IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2023 Vol.:26, Issue:4

An Overview of a Discriminatory *In Vitro* Dissolution Release Method for Novel Drug Delivery System Containing Lincomycin Hydrochloride by RP HPLC

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

*** [1]Palanimuthu Rajeevkumar, [2]Shankaran Vasu Rekha, [1]Shimelis Kumbi, [1]Jemal Abdella**

** [1] School of Pharmacy, College of Health & Medical Sciences, Haramaya University, P.Box. No: 235, Harar, Ethiopia, East Africa.*

[2] College of Health & Medical Sciences, Dire Dawa University, Dire Dawa, Ethiopia, East Africa.

Submitted: 22 February 2023
Accepted: 28 February 2023
Published: 30 March 2023

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: *In vitro* release, Lincomycin Hydrochloride, LMH, validation, Reversed-phase high performance liquid chromatography

ABSTRACT

An easy, efficient, selective and precise reversed-phase high performance liquid chromatographic (RP-HPLC) technique for the evaluation of dissolution release of Lincomycin Hydrochloride (LMH) in tablets has been established and validated. The Chromatographic analysis was performed on an isocratic programming, fine pack SIL RPC_{18} (250x4.6mm,5µ) column using the mobile phase consisting of acetonitrile-methanol: water (14:86 v/v) adjusted with 1% OPA to pH 6.8 at a flow rate of 0.8 mL/min. UV detection was performed at 205nm and the retention time for LMH was 5.1min. The optimized dissolution conditions include the USP apparatus 2 at a paddle rotation rate of 50rpm and 900mL of phosphate buffer (pH 6.8) with 0.03% of polysorbate 80 as dissolution medium, at 37.0 ± 0.5°C. The method was validated according to ICH guidelines. The method was linear (correlation coefficient = 0.9999) in the selected range of LMH. Validation parameters like accuracy, precision, linearity, range, limit of detection, limit of quantification and robustness all were within the limits. The system suitability parameters, such as theoretical plate, tailing factor and relative standard deviation (RSD) between six standard replicates were well within the limits. The stability result illustrated that the drug is stable in the prescribed dissolution medium. The LOD and LOQ were found to be 0.08648µg/ml and 0.262µg/ml respectively. The wide range of linearity, accuracy, short retention time and isocratic elution imply that the method is suitable for routine determination of lincomycin hydrochloride in bulk and matrix formulation with good accuracy and precision.

INTRODUCTION

Class III drugs are increasingly becoming a problem in terms of obtaining the satisfactory dissolution and permeation within the gastrointestinal tract that is necessary for good bioavailability. It is not only existing drugs that cause problems but it is the challenge of medicinal chemists to ensure that new drugs are not only active pharmacologically but have enough solubility to ensure fast enough dissolution at the site of administration, often the gastrointestinal tract [1]. Drug absorption from a dosage form after oral administration depends on the release of the drug from the pharmaceutical formulation, the dissolution and/or its solubilization under physiological conditions and the permeability across the gastrointestinal tract [2]. Because of inherently slow dissolution, are good candidates for developing *in vitro* and *in vivo* correlations (*IVIVCs*) if intestinal permeability is high and drug dissolution is the controlling mechanism for the release of drug from the dosage form. The dissolution rate of solid dosage forms is usually studied for evaluating the drug release. The *in vivo* performance of a drug product could be predicted properly by dissolution test. A reproducible dissolution condition is necessary for carrying out dissolution test effectively [3].

Over the previous decade numerous published literatures stated that there is no validated method for dissolution and furthermore no official monograph was available for LMH. HPLC is acknowledged as the most versatile and useful technique in pharmaceutical analysis and the method of choice in pre-formulation stability assessment. Therefore, the aim of the current study was to develop and validate a dissolution test condition for LMH tablets, using a RP-HPLC method for the quantitative study of the drug molecule in the dissolution medium, contributing to develop the quality control and to guarantee the therapeutic value of the pharmaceutical product. Importantly, the present work defines the estimation and validation of a precise and reliable RP-HPLC method for the estimation of LMH release in tablet dosage form. The paramount dissolution conditions were used to estimate the development and validation of a dissolution method and this method was used to calculate the dissolution profile for the established drug delivery system.

MATERIALS AND METHODS

Materials

Analytically pure lincomycin hydrochloride was obtained as gift sample from Wallace Pharmaceuticals Pvt Ltd, Goa, (India). Methanol, Acetonitrile and Water (HPLC grade) were purchased from Merck Chemical Company, Mumbai (India). Potassium dihydrogen orthophosphate, and sodium hydroxide used were analytical grade and purchased from S D Fine Chem. Ltd. (Mumbai, India). AR grade of orthophosphate, phosphoric acid was purchased from Sigma Aldrich (Bengaluru, India). All other reagents used were of analytical grade.

Instrumentation

USP type 2 rotating paddle apparatus (Electrolab, TDT-08L) was used to read the drug dissolution profile. The dissolution medium was deaerated by vacuum filtration and the temperature sustained at 37.0 ± 0.5°C throughout the study. Drug release assessment was performed using RP-HPLC equipped quaternary pump (Model PU-2080 HPLC pump) with a variable wavelength detector (UV-2075 model UV–Vis detector) at 205nm and the pH of all solutions were studied by Hanna pH analyzer (AS-1559 model sampler).

Determination of solubility and dissolution optimization

Lincomycin hydrochloride solubility was determined using 900mL of purified water, 0.1N hydrochloric acid, acetate buffer (pH 4.5) and phosphate buffer (pH 6.8) with an amount of drug equivalent to three times of the dose in the pharmaceutical formulation. Drug release was carried out as per USP dissolution general specification at 50rpm. Sampling aliquots of 10mL were withdrawn at pre-determined time intervals (15, 30, 45, 60 min, 2 hr, 4 hrs, 6 hrs, 8 hrs, 10 hrs &12 hrs), and replaced with an equal volume of dissolution medium to maintain a constant total volume of 900mL. To assess the stability of LMH in dissolution medium, samples were diluted by phosphate buffer (pH6.8) with 0.03% polysorbate 80. The prepared solutions kept at different conditions, such as room temperature and at 37.0 ± 0.5°C for 24 and 2h, respectively [4]. The stability of these solutions was studied by comparing the values obtained with freshly prepared sample solutions.

Analytical method validation

RP-HPLC method was used to analyze the LMH samples in phosphate buffer (pH 6.8). Validation was carried out for precision, linearity, specificity, accuracy, limit of quantitation and ruggedness according to US Pharmacopoeia [5] and International Conference on Harmonization (ICH) guideline [6]. The HPLC system consisted of quaternary pumps (Model PU-2080 HPLC pump), auto sample injecting system, and temperature controlled column oven. The UV–Vis detector (UV-2075 model UV–Vis detector and AS-1559 model sampler) was operated at a wavelength of 205 nm. The software used was Chrompass software on RP fine pak SIL C_{18} T-5 Columns used were C_{12}, 250 mm × 4.6 mm, 5.0 µm, (Dr. Maisch GmbH, Germany). Chromatographic separation of LMH was achieved at a temperature of 25 ± 2 °C using a Dr. Maisch, RP C_{18} (250 mm × 4.6 mm, 5 µm) analytical column; the mobile phase consisted of acetonitrile-methanol:water in the ratio of (14:86) v/v and adjusted to pH 6.8 with 1% OPA at a flow rate of 0.8 mL min^{-1}. Before use, the mobile phase was filtered through a 0.22 µm nylon membrane filter and sonicated for 15 min. Injection volume was 200 µL in all experiments.

Evaluation of system suitability

A standard solution of twenty microliters was injected in triplicate before and after the analysis and the chromatograms were recorded. System suitability parameters like theoretical plate, tailing factor were also noted. RSD of six replicates of standard was also recorded. The column efficiency as obtained from the active peak not less than 6000 USP theoretical plates. USP tailing factor for the same peak is not more than 2.0 and RSD of six replicates of the standard solution is not more than 2.0%.

RESULTS AND DISCUSSION

Optimization of dissolution test conditions

The accomplishment of dissolution profile is recommended as a support in the development and optimization of drug formulation and in the establishment of *in vitro*/*in vivo* correlation. When dissolution test is not defined or if unavailable in the monograph, then comparison of drug dissolution profiles is recommended on three different dissolution mediums (pH 1–7.0). *In vitro* dissolution was used to analyse the release rate of drug products and to assure the quality of solid dosage forms by the pharmaceutical industry and regulatory agencies [5]. The sink conditions are determined and expressed as a percentage of drug released. Purified

water, 0.1N hydrochloric acid, acetate buffer (pH 1.2–2.2) and phosphate buffer (pH 4.5–6.8) were used as dissolution medium and selected on the basis of solubility and screening study (Figure 1). From the above reading the phosphate buffer (pH 6.8) provided highest drug release profile with greater stability, ensured excellent sink conditions and was designated as the best dissolution medium. The drug release profiles are shown in Figure 2. Based on the solubility and screening study, phosphate buffer (pH 6.8), was selected as the dissolution medium and USP type 2 rotating paddle apparatus at 50rpm as an instrument. In these conditions, typical acceptance criteria for the quantity of drug dissolved were in the range of 65–83%. In the current study, the percentage of drug released for all three different products were >90% in 12hrs and the suggested acceptance criteria can be [6] in 12 hr. The stability test indicated that LMH is stable in the dissolution medium at room temperature and at 37.0 ± 0.5°C for 24 and 2h, respectively. The results obtained from the initial and final response factors were within the acceptable range and not much difference between the stability and freshly prepared solutions.

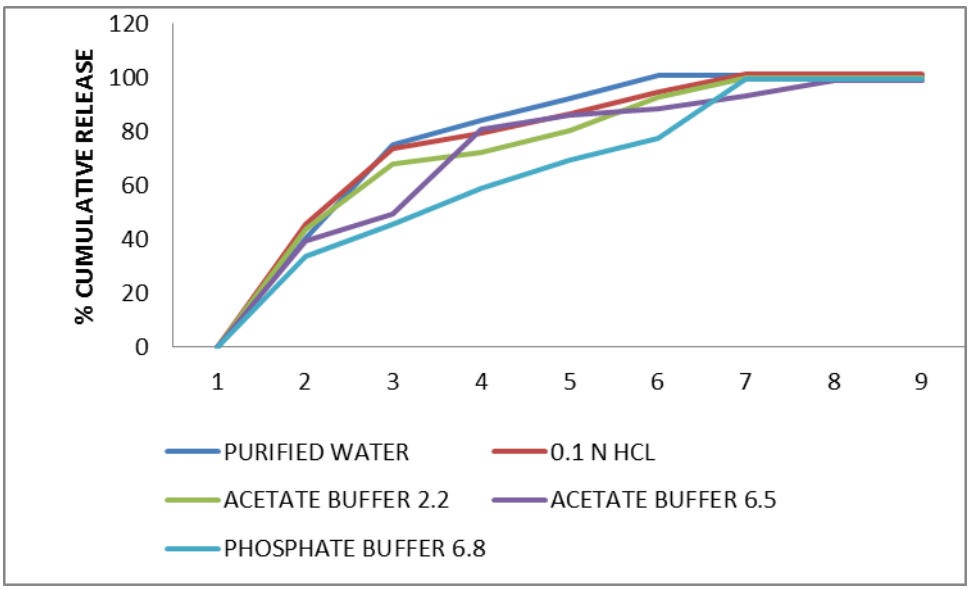

Figure No. 1. The cumulative release curves of lincomycin hydrochloride from tablets at different medium.

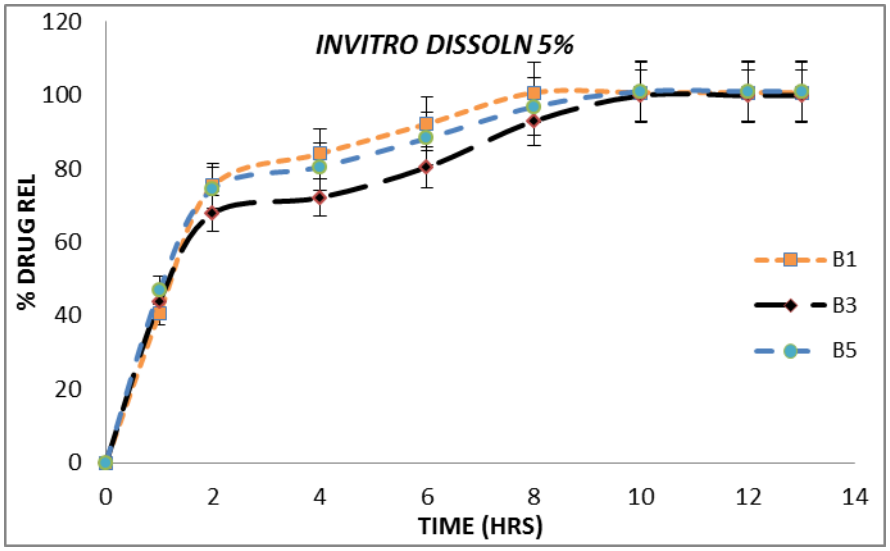

Figure No. 2. *In vitro* release profile (*n* = 6) of three batches of lincomycin hydrochloride tablets.

Analytical method validation

In the current study, RP-HPLC method was used to determine the percentage drug release. HPLC is used to separate, identify and determine the concentration of a specific component in a mixture, moreover that this method is very fast, reproducible and easy to operate. The developed technique was validated to meet the requirements for a universal regulatory filing. The validation parameters such as precision, linearity, specificity, accuracy, limit of quantitation and ruggedness were carried out in accordance with ICH and US Pharmacopoeia guidelines [7, 8].

Linearity

The linearity of LMH response is evaluated from the range of 1–3mcg/mL and showed a good correlation coefficient (r^2) = 0.9999. To validate linearity, the standard curve of LMH was constructed by plotting concentration (mcg/mL) versus area response (mAU) which is shown in Figure 3. The linear regression and slope were calculated and are shown in Table 1, Table 2 and Table 3.

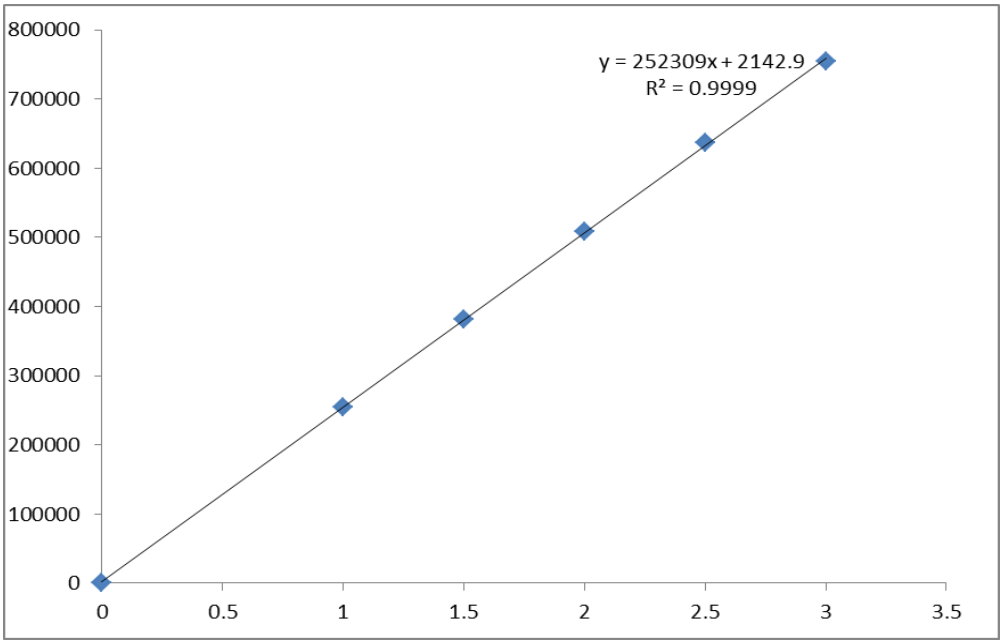

Figure No. 3: Linearity graph of Lincomycin hydrochloride.

Table No. 1. Linear regression of Lincomycin hydrochloride

S. No.	Concentration (mcg/ml)	Area (Average)	Standard deviation	RSD
1	0	0	0	0
2	1	254595	3635.31	0.158
3	1.5	381893	3615.08	0.112
4	2	509190	3606.68	0.092
5	2.5	636488	3625.26	0.258
6	3	753785	3640.82	0.288

[a]Average of six determinations.

Table No. 2: Calculation of regression line

S. No.	*X* (concentration in µg/mL)	*Y* (area obtained in mAU)[a]	*XY*	X^2	Y^2
1	1	254595	254595	1	64818614025
2	1.5	381893	572839.5	2.25	1.45842E+11
3	2	509190	1018380	4	2.59274E+11
4	2.5	636488	1591220	6.25	4.05117E+11
5	3	753785	2261355	9	5.68192E+11

[a]Average of five determinations.

Table No. 3: Slope calculation.

S. No.	*X* axis	*Y* axis[a]	*Y* intercept	Slope
1	1	254595	6000.2	250595.0
2	1.5	381893	6000.2	250592.6
3	2	509190	6000.2	250590.8
4	2.5	636488	6000.2	250594.2
5	3	753785	6000.2	250595.4

[a]Average of five determinations.

Specificity

Specificity is carried out with placebo solution and compared with the standard preparation. The drug release of LMH in the dissolution medium was measured at 205nm and the run time was extended up to 10 min. Two peaks were observed in the chromatogram, the major and minor peaks retention time was 5.1 and 3.3, respectively. The minor peak was due to polysorbate 80 and was confirmed by correlating with the blank peak. The main peak was well separated from the blank peak and the resolution between these two peaks was more than 2 (Figure.4). There were no other additional peaks observed which indicate no interferences by excipients and thus demonstrating that the proposed method is specific for the analysis of LMH.

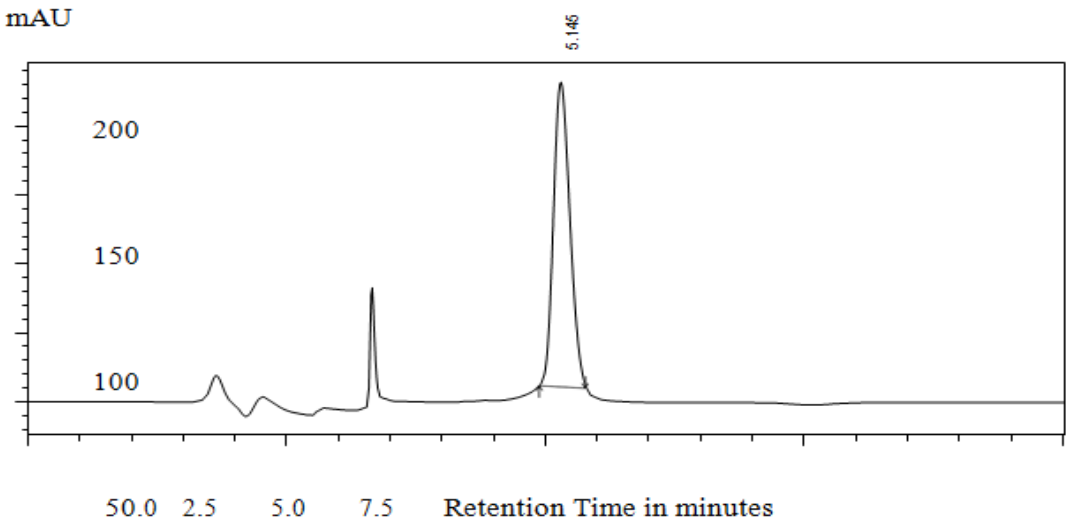

Figure No. 4: Standard chromatogram of Lincomycin hydrochloride.

Precision

The precision of an analytical process expresses the closeness of the agreement (degree of scatter) between a series of measurements achieved from the multiple samples of the same consistent sample under the prescribed conditions. Repeatability is a measure of the precision under the similar working conditions over a short interval of time and it is also known as intra assay precision. A minimum six determinations at 100% of the standard concentration were tested to find out the average, standard deviation and related standard deviation, and all the calculated parameters were well within the prescribed limit. Intra-day precision and intermediate precision were done for ensuring the robustness of the method. The related standard deviation (RSD) of both the tests was well within the desirable limit of NMT 1.8% which is clearly indicated that the developed method is robust. Intraday and intermediate precision results are shown in Table 4.

Table No. 4: Precision, intermediate and intraday precision of Lincomycin hydrochloride.

Parameters	**Lincomycin hydrochloride**
	RSD(%) of peak area
System precision	0.28
Method precision	0.003
Analyst 1 variation	0.12
Analyst 2 variation	0.37
Interday variation	0.39
Intraday variation	0.38

[a]Average of five determinations.

Accuracy

The accuracy of an analytical method is the closeness of agreement between the values that are known either as predictable true values or an accepted reference value. Accuracy is commonly reported as percentage recovery by an assay using the suggested analytical procedure of known quantity of analyte added to the sample. The ICH also recommended assessing a minimum of three determinations over a minimum of three concentration levels covering the specified range. The common way of determining accuracy is to apply the

analytical process to the drug substance and to be quantitated beside the reference standard of known purity. The range for the accuracy limit should be within the linear range. Distinctive accuracy of the recovery of the drug substance in the mixture is estimated to be about 98–102%. Values of accuracy of the recovery data further than this range are to be examined. The precision concentration was 2 mcg/mL, henceforth the linearity range was selected from 1 to 3mcg/mL. The known concentrations of (50%, 75%, 100%, 125% and 150%) were added to the standard preparation (2mcg/mL). The percentage recoveries found were measured under the satisfactory range as per the ICH guidelines [9] (Table 5).

Table No. 5: Accuracy of Lincomycin hydrochloride

Solution	Quantity added (known %)	Area[a]	Average area	Standard deviation	RSD	Recovery	Actual value	Accuracy
Soln-1	50 (1 mcg/ml)	259687 254595 254663	257141	8190.68	0.8614	52	50	96.15
Soln-2	75 (1.5mcg/ml)	381893 374255 378192	378074	5866.35	0.5824	74.66	75	100.56
Soln-3	100(2 mcg/ml)	509190 505248 514282	511736	6240.68	0.9818	100.5	100	99.50
Soln-4	125(2.5mcg/ml)	636488 636995 639034	637761	3835.31	1.018	124.80	125	100.16
Soln-5	150(3mcg/ml)	753785 753881 748760	751272.5	3635.31	0.7162	149.66	150	100.23

[a]Average of three determinations.

Limit of quantitation

Limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under stated experimental conditions. The quantitation limit is expressed as the concentration of analyte in the sample. The standard deviation and related standard deviation for the limit of quantitation was well within the desirable limit of not more than 2.0%.

Ruggedness

Intraday and intermediate precision were determined by analyzing the solutions by two different analysts, using different instruments, using multiple lots of column, in two different labs and on different days. The percentage RSD obtained under different conditions was below 2%. Table 4 represents the intermediate and intraday precision.

System suitability

System suitability is a significant parameter to assure whether the used process was valid or not. The maximum of theoretical plates and tailing factor was made fixed as not less than 6000 and not more than 2, respectively. All the obtained chromatograms, theoretical plates were above 6000 and the tailing factor was less than 1. RSD reports obtained from six replicates indicated adherence to the limits. The above results showed that the developed method is effective and can be followed for routine lab analysis.

ACCERERATED STABILITY STUDY

Samples of LMH tablets were stored for six months under accelerated conditions of temperature and humidity (40 ± 2 °C/75 ± 5% RH) in a climatic chamber. The quantitative analyses of the samples were performed by the previously validated RP-HPLC method, and the dissolution test by the method developed in the present study. Moreover, the LMH tablets were evaluated on the basis of weight variation, disintegration, hardness and friability tests, following the specifications, and the results were compared at the initial and final time points (0 and 6 months, respectively) [10-14].

CONCLUSION

The simple, sensitive and economical isocratic RP-HPLC method was developed to determine the percentage drug release of LMH tablets. The dissolution study indicated that

LMH has good stability and the percentage drug released was satisfactory and the filter suitability was guarantee. The validation results show that the technique is specific, accurate, linear, precise, rugged and robust. The run time is quite short (10.0min) which enables rapid quantification of many samples in routine analysis. Therefore this method is proposed for the quality control studies of LMH modified and conventional pharmaceutical dosage forms contributing to assure the therapeutic efficacy of the drug.

CONFLICT OF INTEREST

There is no Conflict of Interest.

ACKNOWLEDGEMENTS

The authors wish to thank School of Pharmacy, College of Health & Medical Sciences Haramaya University, Harar, Ethiopia, East Africa and College of Medicine & Health Sciences, Dire Dawa University, Dire Dawa, Ethiopia, East Africa for providing necessary facilities and to carry over the work. The authors also wish to express their gratitude to Wallace Pharmaceutical Pvt. Ltd, Goa, India for providing the authentic sample (Lincomycin Hydrochloride).

REFERENCES

[1] Aulton ME. Aulton's pharmaceutics. The design and manufacture of medicines.6th Ed. Edinburgh; Churchill Livingstone; 2007.
[2] Kamalakkannan V., Puratchikody A., Ramanathan L., Jayapraba S. Development and validation of a dissolution test with reversed-phase high performance liquid chromatographic analysis for candesartan cilexetil in tablet dosage forms, Arabian journal of chemistry, 2016, 867-873.
[3] ICH, Draft guidance on specifications: test procedures and acceptance criteria for new drug substances and products: chemical substances. Federal Register (Notices), 2000, 251, 83041–83063.
[4] Effat Souri., Siavash Mottaghi., Mohammad Zargarpoor., Reza Ahmadkhaniha., Hassan Jalalizadeh. Development of a stability-indicating HPLC method and a dissolution test for rivaroxaban dosage forms, Acta Chromatographica, 2016, 347–361.
[5] US Pharmacopoeia FDA. Guidance for industry: dissolution testing of immediate release solid oral dosage forms. US Department of Health and Human Services, Centre for Drug Evaluation and Research (CEDER), 1997.
[6] United States Pharmacopoeia. 31st Ed. United States Pharmacopoeial Convention Inc., Rockville, USA, 2007.
[7] FDA. Analytical procedures and methods validation: chemistry, manufacturing, and controls. Federal Register (Notices), 2000, 65 (169), 52776–52777.
[8] 80 (Q), Pharmacopoeial Forum, 2004. Pharmacopoeial Previews. USP 25-NF 20, 2002. United States Pharmacopoeial Convention, Rockville, MD 2256.1-6.
[9] (ICH) guideline.ICH (Q2R1). Guideline on validation of analytical procedures: text and methodology. In: Proceedings of the International Conference on Harmonization, Geneva, 2007.

[10] Furlanetto S., Maestrelli F., Orlandini S., Pinzauti S., Mura.Optimization of dissolution test precision for a ketoprofen oral extended-release product, Journal of Pharmaceutical and Biomedical Analysis. 2003, 32(1), 65-159.
[11] Ambike A., Mahadik K. R., Paradkar A., Stability study of amorphous valdecoxib, International Journal of Pharmaceutics, 2004, 282(1-2), 62-151.
[12] Shah V. P., Noory A., Noory C., Mc Cullough B., Clarke S., Everett R., Naviasky H., Srinivasan B. N., Fortman D., Skelly J. P. *In vitro* dissolution of sparingly water-soluble drug forms, International Journal of Pharmaceutics, 1995, 125, 99-106.
[13] The United States Pharmacopoeia, 32th Ed., United States Pharmacopoeial Convention: Rockville, 2009.
[14] Azarmi S., Roa W., Löbenberg R. Current perspectives in dissolution testing of conventional and novel dosage forms, International Journal of Pharmaceutics, 2007, 328(1), 12-21.