Journal of Current Pharma Research
(An Official Publication of Human Journals)
An International Peer Reviewed Journal For Pharmacy, Medical & Biological Science
DOI: 10.25166 CODEN: JCPRD6 NLM ID: 101744065
ISSN: Print: 2230-7834 Online: 2230-7842
SJIF Impact Factor: 6.913

Human Journals
Research Article
January 2022 Vol.:13, Issue:4

Analytical Method Validation of Drug Release [Dissolution] by Revers Phase HPLC

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Submitted: 24 December 2021
Accepted: 30 December 2021
Published: 30 January 2022

Keywords: Drug release of Linezolid, analytical parameters.

ABSTRACT

The objective is to develop a method for the determination of percentage drug release of Linezolid formulations. The method, after development, is validated as per ICH guidelines Q2 (R1). Also, the objective of the study is to develop the analytical determination/parameters of drug release rather than the dissolution parameters. Parameters considered for analytical method validation of drug release [dissolution] method for Linezolid Formulations. System suitability, Specificity, Forced degradation, Precision, System precision, Method precision, Stability in analytical solution, Linearity, Accuracy, and Range.

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INTRODUCTION:

Mobile Phase Preparation:

Buffer:

Weigh and transfer 4.7g of monobasic sodium phosphate into l000 mL of water. Dissolve and mix.

Mobile Phase:

Mix 800 mL of buffer and 200 mL of Acetonitrile, Filter, and degas by sonication.

Preparation of Standard

Weigh and transfer about 20 mg of Linezolid standard into 50 mL volumetric flask, dilute to volume with diluent and mix well. Further, dilute 5.0 mL of this solution to 25 ml with diluent.

Preparation of Sample

Weigh accurately 10 tablets and transfer these tablets into200 ml amber colour volumetric flask, add 150 ml of diluent, and sonicate it for 15 minutes; allow it to cool at room temperature. Make up the volume up to the mark with diluent. Dilute 4 ml of this solution to 50 ml with diluent. Filter the sample solution through a 0.45µNylon filter.

Table No. 1: Details of Optimized Chromatographic Conditions

Column	:	YMC Hydrosphere C18, 4.6 x 150 mm, 5µ or equivalent
Wavelength	:	254 nm
Injection volume	:	10 µL
Column Temperature	:	25°C
Sample try Temperature	:	25°C
Flow rate	:	1.0 mL/min
Run Time	:	10 minutes
Diluent	:	Mobile phase

Procedure

Inject Diluent (one injection), then inject five replicate injections of standard preparation and check the system suitability parameters.

Calculation and Formulae:

Calculate the % assay of Linezolid by the following formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{V} \times \frac{P}{100} \times \frac{100}{LA}$$

Where,

AT : The average area of Linezolid peak from the sample chromatogram.

AS : The average area of Linezolid peak from the standard chromatogram

WS : Weight of Linezolid Standard in mg.

DT : Dilution of sample in mL.

DS : Dilution of standard in mL.

V : The volume of a sample taken (mL)

P : % Purity of Linezolid standard

LA : Label Amount

SYSTEM SUITABILITY:

To verify the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set.

Injected diluent (one injection) and Standard preparation (5 injections), recorded chromatograms, and checked the system suitability parameters.

Table No.: 2: Details of System Suitability

Acceptance criteria	Results
The % RSD for the area of Linezolid replicate injections of standard preparation should be NMT 2.0.	0.1
Theoretical plates for the Linezolid peak should be NL T 1500.	10286
The tailing factor for the Linezolid peak should be NMT 2.0.	1.2

Data interpretation:

From the above results, it can be concluded that the system is suitable for analytical method validation.

SPECIFICITY:

Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Performed the specificity parameter of the method by injecting Diluent, Standard preparation, Sample preparation> Placebo preparation, Known impurities, and Sample spiked with impurities into the Chromatographic system and recorded the Retention times.

Acceptance Criteria:

Diluent, placebo, and impurities peaks should not interfere with the Linezolid peak.

The peaks of Impurities and Linezolid should not interfere with each other.

PRECISION:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

SYSTEM PRECISION:

Single-injection of Blank (Diluent) and five replicate injections of standard solution were made on the system. Refer to the below Table. All the data were acceptable as per the system suitability requirements.

Single-injection of Blank (Diluent) and five replicate injections of standard solution were made on the system. Please refer to Table 9.4.2.1. All the data were acceptable as per the system suitability requirements.

Table No. 3: Method Precision

Tablet No.	% Release
1	98
2	99
3	100
4	98
5	97
6	98
Mean	**98.3**
SD	**1.835**
% RSD	**1.82**

METHOD PRECISION:

In method precision, a homogeneous sample of a single batch should be analyzed six times. This indicates whether a method is giving consisting results of a single batch. Analyzed six independent sample solutions prepared and injected on the HPLC. The data shows that % RSD is within the acceptance criteria.

LINEARITY:

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

The Linearity of response was determined by preparing different concentrations of standard stock solution ranging from 10 % to 150 % of the working concentration. The data is summarized below Table. The data shows that the response is found to be linear; the Correlation coefficient is more than 0.999. The Y-intercept is also within the set criterion. The graphical depiction is included in below Figure.

Table No. 4: Linearity Details

Level	Concentration in ppm	Area Response
0	0	0
1	0.0205	422
2	0.0410	1230
3	0.0616	1866
4	0.0821	2436
5	0.1026	3119
6	0.1231	3899
7	0.1539	4797
8	0.2052	6475
9	0.4104	12935
10	0.8208	26007
11	1.3133	42925
12	1.6416	53968
13	1.9700	64841
14	2.4624	79661
15	3.2833	105596
16	4.1041	133281
17	4.9249	160631
Correlation coefficient		**1.000**
Regression coefficient		**1.000**
Slope		**32552.249**
Intercept		**-165.105**
% Intercept		**-0.3**

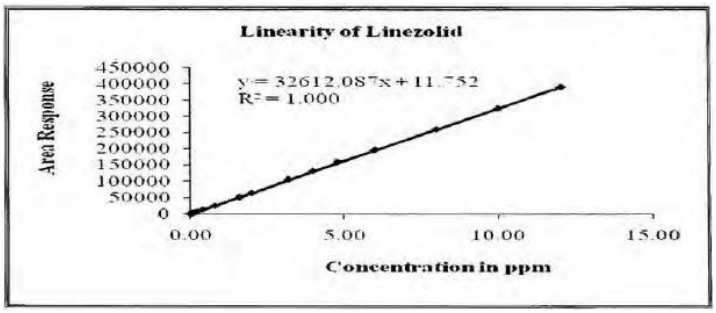

Figure No. 1: Graphical Representation of Linezolid

ACCURACY:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (standard value).

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (standard value).

A spiked known quantity of Linezolid standard at 50%, 80%, 100%, 120%, and 150% of Assay specification limits into the placebo.

Performed precision at the lowest and the highest levels and for the other levels prepared in triplicate and injected in duplicate for all levels.

Calculated the % recovery from the results of Accuracy.

Table No. 5: Details of Recovery of Linezolid

% Level (about)	Sample	Mean Area Response	*mg/mL Added	*mg/mL Recovered	% Recovery	Mean % Recovery	% RSD
50	1	1240140	0.0392	0.0384	98.0	98.0	0.1
	2	1240095		0.0384	98.0		
	3	1243129		0.0385	98.2		
	4	1239626		0.0384	98.0		
	5	1239616		0.0384	98.0		
	6	1240746		0.0384	98.0		
100	1	2505096	0.0784	0.0776	99.0	98.9	0.2
	2	2501077		0.0774	98.7		
	3	2502763		0.0775	98.9		
150	1	3806360	0.1176	0.1178	100.2	100.4	0.1
	2	3815696		0.1181	100.4		
	3	3821258		0.1183	100.6		
	4	3811428		0.1180	100.3		
	5	3817426		0.1182	100.5		
	6	3811600		0.1180	100.3		

Data Interpretation:

From the above results, it can be concluded that the recovery is well within the limit. Hence the Method is accurate.

RANGE:

The range of an analytical method is the interval between the upper and lower levels of analyte that has been demonstrated to be determined with suitable accuracy and linearity. Derived the specified range from the Linearity and Accuracy studies.

Acceptance criteria:

The % RSD obtained for all accuracy level determinations should be NMT 2.0.

The Correlation coefficient should be NLT 0.998 for Linearity and Accuracy level determinations.

RESULTS:

Table No: 6: Details of Linearity Range of Linezolid

% Level	Concentration in ppm	Mean Area response
50	39.2117	1243904
100	80.3839	2511890
150	119.5955	3824363
	Correlation coefficient	1.000

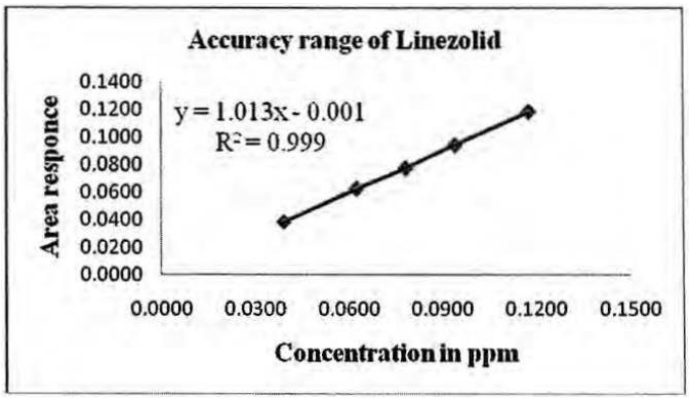

Fig. No. 2: Accuracy Range Graphical Representation

ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and indicates its reliability during normal usage.

Robustness parameters:

Change in column oven temperature ± 5°C.

Change in flow rate ± 0.2 mL/min.

Change the organic phase ratio± 5.0 %

Acceptance criteria:

The System suitability parameters should pass for all the conditions.

Table No. 7: Robustness Parameter Details

System suitability Parameter		% RSD for Area	Theoretical plates	Tailing factor
Limit		NMT 2.0	NLT 1500	NMT 2.0
Original conditions		0.1	10286	1.2
Flow rate	1.2 mL/min	0.0	9213	1.2
	0.8 mL/min	0.1	11423	1.2
Column Temperature	30°C	0.1	10795	1.2
	20°C	0.1	9680	1.2
Organic Phase	+5%	0.2	9938	1.2
	-5%	0.1	10065	1.2

Data Interpretation: From the above results, it can be concluded that the method is robust.

CONCLUSION:

The proposed HPLC method for estimation of Linezolid in the Linezolid formulation by dissolution test methodology is validated. The method is found to be specific. The method is also stability-indicating as evidenced by forced degradation studies. The method is found to be linear in the specified range for Linezolid. The accuracy of this method is established for Linezolid. The method is found to be precise and robust. A system suitability test is established and related parameters are recorded. Hence this method stands validated and can be used for routine and stability analysis.

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