor		
Measuring input	:	1
Sensor	:	Optrode
Wavelength	:	610 nm
Temperature measurement	:	Automatic
Stirrer		
Stirrer	:	1

UNDER PEER REVIEW

Stirring rate	:	6
Switch off automatically	:	on
Start Conditions		
Initial measured value		
Signal drift	:	Off mV/min
Min. waiting time	:	0 s
Max. waiting time	:	1 s
Dosing rate	:	Maximum mL/min
Start measured value		
Start measured value	:	Off mV
Dosing rate	:	5 mL/min
Start Slope		
Start slope	:	Off mV/mL
Dosing rate	:	5 mL/min
Pause		
Pause	:	60 s
Measured value acceptance		
Signal drift	:	50 mV/min
Min. waiting time	:	0 s
Max. waiting time	:	26 s
Dosing of increments		
Volume increment	:	0.1 mL
Dosing rate	:	Maximum mL/min
Temperature		
Temperature	:	25.0°C
Stop Conditions		
Stop volume*	:	15 mL
Stop measured value	:	Off mV
Stop EP	:	Off
Volume after EP	:	Off mL
Stop time	:	Off s
Filling rate	:	Maximum mL/min
Potentiometric Evaluation		
Evaluation without window	:	On
EP criterion	:	5 mV
EP recognition	:	greatest
Evaluation with measured value window (U)	:	off
Evaluation with value window (mL)	:	off

UNDER PEER REVIEW

Table 2. Assay method Parameters for Lanthanum

Sensor		
Measuring input	:	1
Sensor	:	Optrode
Wavelength	:	574 nm
Temperature measurement	:	Automatic
Stirrer		
Stirrer	:	1
Stirring rate	:	6
Switch off automatically	:	on
Start Conditions		
Initial measurement value		
Signal drift	:	Off mV/min
Min. waiting time	:	0 s
Max. waiting time	:	1 s
Dosing rate	:	Maximum mL/min
Start measured value		
Start measured value	:	Off mV
Dosing rate	:	5 mL/min
Start Slope		
Start slope	:	Off mV/mL
Dosing rate	:	5 mL/min
Pause		
Pause	:	60 s
Measured value acceptance		
Signal drift	:	50 mV/min
Min. waiting time	:	0 s
Max. waiting time	:	26 s
Dosing of increments		
Volume increment	:	0.1 mL
Dosing rate	:	Maximum mL/min
Temperature		
Temperature	:	25.0°C
Stop Conditions		
Stop volume	:	12 mL (adjusted based on analyte concentration)
Stop measured value	:	Off mV
Stop EP	:	Off

UNDER PEER REVIEW

Volume after EP	:	Off mL
Stop time	:	Off s
Filling rate	:	Maximum mL/min
Potentiometric Evaluation		
Evaluation without window	:	On
EP criterion	:	5 mV
EP recognition	:	greatest
Evaluation with measured value window (U)	:	off
Evaluation with value window (mL)	:	off

6.2 Reagent Preparation

Reagent preparation for the determination of LAN content involved several steps to ensure accurate and precise results. Firstly, 2.5N Hydrochloric acid solution was prepared by transferring 22.6 mL of concentrated HCl into 100 mL volumetric flask and made upto the volume with purified water. Following this, a NaOH solution with a concentration of 1N was obtained by dissolving 4.0 g of NaOH in 100 mL of water. The hydroxy naphthol blue solution was then prepared by weighing about 0.1g of hydroxy naphthol blue, transferring it into a 100 mL volumetric flask, adding 60 mL of water, sonicating for 15 minutes, and diluting to volume with water. For the EDTA disodium salt (dihydrate) solution, 18.6 g of the EDTA was carefully weighed and transferred into a 1000 mL volumetric flask, dissolved, and diluted to volume with water to achieve a concentration of 0.05M. Finally, 0.01M EDTA solution was prepared by transferring 200 mL of the 0.05M EDTA solution into a 1000 mL volumetric flask and diluting it to volume with water.

6.3 Standardization of 0.01 M EDTA disodium salt (dihydrate)

The standardization of the 0.01 M EDTA disodium salt (dihydrate) was conducted with meticulous steps for accuracy and precision. Firstly, approximately 0.05 g of Chelometric standard calcium carbonate, previously dried at 110 °C for 2 hours and cooled in a desiccator, was weighed into a 250 mL beaker. To this, 10 mL of water was added to form a slurry, and 2 mL of diluted HCl was introduced from a pipette. The contents were swirled until the calcium carbonate dissolved completely.

The beaker and pipette were washed with water, and the solution was diluted to about 100 mL with water. The solution was stirred with a magnetic stirrer, and while stirring, 40 mL of the 0.01 M EDTA solution was added, followed by the addition of 15 mL of 1N NaOH solution. After thorough mixing, 1 mL of Hydroxy Naphthol blue solution was added, and the Potentiometric titration was initiated with the 0.01 M EDTA solution until the pink color turned blue. The total volume of 0.01 M EDTA consumed (V) was recorded, and the results were reported.

The standardization process was performed in triplicate, and the acceptance criteria stated that the relative standard deviation (%RSD) for the three standardizations should not exceed 0.5%. It's important to calculate the results with precision up to 5 decimal points. Additionally, a note specifies that the 0.01 M EDTA solutions can be used for 1 month from the date of preparation. These measures ensure the accuracy, reliability, and consistency of the standardized EDTA solution used in the subsequent determination of LAN content.

6.4 Solution Preparation for the titration

The preparation of solutions for the determination of LAN content involves meticulous steps to ensure accuracy in the analytical process. Xylenol orange indicator solution was prepared by dissolving 0.5g of xylenol orange tetra-sodium salt in 10 mL of water and 0.1 mL of concentrated HCl, then

UNDER PEER REVIEW

diluting to 25 mL with water. Hexamine solution was created by accurately weighing 40.0 g of Hexamine into a 100 mL volumetric flask, adding 60 mL of water, sonicating for 15 minutes, and diluting to volume with water. A 1M HCl solution was prepared by mixing 500 mL of water with 85 mL of concentrated HCl in a 1000 mL volumetric flask. The 50% NaOH solution involved weighing 25.0g of NaOH into a 50 mL volumetric flask, adding 20 mL of water, sonicating for 15 minutes, and diluting to volume with water. Buffer pH 1.2 was made by dissolving 2.0 g of NaCl in 100 mL water, adding 7.0 mL of HCl, and diluting with water to 1 L. Diluent (0.15% Tween 80 in Buffer pH 1.2) was prepared by adding 1.5 mL of Tween 80 to 1 L and diluting with Buffer pH 1.2. The blank solution was created by taking 10 mL of diluent, adding one drop of xylenol orange tetra-sodium salt indicator, 8 mL of Hexamine solution, and 60 mL of water, ensuring meticulous mixing.

6.5 Formulae used for Calculation

The provided formulas are integral to the calculations involved in the determination of lanthanum content through potentiometric titration. The first formula calculates the molarity of EDTA, a crucial component in the titration process. The molarity (M) is determined by dividing the weight of Calcium Carbonate (W) in milligrams by the product of the molecular weight of calcium carbonate (100.09 g/mol) and the volume of EDTA consumed (V) in milliliters.

$$Molarity(EDTA) = \frac{W}{100.09 \times V}$$

The second formula is employed to calculate the percentage content of lanthanum in the sample solution. It takes into account the difference in volume between the sample (V) and blank (V0) EDTA solution, the actual molarity of EDTA (M), the molecular weight of lanthanum (138.91 g/mol), the dilution volume (900 mL), and the volume of the sample solution taken for titration (Vs) in milliliters.

$$\%\text{Content of Lanthanum} = \frac{(V - V0) \times M \times 138.91 \times 900 \times 100}{Vs \times 1000}$$

These formulas play a pivotal role in deriving accurate results from the experimental data obtained during the potentiometric titration process. Additionally, the potentiometric charts visually depict the titration curves for the blank, 0.01M EDTA standardization, and the sample, aiding researchers in interpreting and analyzing the results effectively.

7. RESULTS

The analytical method validation for the assay method of LAN content in lanthanum dioxycarbonate drug substance encompasses a comprehensive set of parameters to ensure the reliability and accuracy of the analysis. Specificity is assessed to confirm the method's ability to distinguish LAN from potential interference. A filter compatibility study is conducted to evaluate the impact of filtration on the results. Precision is evaluated through method precision, examining the repeatability of the method within a laboratory, and intermediate precision, assessing the methods' reproducibility across different laboratories and operators. Stability in analytical solutions is investigated to ensure the robustness of the method over time.

Linearity is established to verify the relationship between analyte concentration and response, while accuracy is assessed by comparing measured values to true values. The range is determined to identify the concentration range over which the method is valid. Finally, robustness is evaluated to assess the methods' reliability under variations in experimental conditions.

7.1 Specificity

In the specificity assessment of the assay method for LAN content in lanthanum dioxycarbonate drug substance, the ability to identify the analyte in the presence of potential interfering substances was evaluated. The blank solution was prepared by pipetting 10.0 mL of diluent into the titration

UNDER PEER REVIEW

vessel, stirring the solution with a magnetic stirrer, adding one drop of xylenol orange tetra-sodium salt indicator, 8 mL of Hexamine solution, and 60 mL of water. The solution was then titrated potentiometrically with 0.01 M EDTA solution. The acceptance criteria included observing blank interference, if any. After the titration, the solution was in yellow color.

Figure 2. Potentiometric graph for Blank

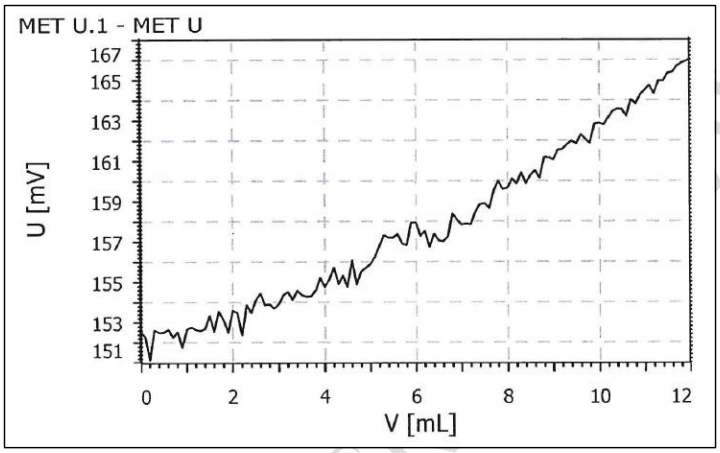


Figure 3. Potentiometric graph for 0.01M EDTA Standardization

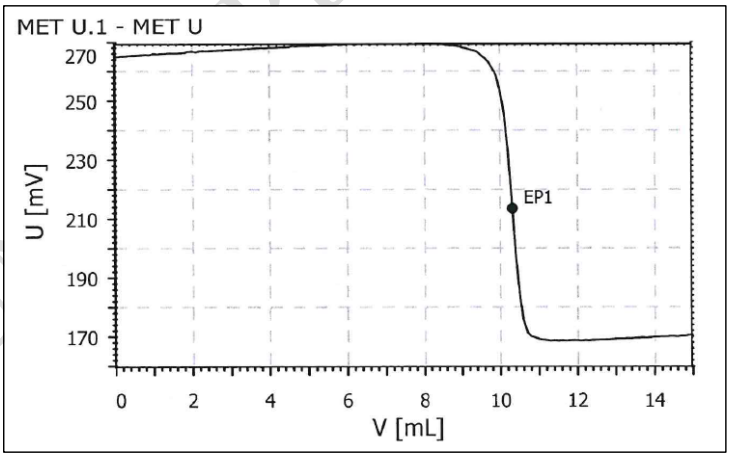
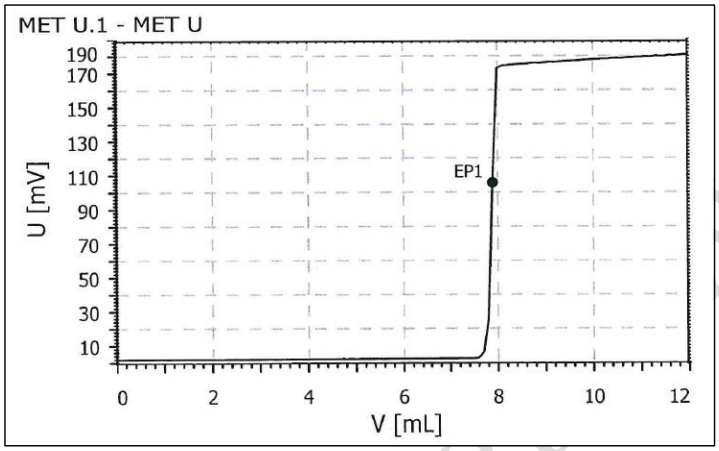


Figure 4. Potentiometric graph for LAN content in Lanthanum dioxycarbonate drug substance



7.2 Filter Compatibility Study

The filter compatibility study for the analytical method aimed to assess the compatibility of the filter with the drug substance when applied for filtration. Centrifuged sample solutions were prepared by centrifuging at 5000 RPM for 10 minutes, followed by pipetting 10.0 mL of the supernatant solution into a titration vessel. The solution was stirred, and one drop of xylenol orange tetra-sodium salt indicator and 8 mL of hexamine solution were added. After adding 60 mL of water, the solution turned purple-red and was titrated using an Optrode electrode with 0.01 M EDTA solution. Similarly, filtered sample solutions were prepared by filtering with 0.45µ PVDF and Nylon syringe filters, discarding the first 3 mL of the filtrate, and following the same titration procedure. The acceptance criteria stipulated that the % difference in Assay results of filtered samples should be within ±2 from the unfiltered (centrifuged) sample. The assay results for centrifuged and filtered samples were within the acceptable limits and tabulated in Table 3.

Table 3. Filter compatibility study for LAN content in Lanthanum dioxycarbonate drug substance

S.No	Filter type		% Lanthanum Content	% Difference
1	Centrifuged	Preparation-1	98.10	As a control
2		Preparation-2	98.38	
3		Preparation-3	99.02	
4	0.45µ PVDF	Preparation-1	98.30	-0.20
5		Preparation-2	98.45	-0.07
6		Preparation-3	99.30	-0.28
7	0.45µ Nylon	Preparation-1	98.35	-0.25
8		Preparation-2	98.38	0.00
9		Preparation-3	99.42	-0.40

UNDER PEER REVIEW

7.3 Precision

Precision in an analytical method refers to the consistency and reproducibility of results obtained when the method is applied repeatedly to multiple samples of a homogeneous substance. It is a measure of the agreement among individual test results. Precision is often quantified using statistical parameters such as standard deviation or relative standard deviation (coefficient of variation). Standard deviation provides a measure of the dispersion or spread of individual results around the mean, while the coefficient of variation expresses this variability as a percentage of the mean.

7.3.1 Method Precision

In the precision assessment of the analytical method for determining LAN content, method precision was evaluated by analyzing a homogeneous sample from a single batch six times. The aim was to determine the consistency of results within a single batch. The acceptance criteria stated that the results should meet specification limits, and the %RSD for the amount of LAN content in the drug substance from the six preparations should not exceed 2.0. The % RSD for the amount of LAN content in the drug substance from six preparations were 0.6% and shown in Table 4.

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Table 4. Method precision for LAN content in Lanthanum dioxycarbonate drug substance

Preparation	Titer value (mL)	Lanthanum Content (%)
1	7.8945	98.30
2	7.9065	98.45
3	7.9745	99.30
4	7.9881	99.47
5	7.9975	99.58
6	7.9868	99.45
Mean		**99.1**
%RSD		**0.6**

7.3.2 Intermediate Precision

In the evaluation of intermediate precision for the analytical method determining LAN content, the aim was to ensure that the results remained unaffected by variations in instruments, analysts, and days. The acceptance criteria included that the results should meet specification limits, the %RSD for the amount of LAN content in drug substances from six preparations should not exceed 2.0, and the %RSD for the amount of LAN content in drug substances from twelve preparations (combining method precision and intermediate precision) should not be more than 3.0 (Table 5)

Table 5. Intermediate precision for LAN content in Lanthanum dioxycarbonate drug substance

Preparation	Titer value (mL)	Lanthanum Content (%)
1	7.8005	97.13
2	7.9805	99.37
3	7.8871	98.21
4	8.0875	100.70
5	8.0006	99.62
6	8.0005	99.62
Mean		**99.1**
%RSD		**1.3**

7.3.3 Comparison between Method Precision and Intermediate Precision

UNDER PEER REVIEW

The comparison between method precision and intermediate precision was conducted by analyzing LAN content in drug substance preparations. For method precision, six preparations were made, yielding LAN content percentages ranging from 98.30% to 99.58%, with a mean of 99.1% and a %RSD of 0.6%. In intermediate precision, an additional six preparations were analyzed, resulting in LAN content percentages between 97.13% and 100.70%, with a mean of 99.1% and a %RSD of 0.9% (Table 6).

Table 6. Comparison between method and intermediate precisions for LAN content in Lanthanum dioxycarbonate drug substance

Preparation		Lanthanum Content (%)
Method precision	1	98.30
	2	98.45
	3	99.30
	4	99.47
	5	99.58
	6	99.45
Intermediate precision	7	97.13
	8	99.37
	9	98.21
	10	100.70
	11	99.62
	12	99.62
Mean		**99.1**
%RSD for 12 determinations		**0.9**

7.4 Stability

The stability of the sample solution in the analytical method for determining LAN content was assessed by analyzing the solution at regular intervals at room temperature (25°C ± 2°C). Blank and sample solutions were prepared following the method description. The acceptance criteria stated that the difference in the % amount of LAN content between the initial measurement and measurements after specified periods should be within ±3.0%. The % differences between initial and subsequent time points were calculated as 0.20% and 0.12% at 24 and 48 hours, respectively (Table 7)

Table 7. Results of Sample solution stability for LAN content in Lanthanum dioxycarbonate drug substance

Sample Solution Stability at Room Temperature (25 °C)		
Time in Hours	**Lanthanum Content (%)**	**% Difference**
Initial	98.30	---
24 hours	98.10	0.20
48 hours	98.18	0.12

7.5 Linearity

The linearity assessment of the analytical method involved preparing a series of lanthanum dioxycarbonate API solutions within the concentration range of 10% to 150% of the working concentration. For linearity solution-1, 133.24 mg of lanthanum dioxycarbonate API was accurately weighed and transferred into a 1000 mL volumetric flask. After adding 900 mL of diluent and sonicating to dissolve, 10.0 mL of this solution was pipetted into the titration vessel. The solution was

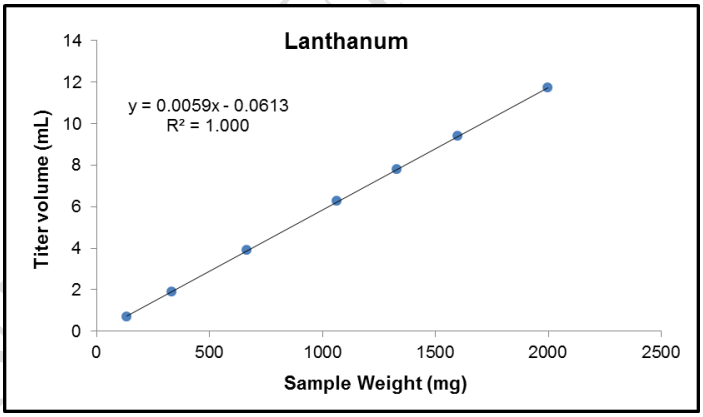
UNDER PEER REVIEW

stirred, and the xylenol orange tetra-sodium salt indicator, along with 8 mL of Hexamine solution and 60 mL of water, was added. The resulting solution exhibited a purple-red color, and titration was performed with 0.01 M EDTA solution using an Optrode electrode. The total volume of 0.01 M EDTA consumed (V) was recorded for each preparation. The recorded data of the 0.01 M EDTA consumed volume was then used to calculate the correlation coefficient. A graph (Figure 2) was plotted with the sample weight on the X-axis and the titer value on the Y-axis to visualize the linearity. The acceptance criterion for linearity was set at a correlation coefficient not less than 0.99. The correlation coefficient was 1.00 (Table 8).

Table 8. Results of Linearity for LAN content in Lanthanum dioxycarbonate drug substance

Linearity Level	Sample Weight (mg)	Titer Volume (mL)	Correlation coefficient
Linearity solution-1 (10%)	133.24	0.7025	1.000
Linearity solution-2 (25%)	332.99	1.9005	
Linearity solution-3 (50%)	665.65	3.8896	
Linearity solution-4 (80%)	1065.07	6.2678	
Linearity solution-5 (100%)	1331.54	7.7995	
Linearity solution-6 (120%)	1597.59	9.3959	
Linearity solution-7 (150%)	1997.89	11.7191	

Figure 5 Linearity graph for the LAN content in Lanthanum dioxycarbonate in drug substance:



7.6 Accuracy

The accuracy assessment of the analytical method involved determining the LAN content of lanthanum dioxycarbonate drug substance at three concentration levels, namely 10%, 100%, and 150% of the test concentration. This was achieved by preparing solutions in triplicates, resulting in a total of 9 determinations. For the 10% recovery level, 132.1 mg of lanthanum dioxycarbonate API was accurately transferred into a 1000 mL volumetric flask. After adding 900 mL of diluent and sonicating to dissolve, 10.0 mL of the sample solution was pipetted into the titration vessel. The solution was stirred, and the xylenol orange tetra-sodium salt indicator, along with 8 mL of hexamine solution and

UNDER PEER REVIEW

60 mL of water, was added. The resulting purple-red solution was titrated using an Optrode electrode with 0.01 M EDTA solution until the color changed from purple-red to yellow. The total volume of 0.01 M EDTA consumed (V) was recorded for this preparation. Similar procedures were followed for the remaining samples at the 100% and 150% recovery levels. All determinations were carried out in triplicates for each concentration level. The acceptance criteria for accuracy were defined as follows. Individual and mean % recovery at the 10% level shall be between 85.0% and 115.0%. Individual and mean % recovery at other levels (100% and 150%) shall be between 95.0% and 105.0%. At the 10% recovery level, the individual and mean % recovery ranged from 89.1% to 97.8%, resulting in a mean % recovery of 92.4%. Similarly, at the 100% recovery level, individual and mean % recovery fell between 98.3% and 99.1%, leading to a mean % recovery of 98.6%. Finally, for the 150% recovery level, individual and mean % recovery ranged from 99.2% to 100.0%, resulting in a mean % recovery of 99.6%. The average % recovery for all levels was calculated as 96.9%, and the %RSD of all levels was determined as 4.2% (Table 9)

Table 9. Results of Accuracy for LAN content in Lanthanum dioxycarbonate drug substance

Set	% Levels	Weight of LAN API (mg)	Lanthanum Added (mg)	Lanthanum Recovered (mg)	Lanthanum Recovery (%)	Mean % Recovery
1	10	132.1	1.08909	0.97056	**89.1**	92.4
2	10	132.6	1.09321	1.06879	**97.8**	
3	10	133.2	1.09816	0.99242	**90.4**	
1	100	1331.5	10.97748	10.80949	**98.5**	98.6
2	100	1331.1	10.97418	10.87991	**99.1**	
3	100	1332.0	10.98160	10.79233	**98.3**	
1	150	1996.7	16.46168	16.46486	**100.0**	99.6
2	150	1996.4	16.45921	16.37175	**99.5**	
3	150	1996.2	16.45756	16.32983	**99.2**	
Average of All levels					**96.9**	
%RSD of all levels					**4.2**	

7.7 Range

The analytical methods' range, indicative of the interval in which the analyte can be accurately determined, was derived through a thorough examination of linearity and accuracy studies. To ascertain the suitability of this range, two crucial acceptance criteria are employed. Firstly, the %RSD was calculated based on a minimum of 9 recovery determinations, spanning three concentration levels with three repetitions each. The calculated %RSD must not exceed 10.0%, emphasizing the methods' consistency and reliability across diverse concentration ranges. Secondly, the correlation coefficient, a pivotal parameter denoting the linear relationship between sample weight and titer value, was scrutinized. This coefficient, evaluated for both linearity and accuracy studies, should not be less than 0.99, underscoring the methods' ability to produce results directly proportional to the analyte concentration. The specified range, once established, enhances the methods' robustness and reliability, ensuring accurate and precise results across a spectrum of analyte concentrations in routine analytical applications. The accuracy and linearity ranges of LAN content were comprehensively assessed to validate the robustness and reliability of the analytical method. In the accuracy range study, average titer volumes were determined at different concentration levels: 0.7304 mL at 10%, 7.8257 mL at 100%, and 11.8455 mL at 150% (Table 10-11). These values illustrated the precision and consistency of the method across a range of LAN concentrations. The correlation coefficient, a key parameter indicating the linear relationship between sample weight and titer value, was found to be 1.000,

UNDER PEER REVIEW

denoting a perfect linear correlation. The %RSD of levels, calculated as 4.2%, further confirmed the methods' accuracy and reliability in recovering LAN content (Figure 6-7).

Simultaneously, the linearity range study involved determining the titer volumes at 10%, 100%, and 150% concentration levels, resulting in 0.7025 mL, 7.7995 mL, and 11.7191 mL, respectively.

Table 10. Range of LAN content in Lanthanum dioxycarbonate drug substance

% Levels	Average Titer Volume from Accuracy (mL)
10	0.7304
100	7.8257
150	11.8455
Correlation coefficient	**1.000**
%RSD of levels (% of Lanthanum Recovery)	**4.2**

Figure 6. Range graph of LAN content in Lanthanum dioxycarbonate drug substance

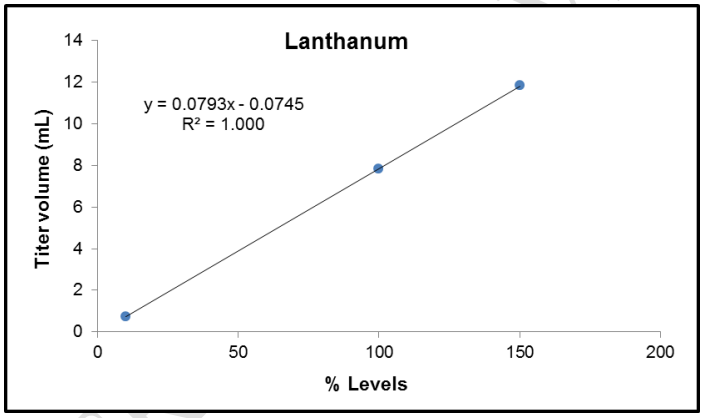
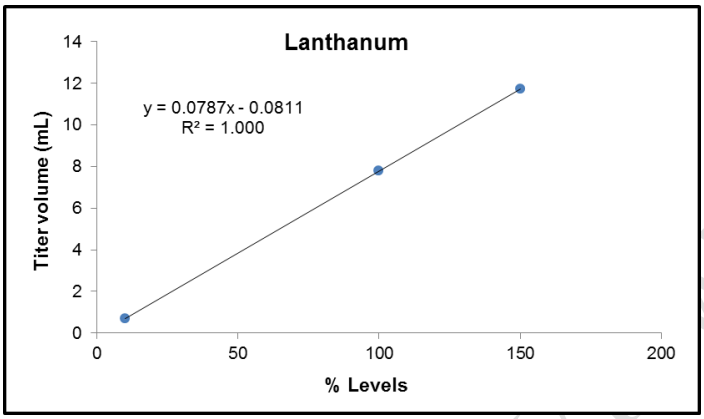


Table 11. Linearity Range of LAN content in Lanthanum dioxycarbonate drug substance

% Levels	Titer Volume (mL)
10	0.7025
100	7.7995
150	11.7191
Correlation coefficient	1.000

Figure 7. Linearity Range graph of LAN content in Lanthanum dioxycarbonate

UNDER PEER REVIEW



7.8 Robustness

The robustness of the analytical method for determining LAN content was evaluated by testing its capacity to remain unaffected by small but deliberate variations in method parameters. The robustness parameters included changes in Tween-80 concentration (± 5%) and changes in buffer pH (± 0.2). Blank and sample solutions were prepared under the original conditions and in the specified robustness conditions. The acceptance criteria were set based on the labeled amount of LAN that should dissolve within 60 minutes, and the results were required to be within specification limits. The original conditions, representing the standard method, demonstrated a mean LAN content ranging from 98.30% to 99.58%, with an overall mean of 99.1%. Subsequent robustness testing involved intentional variations in Tween-80 concentration by ± 5%, specifically at 14.25 mL and 15.75 mL. The results indicated LAN contents within the range of 96.75% to 99.62%, affirming the methods' robustness under these altered conditions. Similarly, variations in buffer pH, both at pH 1.00 and pH 1.40, exhibited LAN contents between 93.01% and 98.38%, further confirming the methods' robust performance (Table 12)

Table 12. Results of Robustness

	Not less than 75% (Q) of the labeled amount of Lanthanum should dissolve in 60 minutes		Sample No.	Lanthanum Content (%)	Mean Lanthanum Content (%)
Robustness	Original Condition		1	98.30	99.1
			2	98.45	
			3	99.30	
			4	99.47	
			5	99.58	
			6	99.45	
	Change in Tween-80 concentration ± 5 %	14.25 mL	1	96.75	98.8
			2	99.61	
			3	98.21	
			4	99.56	

UNDER PEER REVIEW

			5	99.61	
			6	99.24	
		15.75 mL	1	99.62	98.8
			2	99.33	
			3	99.70	
			4	97.22	
			5	98.49	
			6	98.38	
	Change in buffer pH ± 0.2	pH 1.00	1	98.38	98.1
			2	98.62	
			3	97.34	
			4	98.38	
			5	98.45	
			6	97.25	
		pH 1.40	1	93.01	93.3
			2	93.52	
			3	94.67	
			4	90.37	
			5	93.40	
			6	94.70	

8 DISCUSSION

8.1 Specificity

In the data interpretation, the yellow color of the solution at the endpoint indicated the absence of the active moiety (LAN). As no purple-red color formation occurred, which was specific to the reaction mixture, titration cannot be performed. The absence of blank interference was confirmed as there was no titrant consumption. Therefore, the method demonstrated specificity, as the color changes were specific to the reaction mixture, ensuring accurate identification and quantification of LAN content in the drug substance.

8.2 Filter Compatibility Study

Results indicated that the % LAN content for centrifuged and filtered samples was within the acceptable limits. For 0.45µ PVDF filters, the % difference ranged from -0.28 to -0.07, and for 0.45µ Nylon filters, it ranged from -0.40 to 0.00. Therefore, it was concluded that the results obtained from centrifuged samples and both types of filtered samples met the acceptance criteria (Table 3). Consequently, the sample solution can be filtered using 0.45 µ PVDF or 0.45 µ Nylon syringe filters for regular analysis, ensuring compatibility with the analytical method.

8.3 Precision

8.3.1 Method Precision

The obtained results demonstrated high consistency, with titer values ranging from 7.8945 to 7.9868 mL and corresponding LAN content percentages from 98.30% to 99.58%. The mean LAN

UNDER PEER REVIEW

content was calculated as 99.1%, and the %RSD was determined as 0.6%. These results indicated that the method exhibited precision, meeting the specified acceptance criteria (Table 4). The low %RSD suggests that the method provides reliable and consistent results for the LAN content in the drug substance, reinforcing its suitability for analytical purposes.

8.3.2 Intermediate Precision

The results from the six intermediate precision preparations, with titer values ranging from 7.8005 to 8.0875 mL and corresponding LAN content percentages from 97.13% to 100.70%, demonstrated a mean LAN content of 99.1%. The %RSD was calculated as 1.3%, meeting the specified acceptance criteria (Table 5). These findings indicate that the analytical method exhibited intermediate precision, ensuring consistent and reliable results even with variations in instruments, analysts, and days. The overall %RSD for both method precision and intermediate precision falls within the acceptable limit, confirming the robustness and reliability of the method for determining LAN content in the drug substance.

8.3.3 Comparison between Method Precision and Intermediate Precision

The %RSD for the combined twelve determinations was calculated as 0.9%. This comparison led to the conclusion that the method was not only precise but also rugged, demonstrating consistency and robustness even in the presence of variations in instruments, analysts, and days (Table 6). The %RSD values for both method precision and intermediate precision fall within acceptable limits, confirming the reliability of the analytical method for determining LAN content in the drug substance.

8.4 Stability

The results indicated the following LAN content percentages: initial - 98.30%, 24 hours - 98.10%, and 48 hours - 98.18%. These results lead to the conclusion that the sample solution was stable for up to 48 hours at room temperature, with a maximum % difference of 0.20% (Table 7). This stability assessment ensured the reliability and consistency of the analytical method over a defined period, contributing to the robustness of the overall analysis for LAN content in the drug substance.

8.5 Linearity

Data interpretation revealed that the statistical treatment of the linearity data demonstrated a linear relationship between the titer values of LAN content and the sample weight. The correlation coefficient, exceeding 0.99 (Figure 5), indicated that the methods' response was directly proportional to the concentration of LAN in the samples across the specified range of 10% to 150% (Table 8). Consequently, the method was considered linear, meeting the acceptance criteria for linearity. This finding underscored the methods' capability to provide accurate and proportional results for the determination of LAN content in lanthanum dioxycarbonate within the defined concentration range.

8.6 Accuracy

Data interpretation revealed that the recovery values for all concentration levels were well within the specified limits. (Table 9). These results collectively indicated that the method exhibited accuracy, meeting the acceptance criteria for recovery at all three concentration levels. The accuracy assessment confirmed the closeness of the test results obtained by the method to the true values, highlighting the reliability of the analytical method for determining LAN content in the drug substance.

8.7 Range

UNDER PEER REVIEW

The correlation coefficient for the linearity range was also determined as 1.000, indicating a strong linear relationship across these concentration levels (Table 10-11 and Figure 6-7). These findings collectively established the methods' capability to produce accurate and consistent results for LAN content determination, supporting its suitability for pharmaceutical quality control and ensuring compliance with stringent validation criteria.

8.8 Robustness

The robustness of the analytical method was evaluated by subjecting it to variations in specific parameters, including Tween-80 concentration and buffer pH, to assess its resilience and reliability under different conditions (Table 12).

The data interpretation emphasized that the methods' ability to withstand deliberate variations in these critical parameters ensured its robustness. The LAN content percentages, even under varied conditions, aligned closely with the original conditions, demonstrating the methods' reliability and consistency. This robustness assessment enhanced confidence in the methods' applicability and effectiveness for determining LAN content in the drug substance, contributing to its suitability for routine pharmaceutical analysis.

9 LIMITATION

This procedure may not be used for the determination of impurities in Lanthanum dioxycarbonate which may be the limitation of this study.

10 CONCLUSION

To conclude, the comprehensive analytical method validation for determining LAN content in lanthanum dioxycarbonate drug substance through potentiometric titration has yielded robust and reliable results. The validation encompassed various crucial parameters in this study. The method exhibited high specificity, as evidenced by the absence of interference and distinct color changes during titration, ensuring accurate identification and quantification of LAN. Precision studies, including method precision and intermediate precision, showed consistent and reliable results, highlighting the methods' reproducibility and ruggedness. Stability assessments demonstrated the sample solutions' reliability for up to 48 hours at room temperature. Linearity studies confirmed the methods' ability to provide proportional results across a concentration range of 10% to 150%, with correlation coefficients exceeding 0.99. Accuracy studies revealed precise recovery values within acceptable limits at various concentration levels. The derived range from linearity and accuracy studies, coupled with the robustness evaluation under varying conditions, further underscored the methods' suitability for routine analysis. The overall success of this analytical method validation strengthens its applicability in pharmaceutical quality control, ensuring the accurate determination of LAN content in Lanthanum Dioxycarbonate drug substance.

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UNDER PEER REVIEW

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