1. Introduction

1.1 Levocetirizine

Levocetirizine dihydrochloride is chemically dihydrochloride salt of (R) 2-(2-(4-((4-chlorophenyl) phenyl methyl) pipeyl) ethoxy) acetic acid and its chemical structure is shown in Figure 1(Grant *et al.*, 2002). It is used as antihistamine mediated via selective inhibition of H1 receptors. Levocetirizine dihydrochloride is available in number of combinations with montelukast, diethylcarbamazine, nimesulide, pseudoephedrine, cefpirome, while multi-component combination of gliquidone, fexofenadone, buclizine and phenylephrine hydrochloride, guaiphenesin, ambroxol hydrochloride with levocetirizine dihydrochloride is also available (Brij *et al.*, 2013).

Levocetirizine diydrochloride works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents its binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area, and provides relief from the typical symptoms of hayfever (Sunil *et al.*, 2011).

Levocetirizine diydrochloride is the most active enantiomer of cetirizine and has a favorable pharmacokinetic profile.

Levocetirizine is rapidly and extensively absorbed, minimally metabolized and has a volume of distribution (Vd) which is lower than other compounds from the same group. Literature shows that Levocetirizine can be estimated by different analytical methods including High performance liquid chromatography HPLC and Spectrophotometric method (Hashem *et al.*, 2013).
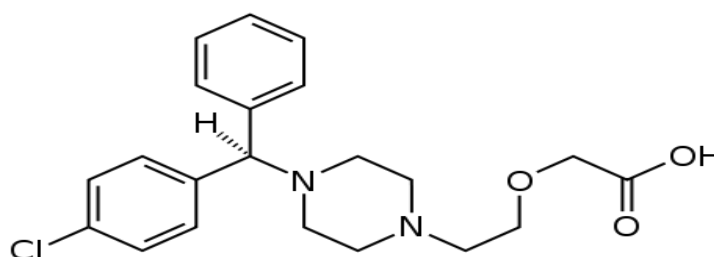

Figure (1.1): structure of Levocetirizine diydrochloride

1.2 Ultraviolet and visible absorption spectroscopy

Ultraviolet-Visible (UV-VIS) spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of compounds and technologically important materials, such as pigments, coatings etc. The UV-VIS spectra have broad features that are of some use for sample identification but are very useful for quantitative measurements (Pavia *et al*., 2001).

1.3 Qualitative analysis

UV-VIS spectroscopy studies the electronic transitions of molecules as they absorb light in the UV and visible regions of the electromagnetic spectrum. The data is used to produce absorbance spectra (Silverstein, 1996). The visible region of the spectrum comprises photon energies of 36 to 72 Kcal/mole, and the near ultraviolet region, out to 200 nm, extends this energy range to 143 kcal/mole.

This energy is enough to promote the outer electrons to higher energy levels. As a rule, the energetically favored electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The resulting species is said to be in an excited state. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength to produce a spectrum (Silverstein, 1996). For example for isoprene, wavelength of maximum absorption is caused by the π- π * electronic transition within the conjugated system present in the molecule. UV absorptions of molecules are generally broad because vibrational and rotational levels are "superimposed" on top of the electronic levels. In addition to undergoing electronic transitions the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels. This large number of available levels produces multiple absorptions and appearance of broad bands

in an UV/VIS spectrum, rather than narrow peaks. For this reason, the wavelength of maximum absorption (λ_{max}) is usually reported (Silverstein; 1996).

1.4 Quantitative UV/Vis analysis

Absorption spectroscopy is one of the most useful tools available to the chemist for quantitative analysis and its applications are not only numerous but also touch upon every field in which chemical quantitative analysis is required.

Quantitative UV/vis analysis is used to determine the concentration of an analyte, which can absorb in this region, in a solution. Beer Lambert Law, which gives a linear relationship between absorbance and concentration for dilute solutions, is used. A calibration plot is formed by measuring the absorbance of a series of analyte solutions with different known concentrations and the concentration of the target analyte in the sample under study is determined from the plot (Williams *et al*., 2004).

1.5 Spectrophotometry

Beer-Lambert Law is central in spectrophotometry and for many current applications a spectrometer is increasingly becoming the measurement device of choice. Simply stated, the law claims that when a sample is placed in the beam of a spectrometer, there is a direct and linear relationship between the amount (concentration) of its constituent(s) and the amount of energy it absorbs (Williams *et al*., 2004).

In mathematical terms:

Absorbance (A) = -log (I/Io) = εbc

Where **A** is the sample's Absorbance value at specific wavelength (or frequency), **I_o** is the intensity of incident light**, I** is the intensity of transmitted light , **ε** is the absorptivity coefficient of the material (constituent) at that wavelength, **b** is the path length through the sample and **c** is the concentration (Williams *et al*., 2004).

1.6 The dissolution procedure: development and validation

1.6.1 Dissolution

The definition of dissolution is deceptively simple. It is the process in which a solid substance goes into solution. For dosage forms containing an active solid ingredient, the rate of dissolution may be critical to absorption. Obviously, in most instances, dissolution of the active solid material is affected by a variety of factors such as the media in which the drug is dissolving, the temperature of the media, and the affinity for the solid particles to dissolve in the media. There are numerous other factors, such as excipients, coatings, and pH, which have an effect on the rate of dissolution. While the most rapid absorption is from a solution, most dosage forms are solids, either tablets or capsules. One must also consider dissolution from suspensions and suppositories. The theory is the same regardless of the dosage form design, but obviously, the rate of dissolution and the limitations are different for each individual dosage form (Anthony Palmieri; 2007).

The dissolution test is performed to determine compliance with the dissolution requirements. Where stated in the individual monograph for dosage forms administered orally. Dosage unit is defined as 1 tablet or 1 capsule or the amount specified for thereby active ingredient. Of the types of apparatus described herein, use the one specified in the individual monograph is used. Where the label states that an article is enteric-coated, and where a dissolution or disintegration test that does not specifically state that it is to be applied to delayed-release articles is included in the individual monograph, the procedure and interpretation given for delayed-Release dosage forms is applied unless otherwise specified in the individual monograph. For hard or soft gelatin capsules and gelatin-coated tablets that do not conform to the dissolution specification, the test is repeated as follows. Where water or a medium with a pH of less than 6.8 is specified as the Medium in the individual monograph, the same Medium specified may be used with the addition of purified pepsin that results in an activity of 750,000 Units or less per 1000 ml. For

media with a pH of 6.8 or greater, pancreatin can be added to produce not more than 1750 USP Units (United States pharmacopeia) of protease activity per 1000-ml. Drug dissolution (or release) testing is an analytical technique used to assess release profiles of drugs in pharmaceutical products, generally solid oral products such as tablets and capsules. This test gains its significance from the fact that if a drug from a product is to produce its effect, it must be released from the product and should generally be dissolved in the fluids of the gastrointestinal (GI) tract. Thus, a drug dissolution test may be considered as an indicator of potential drug release and absorption characteristics of a product in humans as well as in animals. Therefore, a dissolution test is often considered a surrogate for the assessment of availability of drugs in the body (Saeed A. Qureshi; 2006).

The dissolution procedure requires an apparatus, a dissolution medium, and test conditions that provide a method that is discriminating yet sufficiently rugged and reproducible for day-to-day operation and capable of being transferred between laboratories (USP;2014).

The acceptance criteria should be representative of multiple batches with the same nominal composition and manufacturing process, typically including key batches used in pivotal studies, and representative of performance in stability studies (FDA; 2000).

With regard to stability, the dissolution test should appropriately reflect relevant changes in the drug product over time that are caused by temperature, humidity, photosensitivity, and other stresses.

A properly designed test should result in data that are not highly variable and should not be associated with significant analytical solution stability problems. High variability in results can make it difficult to identify trends or effects of formulation changes. Dissolution results may be considered highly variable if the relative standard deviation (RSD) is greater than 20% at time points of 10 minutes or less and greater than 10% RSD at later time points.(FDA;2000) .However, most dissolution results exhibit less variability

than this. The source of the variability should be investigated when practical, and attempts should be made to reduce variability whenever possible. The two most likely causes are the formulation itself (e.g., drug substance, excipients, or manufacturing process) or artifacts associated with the test procedure (e.g., coning, tablets sticking to the vessel wall or basket screen). Visual observations are often helpful for understanding the source of the variability and whether the dissolution test itself is contributing to the variability. Any time the dosage contents do not disperse freely throughout the vessel in a uniform fashion, aberrant results can occur. Depending on the problem, the usual remedies include changing the apparatus type, speed of agitation, or de aeration; consideration and/or examination of sinker type; and changing the composition of the medium. Modifications to the apparatus may also be useful, with proper justification and validation.

Many causes of variability can be found in the formulation and manufacturing process. For example, poor content uniformity, process inconsistencies, a reaction taking place at different rates during dissolution, excipient interactions or interference, film coating, capsule shell aging, and hardening or softening of the dosage form on stability may be sources of variability and interferences. During routine testing of the product, variability outside the expected range should be investigated from analytical, formulation, and processing perspectives.

1.6.1.1. Medium

Physical and chemical data for the drug substance and dosage unit need to be determined before selecting the dissolution medium. Two key properties of the drug are the solubility and solution state stability of the drug as a function of the pH value. When selecting the composition of the medium, the influence of buffers, pH value, and surfactants on the solubility and stability of the drug need to be evaluated. Key properties of the dosage unit that may affect dissolution include release mechanism (immediate, delayed, or modified) and disintegration rate as affected by hardness, friability, presence of solubility enhancers, and presence of other excipients.

Generally, when developing a dissolution procedure, one goal is to have sink conditions, defined as the volume of medium at least three times that required in order to form a saturated solution of drug substance. When sink conditions are present, it is more likely that dissolution results will reflect the properties of the dosage form. A medium that fails to provide sink conditions may be acceptable if it is shown to be more discriminating or otherwise appropriately justified (FDA; 2000).

Using an aqueous–organic solvent mixture as a dissolution medium is discouraged; however, with proper justification this type of medium may be acceptable.

Purified water is often used as the dissolution medium, but is not ideal for several reasons. First, the quality of the water can vary depending on the source of the water, and the pH value of the water is not controlled. Second, the pH value can vary from day to day and can also change during the run, depending on the active substance and excipients. Despite these limitations, water is inexpensive, readily available, easily disposed of, ecologically acceptable, and suitable for products with a release rate independent of the pH value of the medium. The dissolution characteristics of an oral formulation should be evaluated in the physiologic pH range of 1.2 to 6.8 (1.2 to 7.5 for modified-release formulations). (USP; 2014).

1.6.1.2. Volume

Normally, for basket and paddle apparatus, the volume of the dissolution medium is 500 mL to 1000 ml, with 900 ml as the most common volume. The volume can be raised to between 2 and 4 L, using larger vessels and depending on the concentration and sink conditions of the drug; justification for this procedure is expected (USP; 2014).

1.6.1.3. Deaeration

The significance of de aeration of the medium should be determined, because air bubbles can interfere with the test results, acting as a barrier to dissolution if present on the dosage unit or basket mesh. Further, bubbles can cause particles to cling to the apparatus and vessel walls. On the other hand, bubbles on the dosage unit may increase buoyancy,

leading to an increase in the dissolution rate, or may decrease the available surface area, leading to a decrease in the dissolution rate. Typical steps include heating the medium, filtering, and drawing a vacuum for a short period of time. Other methods of de aeration are available and in routine use throughout the industry. Media containing surfactants are not usually deaerated because the process results in excessive foaming. To determine whether deaeration of the medium is necessary, results from dissolution samples run in non de aerated medium and de aerated medium should be compared (USP, 2014).

1.6.1.4. Agitation

For immediate-release capsule or tablet formulations, Apparatus 1 (baskets) at 100 rpm or Apparatus 2 (paddles) at 50 or 75 rpm are most commonly used. Other agitation speeds and apparatus are acceptable with appropriate justification.

Rates outside 25 to 150 rpm are usually inappropriate because of the inconsistency of hydrodynamics below 25 rpm and because of turbulence above 150 rpm. Agitation rates between 25 and 50 rpm are generally acceptable for suspensions. For dosage forms that exhibit coning (mounding) under the paddle at 50 rpm, the coning can be reduced by increasing the paddle speed to 75 rpm, thus reducing the artifact and improving the data. If justified, 100 rpm may be used, especially for extended-release products. Decreasing or increasing the apparatus rotation speed may be justified if the profiles better reflect in *vivo* performance and/or the method results in better discrimination without adversely affecting method reproducibility.

Selection of the agitation and other study design elements for modified release dosage forms is similar to that for immediate-release products. These elements should conform to the requirements and specifications given in dissolution when the apparatus has been appropriately calibrated (USP; 2014).

1.6.1.5. Study design

1.6.1.5.1. Time points

For immediate-release dosage forms, the duration of the procedure is typically 30 to 60 minutes; in most cases, a single time point specification is adequate for Pharmacopeia purposes. Industrial and regulatory concepts of product comparability and performance may require additional time points, which may also be required for product registration or approval. A sufficient number of time points should be selected to adequately characterize the ascending and plateau phases of the dissolution curve. According to the Biopharmaceutics Classification System referred to in several Food and Drug Administration FDA Guidance's, highly soluble, highly permeable drugs formulated with rapidly dissolving products need not be subjected to a profile comparison if they can be shown to release 85% or more of the active drug substance within 15 minutes. For these types of products, a one-point test will suffice. However, most products do not fall into this category. Dissolution profiles of immediate release products typically show a gradual increase reaching 85% to 100% at about 30 to 45 minutes. Thus, dissolution time points in the range of 15, 20, 30, 45, and 60 minutes are usual for most immediate-release products. For rapidly dissolving products, including suspensions, useful information may be obtained from earlier points, e.g., 5 to 10 minutes. For slower-dissolving products, time points later than 60 minutes may be useful. Dissolution test times for compendia tests are usually established on the basis of an evaluation of the dissolution profile data. So-called infinity points can be useful during development studies. To obtain an infinity point, the paddle or basket speed is increased at the end of the run for a sustained period (typically 15 to 60 minutes), after which time an additional sample is taken. Although there is no requirement for 100% dissolution in the profile, the infinity point can provide data that may supplement content uniformity data and may provide useful information about formulation characteristics during initial development or about method bias.

For an extended-release dosage form, at least three test time points are chosen to characterize the in *vitro* drug release profile for Pharmacopeia purposes. Additional sampling times may be required for drug approval purposes. An early time point, usually 1 to 2 hours, is chosen to show that there is little probability of dose dumping. An intermediate time point is chosen to define the in *vitro* release profile of the dosage form, and a final time point is chosen to show the essentially complete release of the drug. Test times and specifications are usually established on the basis of an evaluation of drug release profile data. For products containing more than a single active ingredient, drug release is to be determined for each active ingredient (USP; 2014).

1.6.1.5.2. Observations

Visual observations and recordings of product dissolution and disintegration behavior are very useful because dissolution and disintegration patterns can be indicative of variables in the formulation or manufacturing process. To accomplish visual observation, proper lighting (with appropriate consideration of photo degradation) of the vessel contents and clear visibility in the bath are essential. Documenting observations by drawing sketches and taking photographs or videos can be instructive and helpful for those who are not able to observe the real time dissolution test. Observations are especially useful during method development and formulation optimization. Examples of typical observations include, but are not limited to, the following: uneven distribution of particles throughout the vessel. This can occur when particles cling to the sides of the vessel, when there is coning or mounding directly under the apparatus, when particles float at the surface of the medium, when film-coated tablets stick to the vessel, and/or when off-center mounds are formed.

1.6.1.6. Sampling

Manual— manual sampling uses plastic or glass syringes, a stainless steel cannula that is usually curved to allow for vessel sampling. The sampling site must conform to specifications under Dissolution.

Auto sampling— Auto sampling is a useful alternative to manual sampling, especially if the test includes several time points. However, because regulatory labs may perform the dissolution test using manual sampling, auto sampling requires validation with manual sampling.

There are many brands of auto samplers, including semi-automated and fully automated systems. Routine performance checks, cleaning, and maintenance as described in the pertinent standard operating procedures or metrology documents are useful for reliable operation of these devices.

Some instruments are equipped with sampling through the basket or paddle shaft. Proper validation (e.g., demonstrated equivalence to results with the usual sampling procedure) may be required.

The disturbance of the hydrodynamics of the vessel by sampling probes should be considered and adequate validation performed to ensure that the probes are not introducing a significant change in the dissolution rate.

Comparison of manual and automated procedures should be performed to evaluate the interchangeability of the procedures. This can be accomplished by comparing data from separate runs or, in some cases, by sampling both ways from the same vessel. Results should be consistent with the requirements for intermediate precision if the procedures are to be considered interchangeable.

Other aspects of automation validation may include carryover of residual drug, effect of an in-residence probe (simultaneous sampling as mentioned above may not be suitable in this case), adsorption of drug, and cleaning and/or rinse cycles (USP; 2014).

1.6.1.7. Filters

Filtration of the dissolution samples is usually necessary to prevent undissolved drug particles from entering the analytical sample and further dissolving. Also, filtration removes insoluble excipients that may otherwise cause high background or turbidity. Pre wetting of the filter with the medium may be necessary.

Filters can be in-line or at the end of the sampling probe or both. The pore size can range from 0.45 to 70 µm. The usual types of filters are depth, disk, and flow-through. However, if the excipient interference is high, if the filtrate has a cloudy appearance, or if the filter becomes clogged, an alternative type of filter or pore size should be evaluated.

Adsorption of the drug(s) onto the filter needs to be evaluated. If drug adsorption occurs, the amount of initial filtrate discarded may need to be increased. If results are still unsuitable, an alternative filter material may be sought.

Filter validation may be accomplished by preparing a suitable standard solution or a completely dissolved sample solution (e.g., prepared as a typical sample in a vessel or a sample put in a beaker and stirred with a magnetic stirrer for 1 hour). For standard solutions, compare the results for filtered solutions (after discarding the appropriate volume) to those for the unfiltered solutions. For sample solutions, compare the results for filtered solutions (after discarding the appropriate volume) to those for centrifuged, unfiltered solutions (USP; 2014).

1.6.1.8. Centrifugation

Centrifugation of samples is not preferred, because dissolution can continue to occur and because there may be a concentration gradient in the supernatant. A possible exception might be for compounds that adsorb onto all common filters (USP; 2014).

1.6.1.9. Assay

The usual assay for a dissolution sample is either spectrophotometric determination or HPLC. The preferred method of analysis is

Spectrophotometric determination because results can be obtained faster, the analysis is simpler, and fewer solvents are used. HPLC methods are used when there is significant interference from excipients or among drugs in the formulation to improve analytical sensitivity and/or when the analysis can be automated. It may be useful to obtain data for the drug with a stability indicating assay (e.g., HPLC chromatograms) in the medium of choice, even if the primary assay is based on a spectrophotometric method (USP, 2014).

1.6.2 Validation

The development of a drug product is a lengthy process involving drug discovery, laboratory testing, animal studies, clinical trials and regulatory registration. To further enhance the effectiveness and safety of the drug product after approval, many regulatory agencies such as the United States Food and Drug Administration FDA also require that the drug product be tested for its identity, strength, quality, purity and stability before it can be released for use. For this reason, pharmaceutical validation and process controls are important in spite of the problems that may be encountered (Elsie Jatto et al, 2002).

The validation topics are typical but not all-inclusive. The validation elements addressed may vary, depending on the phase of development or the intended use for the data (Boureau *et al*., 2004).

The acceptance criteria are presented as guidelines only and may differ for some products. Firms should document the appropriate acceptance criteria for their products in pertinent standard operation procedures SOPs. Other considerations may be important for special dosage forms. The extent of validation depends on the phase of the product development. Full validation takes place by the time of the phase of clinical studies. Validation studies should address the variations associated with different profile time points. For products containing more than a single active ingredient, the dissolution method needs to be validated for each active ingredient (USP; 2014).

1.6.2.1. Specificity/placebo interference

Specificity is the ability to measure accurately and specifically the analyze of interest in the presence of other components that may be expected to be present in the sample matrix (Chandran *et al*, 2007).

It is necessary to demonstrate that the results are not unduly affected by placebo constituents, other active drugs, or degradants. The placebo consists of all the excipients and coatings (inks, sinker, and capsule shell are also included when appropriate) without the active ingredient. Placebo interference may be determined by weighing samples of the placebo blend and dissolving or dispersing them in dissolution medium at concentrations that would be encountered during testing. It may be desirable to perform this experiment at $37°$ by comparing it to the 100% standard by the formula:

$$100C \, (AP \, /AS) \, (V/L)$$

In which C is the concentration, in g /L, of the standard; AP and AS are the absorbance's of the placebo and the standard, respectively; V is the volume, in ml, of the medium; and L is the label claim, in mg. The interference should not exceed 2%.

Note— for extended-release products, a placebo version of the finished dosage form may be more appropriate to use than blends, because this placebo formulation will release the various excipients in a manner more nearly reflecting the product than will a simple blend of the excipients. In this case, it may be appropriate to evaluate potential interference at multiple sampling points in the release profile. If the placebo interference exceeds 2%, then method modification—such as (1) choosing another wavelength, (2) baseline subtraction using a longer wavelength, or (3) using HPLC—may be necessary in order to avoid the interference. When other active drugs or significant levels of degradates are present, it is necessary to demonstrate that these do not significantly affect the results. One procedure for doing this is to measure the matrix in the presence and absence of the other active drug or degradate: any interference should not exceed 2 %(USP; 2014).

1.6.2.2. Linearity and range

Linearity and range are typically established by preparing solutions of the drug, ranging in concentration from below the lowest expected concentration to above the highest concentration during release. This may be done in conjunction with accuracy/recovery determination. The scheme may be altered if different flow-cell sizes or injection volumes are used (Green; 1996).

Typically, solutions are made from a common stock if possible. For the highest concentration, the determination may not exceed the linearity limits of the instrument.

Organic solvents may be used to enhance drug solubility for the preparation of the standard solutions; however, no more than 5% (v/v) of organic solvent in the final solution should be used, unless validated.

Linearity is typically calculated by using an appropriate least-squares regression program. Typically, a square of the correlation coefficient ($r2 \geq 0.98$) demonstrates linearity. In addition, the y-intercept must not be significantly different from zero (USP; 2014).

1.6.2.3. Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by established the maximum level at which the analyte can be reliably detected and determined by established the lowest concentration that can be measured according ICH.

1.6.2.4. Accuracy/recovery

Accuracy/recovery are typically established by preparing multiple samples containing the drug and any other constituents present in the dosage form (e.g., excipients, coating materials, capsule shell) ranging in concentration from below the lowest expected concentration to above the highest concentration during release.

In cases of poor drug solubility, it may be appropriate to prepare a stock solution by dissolving the drug substance in a small amount of organic solvent (typically not exceeding 5%) and diluting to the final concentration with dissolution medium. An

amount of stock solution equivalent to the targeted label claim may be added to the vessel instead of the drug powder. Similarly, for very low strengths, it may be more appropriate to prepare a stock solution than to attempt to weigh very small amounts. The measured recovery is typically 95% to 105% of the amount added. Bracketing or matrixing of multiple strengths may be useful.

A special case for validation is the Acid Stage procedure described in Delayed-Release Dosage Forms under Dissolution. The limit of not more than 10% needs to be validated. If the compound degrades in acid, the validation experiment must address this fact (USP; 2014).

1.6.2.5. Precision

Repeatability— repeatability is determined by replicate measurements of standard and/or sample solutions. It can be measured by calculating the RSD of the multiple injections or spectrophotometric readings for each standard solution, or from the accuracy or linearity data.

Intermediate Precision— Intermediate precision may be evaluated to determine the effects of random events on the precision of the analytical procedure. This evaluation is typically done later in the development of the drug product. The precision can be across the range of product strengths. Typical variations to study include days, analysts, and equipment. The use of an experimental matrix design is encouraged for evaluation of intermediate precision. If possible, intermediate precision can be evaluated using a well characterized lot of drug product of tight content uniformity. In cases where a well-characterized product is not available, placebo and active ingredient may be used to identify intermediate precision.

The dissolution profiles on the same sample may be run by at least two different analysts, each analyst preparing the standard solutions and the medium. Typically, the analysts use different dissolution baths, spectrophotometers or HPLC equipment (including columns), and auto samplers; and they perform the test on different days. This procedure may not

need to be performed for each strength; instead, bracketing with high and low strengths may be acceptable.

A typical acceptance criterion is that the difference in the mean value between the dissolution results at any two conditions using the same strength does not exceed an absolute 10% at time points with less than 85% dissolved and does not exceed 5% for time points above 85%. Acceptance criteria may be product-specific, and other statistical tests and limits may be used (USP; 2014).

1.6.2.6. Robustness

The evaluation of robustness, which assesses the effect of making small, deliberate changes to the dissolution conditions, typically is done later in the development of the drug product. The number of replicates (typically 3 or 6) is dependent on the intermediate precision. Parameters to be varied are dependent on the dissolution procedure and analysis type. They may include medium composition (e.g., buffer or surfactant concentration), pH, volume, agitation rate, and temperature. For HPLC analysis, parameters may include mobile phase composition (percentage organic, buffer concentration, pH), flow rate, wavelength, column temperature, and multiple columns (of the same type). For spectrophotometric analysis, the wavelength may be varied (USP; 2014).

1.6.2.7 Standard and sample solution stability

The standard solution is stored under conditions that ensure stability. The stability of the standard is analyzed over a specified period of time, using a freshly prepared standard solution at each time interval for comparison. The acceptable range for standard solution stability is typically between 98% and 102%.

The sample solution is typically stored at room temperature. The sample is analyzed over a specified period of time using the original sample solution response for comparison. The typical acceptable range for sample solution stability may be between 98% and 102%

compared with the initial analysis of the sample solutions. If the solution is not stable, aspects to consider could be temperature (refrigeration may be needed), light protection, and container material (plastic or glass).

The procedure may state that the standards and samples need to be analyzed within a time period demonstrating acceptable standard and sample solution stability (USP, 2014).

Stability testing thus evaluates the effect of environmental factors on the quality of a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation (Sanjay *et al*., 2012).

1.6.2.8. Spectrophotometric Analysis

Samples may be automatically introduced into the spectrophotometer using auto sippers and flow cells. Routine performance checks, cleaning, and maintenance as described in the standard operating procedures or metrology documents are useful for reliable operation of these instruments. Cells with path lengths ranging from 0.02 to 1 cm are typically used. Cell alignment and air bubbles could be sources of error. The smaller path length cells are used to avoid diluting the sample; however, acceptable linearity and standard error need to be demonstrated. During analysis, standard solutions are typically prepared and analyzed at just one concentration at 100% (or the selected Q% value) of the dosage strength. During profile analysis, other concentrations may be useful. A typical blank, standard, and sample may be analyzed in a sequence that brackets the sample with standards and blanks, especially at the beginning and end of the analysis. In most cases, the mean absorbance of the dissolution medium blank may not exceed 1% of the standard. Values higher than 1% must be evaluated on a case-by-case basis. The typical RSD for UV analysis is usually not more than 2%. The absorptivity is calculated by dividing the mean standard absorbance by the concentration, in mg per mL, divided by the flow-cell path length in cm. After enough historical data are accumulated, an acceptable

absorptivity range for the analyze (using the appropriate flow cell) may be determined. This value may be useful in troubleshooting aberrant data. Fiber optics as a sampling and determinative method, with proper validation, is an option. It may be useful to examine the UV spectrum of the drug in solution to select the optimum wavelength (USP; 2014).

1.6.2.9. HPLC

For HPLC analysis, the compatibility of dissolution media and mobile phase may be examined, especially if large injector volumes (over 100 µL) are needed. Samples are normally analyzed with HPLC using a spectrophotometric detector and an auto-injector. Single injections of each vessel time point with standards throughout the run constitute a typical run design. System suitability tests include, at a minimum, the retention window and injection precision. Typically, the repeatability of an HPLC analysis should be less than or equal to 2% RSD for five or six standard determinations. The standard level is typically at the 100% label claim level, especially for a single-point analysis (USP, 2014). Preparation of the placebo samples for the HPLC analysis is to be performed in the same way as in the spectrophotometric analysis. Examine the chromatogram for peaks eluting at the same retention time as the drug. If there are extraneous peaks, inject the standard solution, and compare retention times. If the retention times are too close, the placebo solution with the drug. Chromatograms may also be obtained over an extended run time using the blank (dissolution medium), standard, and sample solution to identify late elutes that may interfere with subsequent analyses.

The validation documentation may include overlaid representative chromatograms or spectra of blank dissolution medium, a filtered placebo solution, a standard solution, and a filtered dissolution sample. Absence of interfering peaks in the placebo chromatogram or lack of absorbance by the placebo at the analytical wavelength demonstrates specificity (USP; 2014).

1.6.2.10. Acceptance criteria

Typical acceptance criteria for the amount of active ingredient dissolved, expressed as a percentage of the labeled content (Q), are in the range of 75% to 80% dissolved. A Q value in excess of 80% is not generally used, because allowance needs to be made for assay and content uniformity ranges (FDA; 2000).

Acceptance criteria including test times are usually established on the basis of an evaluation of the dissolution profile data. Acceptance criteria should be consistent with historical data, and there is an expectation that acceptable batches (e.g., no significant differences in in vivo performance, composition, or manufacturing procedure) will have results that fall within the acceptance criteria.

Acceptance criteria could be the precision and accuracy of the dilution integrity QCs is ≤15% and within ± 15% of the nominal concentrations respectively (Rama *et al*, 2009).

1.7 Objectives

General objectives are:

- To assess the release profile of the third-generation H1-antihistamaines in solid oral pharmaceutical product such as tablets and capsules.

- To develop and validate dissolution method for Levocetirizine dihydrochloride dosage form using UV/VIS spectrophotometry.

Specific objectives are:

- To evaluate the influence of buffer, pH and surfactants on solubility of levocitrizine dihydrochloride (LCTZ).

- To develop and validate a dissolution method for Levocetirizine dihydrochloride using UV spectrophotometric assay method

2. Materials and methods

2.1 Apparatus

2.1.1 Dissolution apparatus

When Apparatus 1 (basket apparatus) or 2(paddle apparatus) is not appropriate, another official apparatus may be used.

2.1.1.1. Apparatus 1 (Basket Apparatus)

The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material, a motor, a metallic drive shaft, and a cylindrical basket (figure 2.1). The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at $37 \pm 0.5°$ during the test and keeping the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating stirring element. An apparatus that permits observation of the specimen and stirring element during the test is preferable. The vessel is cylindrical, with a hemispherical bottom and with one of the following dimensions and capacities: for a nominal capacity of 1 L, the height is 160 to 210 mm and its inside diameter is 98 to 106 mm; for a nominal capacity of 2 L, the height is 280 to 300 mm and its inside diameter is 98 to 106 mm; and for a nominal capacity of 4 L, the height is 280 to 300 mm and its inside diameter is 145 to 155 mm. Its sides are flanged at the top. A fitted cover may be used to retard evaporation. The shaft is positioned so that its axis is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. A speed-regulating device is used that allows the shaft rotation speed to be selected and maintained at the specified rate given in the individual monograph within ±4 %.
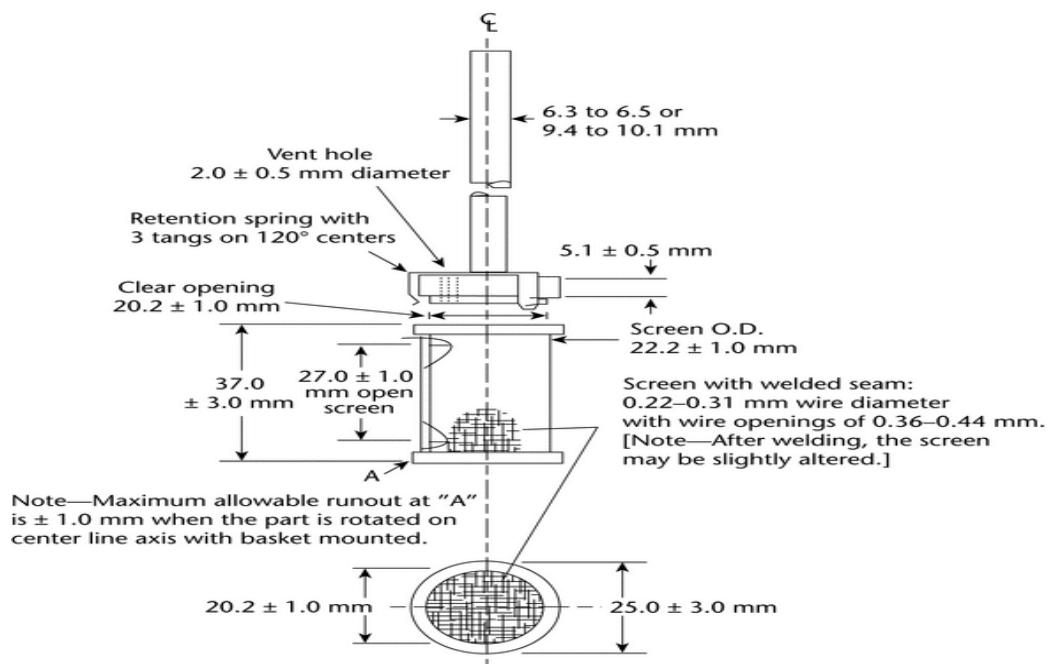
Figure 2.1. Basket stirring element.

2.1.1.2. Apparatus 2 (Paddle Apparatus)

In this work the paddle apparatus was applied, and the assembly from Apparatus 1 was used, except that a paddle formed from a blade and a shaft is used as the stirring element (Figure 2.2). The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel at any point and rotates smoothly without significant wobble that could affect the results. The vertical center line of the blade passes through the axis of the shaft so that the bottom of the blade is flushed with the bottom of the shaft. The paddle conforms to the specifications shown in Figure 2.2. The distance of 25 ± 2 mm between the bottom of the blade and the inside bottom of the vessel was maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used provided the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of nonreactive material, such

as not more than a few turns of wire helix, may be attached to dosage units that would otherwise float. Other validated sinker devices may be used.
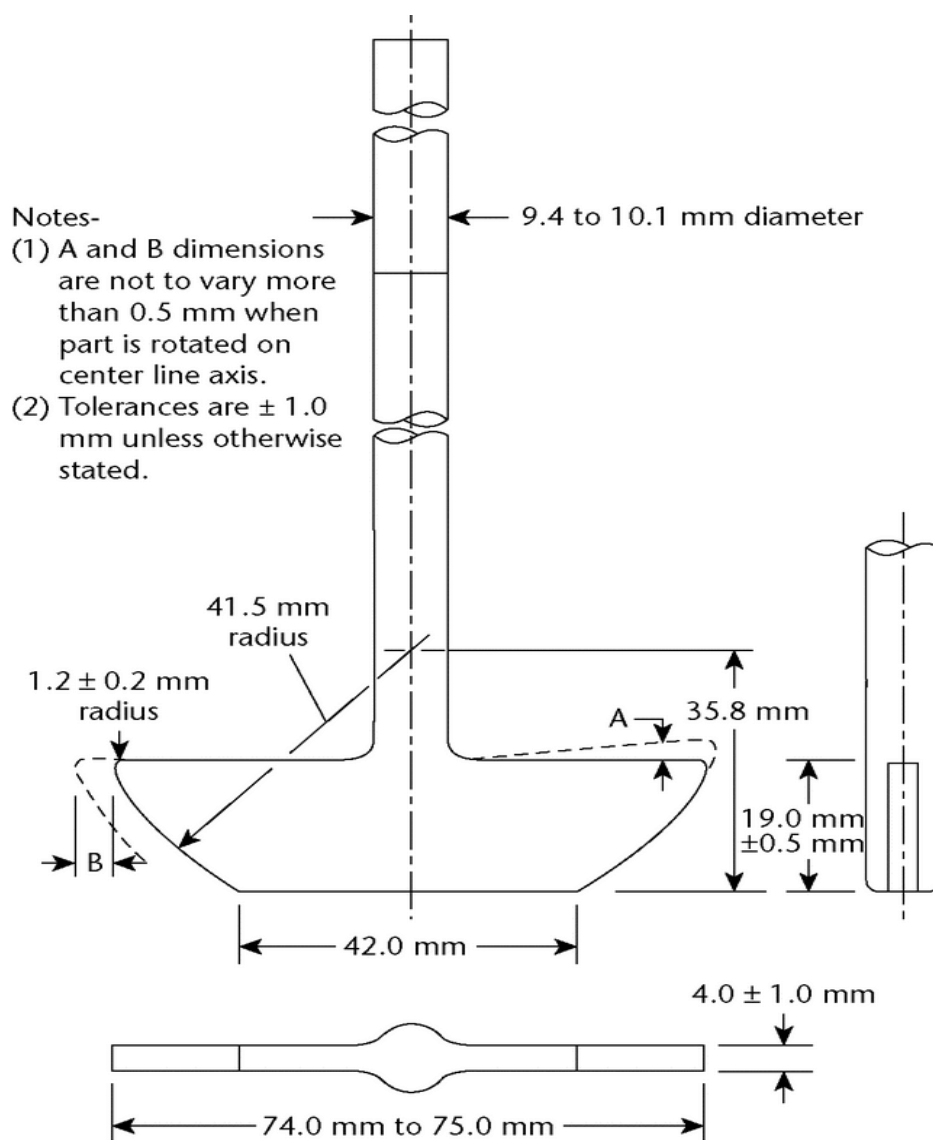

Figure 2.2. Paddle stirring element.

2.1.1.3 Procedure

Apparatus 1 and Apparatus 2

Immediate-release dosage forms

The stated volume of the Dissolution Medium (±1%) was placed in the vessel of the specified apparatus given in the individual monograph, the apparatus was assembled, the dissolution medium was equilibrated to $37 \pm 0.5°C$, and the thermometer were removed.

1 dosage unit was placed in the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately the apparatus was operated at the specified rate given in the individual monograph. Within the time interval specified, or at each of the times stated, a specimen was withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating basket or blade, not less than 1 cm from the vessel wall. The analysis was performed as directed in the individual monograph using a suitable assay method. The test was repeated with additional dosage form units.

Dissolution medium— a suitable dissolution medium was used. The solvent specified in the individual monograph was used. The volume specified refers to measurements made between 20° and 25°. If the dissolution medium was a buffered solution, the solution was adjusted so that its pH is within 0.05 unit of the specified pH given in the individual monograph.

Time— where a single time specification was given, the test might be concluded in a shorter period if the requirement for minimum amount dissolved was met. Specimens were to be withdrawn only at the stated times within a tolerance of ±2 %.

2.1.2 Glass apparatus

- Volumetric flasks 50ml, 100ml and 1000ml, class A, ISO LAB, Germany.
- Beaker, 40ml, 100ml and 400ml, BORA, Germany.
- Measuring cylinder, 10ml, BORA, Germany.
- Weight bottle, 40ml, BORA, Germany.
- Volumetric pipettes class a 5ml, 10ml, ISO LAB, Germany.

2.1.3 Plastic apparatus

- Syringe 20ml.
- Syringe filter 0.45µm, Oilm peak .Teknokroma.

2.2 Instrumentals

- Analytical balance, model: ED2245, Sartorius, Germany.
- UV-visible spectrophotometer, model UV-1800 240V, Shimadzu Corporation, Japan.
- -Ultrasonic, model, WUC-A10H, Wise clean, Korea.
- -Microprocessor tablet dissolution, Pharma test, model, PTWS1000, Germany.
- Microprocessor tablet dissolution, ELETROLAB, model, EDT-08LX, India.
- -Horizontal flow oven, model WOF-155, Wise oven, Korea.
- -Water purification system, model, NW10UV.Heal force, China.

2.3 Chemicals

- Levocitrizine dihydrochloride (working standard), METROCHEM, India.
- Hydrchloric acid 35-38%, SDFCL-Mumbai.
- Levohist tablets, AZAL Pharmaceutical Industries Co. LTD.-Khartoum Bari - Sudan.
- Lactose Anhydrous, DFE Pharma, Germany.
- Microcrystalline cellulose NF, Pharma –EUR, India.
- Colloidal Silicon Dioxide, Evonik industries, Germany.
- Magnesium Stearate, Graven Pharma, India.
- Sodium starch glycol ate, JRS Pharma, USA-Canada.

2.4 Methods

The following dissolution system apparatus, medium and its volume, dissolution system is the process in which a solid substance goes into solution. For dosage forms containing an active solid ingredient, the rate of dissolution may be critical to absorption.

2.4.1 Dissolution method

2.4.1.1 Dissolution System:

- Medium: 0.01 M-HCL; 500 ml.

- Apparatus: Paddle.

- Speed: 50 rpm (revolution per minute).

- Time: 30 minutes.

- Temperature 37˚C±5˚C.

- Manual sampling by syringe.

2.4.1.2 Standard solution preparation :

Levocetrizine dihydrochloride working standard, LCTZ-W.S (10.0 mg) was weighed accurately and transferred to 100-ml volumetric flask which- was half-filled with dissolution medium .The mixture was sonicated for 10 minutes to dissolved the LCTZ powder, cooled to room temperature and completed to the mark with dissolution medium, from which 5ml was diluted to the mark in 50-ml volumetric flask   (0.01 mg ml^{-1}).

2.4.1.3 Sample preparation :

One tablet was placed into each six dissolution vessels individually containing 500ml of the dissolution medium placed in the dissolution tester after the dissolution system was conditioned (equilibrating temperature at 37˚C±5˚C, using Paddle and adjusting speed at 50 rpm)for 30mintues .

After 30 minutes using syringe with syringe filter, 20ml was taken from each vessel and without any further dilution an aliquot of the sample solution was withdrawn (0.01 mg ml^{-1}).

2.4.1.4 Procedure:

Development of the dissolution method of LCTZ was assessed by UV/vis spectrophotometry .The amount of the LCTZ dissolved in the test solution compared to that of the standard solution was determined by measuring the absorbance at wavelength 236.5 nm of each test solution and standard solution against the dissolution medium as blank.

2.4.2 Validation test

The following validation parameters for the dissolution method for LCTZ were determined by UV spectrophotometry:

1. Specificity /Selectivity
2. Linearity and range
3. Limit of detection (LOD) and limit of quantitation (LOQ)
4. Accuracy
5. Precision
6. Robustness
7. Stability of solution

2.4.2.1 Specificity /selectivity

2.4.2.1.1 Standard solution:

Standard solution of LCTZ – W.S was prepared in100-ml volumetric flask. 10 mg of LCTZ W.S were weighed accurately and transferred quantitatively to 100-ml volumetric flask which was half -filled with dissolution medium, the mixture sonicated for 10 minutes to dissolve the LCTZ– W.S powder, and the volume was completed to the mark with the same dissolution medium from which 5ml was diluted to the mark in 50-ml volumetric flask.

2.4.2.1.2 Placebo Solutions:

Placebo equivalents, 100.0mg weight of one tablet (5mg) were accurately weighed and transferred to 100-ml volumetric flask which was half-filled with dissolution medium .The mixture was sonicated for 10 minutes, to dissolved, cooled to room temperature and completed to the mark with dissolution medium from which 5ml aliquot was diluted to the mark in 50-ml volumetric flask (0.01 mg ml^{-1}).

2.4.2.1.3 Test Solutions:

20 tablets from Levohist were weighed and crushed 300mg of the powder sample equivalent to 10 mg of LCTZ was transferred to 100- ml volumetric flask which was half-filled with dissolution medium the mixture was sonicated for 10 minutes to dissolved LCTZ powder, cooled to room temperature and complete to the mark with dissolution medium from which 5ml aliquot was diluted to the mark in 50-ml volumetric flask (0.01 mg ml^{-1}).

2.4.2.2 Linearity and range

Taking into considering that the concentration of sample used for validation, was 5mg of LCTZ, the calibration curve was prepared to cover the range from down 50% up to 300% of the sample concentration.

LCTZ (10.0mg) was weighed accurately and transferred quantitatively to100-ml volumetric flask which was half -filled with dissolution medium, the mixture was sonicated for 10 minutes to dissolve the LCTZ powder, cooled to room temperature and the volume was completed to the mark with the same dissolution medium to give concentration 0.1mg ml^{-1} of LCTZ. From this standard stock solution subsequent dilutions were made with the dissolution medium to give concentration of 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 18.0 and 20.0 µg ml^{-1} of LCTZ. The absorbance of each solution was measured at 236.5nm against the dissolution medium. Range was established

through linearity measurement to be from 40.0 % to 400.0% and from 2.0 mg to 20.0 mg LCTZ.

2.4.2.3. Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by established the maximum level at which the analyte can be reliably detected and determined by established the lowest concentration that can be measured according ICH. The limit of detection (LOD) and limit of quantitation (LOQ) were determined according to the following formula

$$LOD=3.3*STDEVa/b$$
$$LOQ=10*SDEVa/b$$

STDEVa: standard deviation of the intercept

b: slope

2.4.2.4 Accuracy /recovery

The accuracy /recovery of the dissolution method LCTZ was done in conjunction with the linearity by determining different concentrations from 2.0 to $20.0 \mu gml^{-1}$, LCTZ. The absorbance of each solution was measured at 236.5nm against the dissolution blank. The Recovery of the dissolution method for LCTZ was calculated by using the absorbance of each solution from the linearity determination.

2.4.2.5. Precision

2.4.2.5.1 Repeatability

The repeatability of the dissolution of LCTZ tablets was conducted by performing triplicate dissolution steps on equivalent composites of the ingredient of the table three composites of the standards and placebo required to produce 50%, 100%, 150%" of the tablet content were weighed accurately ,transferred quantitatively to 100-ml volumetric flask which was half -filled with dissolution medium. The mixture was sonicated for 10 minutes to dissolve LCTZ powder, cooled to room temperature and complete to the mark

with dissolution medium from which 5ml aliquot was diluted to the mark in 50-ml volumetric flask. The absorbance of each of these solutions were measured at 236.5 nm.

2.4.2.5.2 Intermediate precision / ruggedness

2.4.2.5.2.1 Analyst –to- analyst

From the same batch, two analysts working in the same laboratory and using the same dissolution apparatus and UV/vis spectrophotometer independently prepared test and standard solution of LCTZ and measured their absorbance's at 236.5nm on the same day .The percentage of quantities dissolved (Q%) were calculated representing analyst-to-analyst precision, respectively.

2.4.2.5.2.2 Day-to-day

From same batch, in the same laboratory and using the same dissolution apparatus and UV/vis spectrophotometer, the absorbance of prepared test and standard solution of LCTZ was measured at 236.5nm after two days. The percentage of quantities dissolved (Q %) were calculated representing day-to-day precision, respectively.

2.4.2.6. Robustness

Evaluation of robustness, which assesses the effect of making small, deliberate changes to the dissolution conditions

1. medium (water, 0.01 M-HCL)
2. Speed: (50 rpm and100rpm) (revolution per minute).
3. Time (30 and45) minutes.

Robustness for UV spectrophotometric analysis was evaluated by assessing the effect of slightly varying the absorption wavelength. 10 mg of LCTZ W.S were of weighed accurately and transferred quantitatively to 100-ml volumetric flask which was half -filled with dissolution medium. The mixture was sonicated for 10 minutes to dissolve the LCTZ– W.S powder, and the volume was completed to the mark with the same dissolution medium from which 5ml aliquot was diluted to the mark in 50-ml volumetric

flask. The absorbance of standard solution was recorded at wave length 238.5, 236.5and 234.5nm.

2.4.2.7 Stability of solution

2.4.2.7.1 Dissolution System:

- Medium: 0.01 M-HCL; 500 ml.
- Apparatus: Paddle.
- Speed: 50 rpm (revolution per minute).
- Time: 30 minutes.
- Temperature 37˚C±5℃.
- Manual sampling by syringe.

2.4.2.7.2 Standard solution preparation:

10.0 mg of levocetrizine dihydrochloride working standard, LCTZ-W.S which was weighed accurately and transferred to 100-ml volumetric flask which was half-filled with dissolution medium .The mixture was sonicated for 10 minutes, cooled to room temperature and completed to the mark with dissolution medium from which 5ml was diluted to the mark in 50-ml volumetric flask   (0.01 mg ml^{-1}).

2.4.2.7.3 Sample preparation:

One tablet was placed into each six dissolution vessels, individually containing 500-ml of the dissolution medium placed in the dissolution tester,   after the dissolution system was conditioned (temperature equilibrated to 37˚C, using Paddle and speed 50 rpm) for 30 minutes. After 30 minutes using syringe with syringe filter, 20ml was taken from each vessel and without any further dilution an aliquot of the sample solution was withdrawn (0.01mg ml^{-1}).

2.4.2.7.4 Procedure:

Development of the dissolution method of LCTZ was assessed by UV spectrophotometry .the amount of the LCTZ dissolved in the test solution compared to that of the standard

solution was determined by measuring the absorbance at wavelength 236.5 nm of each test solution and the standard solution against the dissolution medium as a blank. The percentage of quantities dissolved (Q %) were calculated.

The standards and sample solutions were stored for two days and their absorbance were remeasured at 236.5nm of each test solution and the standard solution against the dissolution medium as blank. The percentage of quantities dissolved (Q %) were calculated.

3. Results and discussion

3.1 Dissolution

Table 3.1 describes in details the first dissolution test in the developed analytical test method for UV spectrophotometric determination of the percentage of quantities dissolved (Q %) of LCTZ in Levohist 5 mg tablet.

Table 3.1 Result of dissolved percentage (Q %) of LCTZ

No of tests	Weight of tablet	Absorbance Average	Q %	Average of Q %	RSD%
T1	155.0	0.287	103.38		
T2	157.2	0.290	103.00		
T3	156.4	0.296	105.67	103.97	0.88
T4	162.3	0.302	103.89		
T5	160.5	0.299	104.01		
T6	157.0	0.292	103.84		

3.2 Dissolution validation

The dissolution validation parameters in the developed analytical test method for determination of the percentage of quantities dissolved (Q %) LCTZ in Levohist 5 mg tablet using UV spectrophotometry were determined.

3.2.1 Specificity/ selectivity

The specificity of LCTZ validation method was demonstrated by absence of main peak in placebo solution, prepared in 100 ml volumetric flask, and presence of it in standard solution, the interference between placebo and standard solution is 0. 0%. STD1 absorbance was used to calculate the placebo interference, figure 3.2.1, 3.2.2, 3.2.3, 3.2.4 and 3.2.5 show the UV spectra of identification test, sample, standard and blank respectively.
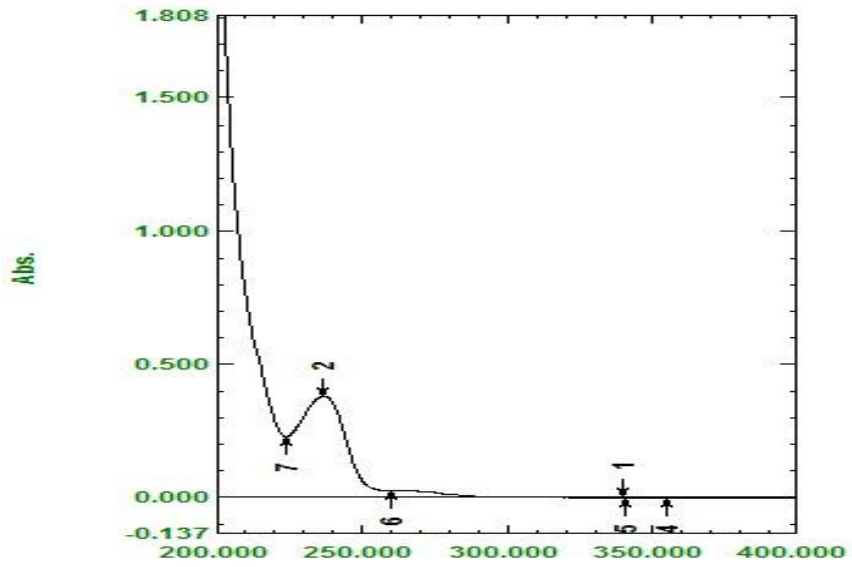
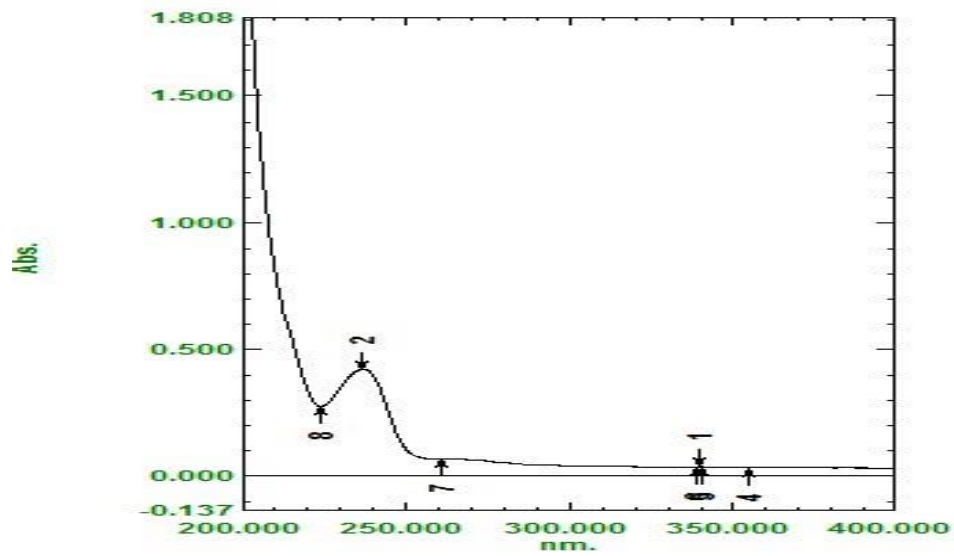**Figure (3.2.1): UV spectrum of Identification test**

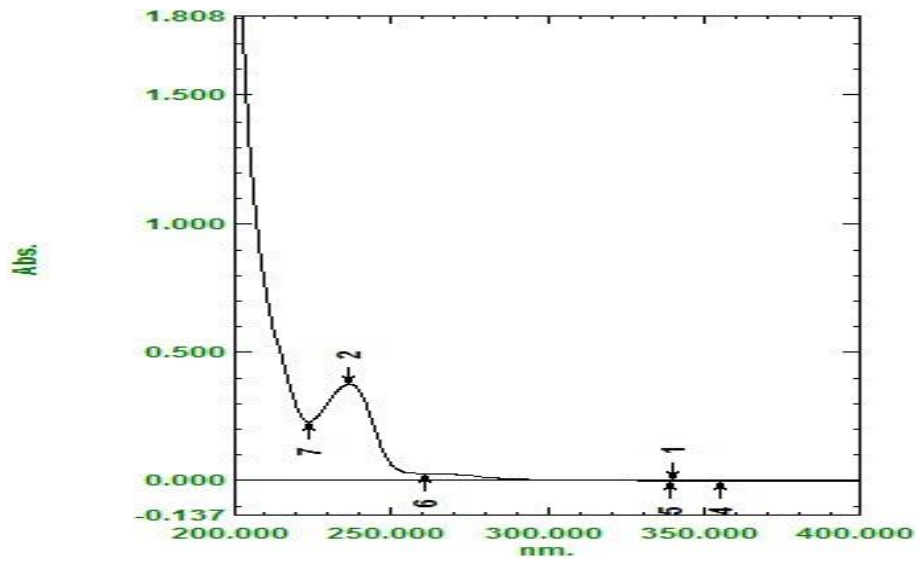**Figure (3.2.2): UV spectrum of sample**

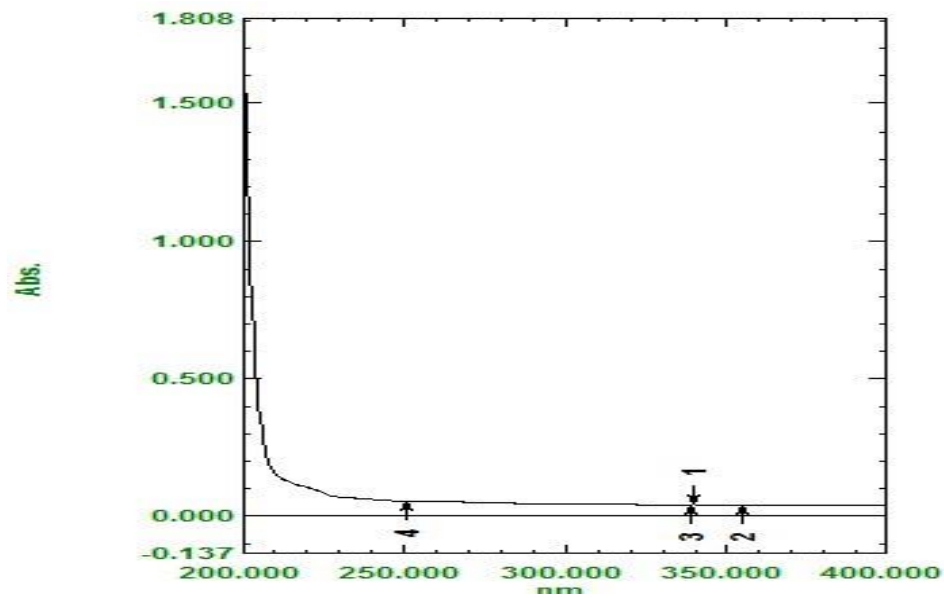**Figure (3.2.3): UV spectrum of standard**

Figure (3.2.4): UV spectrum of blank
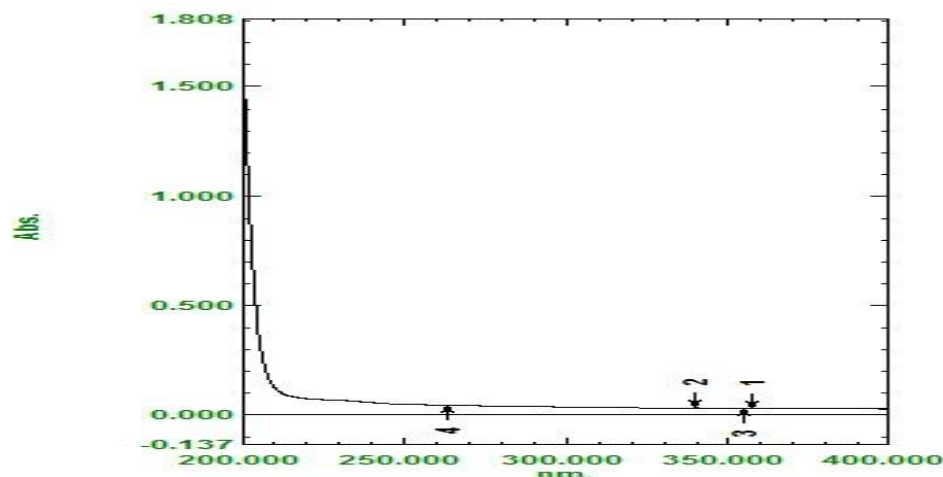
Figure (3.2.5): UV spectrum of placebo

Table (3.2.1) shows the result of the Specificity (selectivity) validation method of LCTZ tablet

Table (3.2.1): Specificity (selectivity) of LCZT-working standard, tests and placebo

Sample ID at wavelength 236.5nm	Actual weight	Average of Absorbance
LCZT-WS	10.37	0.422
Test	314.3	0.399
Placebo	104.05	0.000

3.2.2 Linearity and range

3.2.2.1 Linearity

Linearity of LCTZ validation method was demonstrated by drawing Beer's calibration curve (Fig 3.2.6).Following the regression equation:

$$Y = mX + C$$

$$y = 35.868x - 0.0018$$

Linearity range from concentration with 2.0 µg ml^{-1} to 20.0 µg ml^{-1}, correlation coefficient of determination "R²" = 0.9998, equation X= (y- 0.0018/35.868). The linearity results are shown below in Table (3.2.2.1).

The UV spectrophotometric validation method for the determination of linearity range of LCTZ was found to be from concentration of 2.0 µg ml^{-1} to 20.0 µg ml^{-1}

Table (3.2.2.1): The linearity sited of LCZT samples

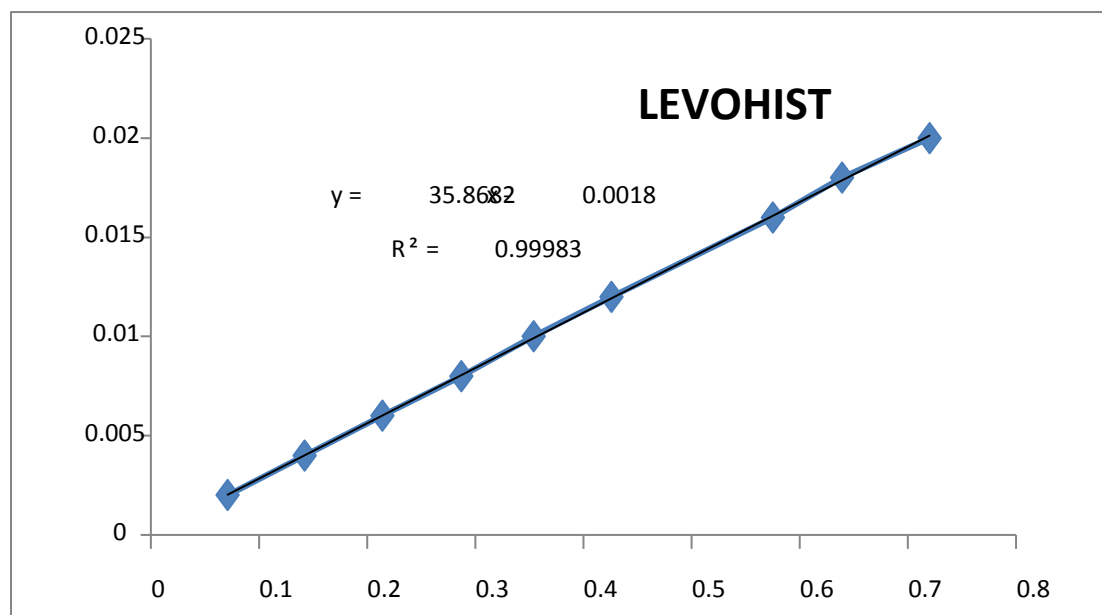| Conc.(µg ml^{-1})	Abs	Conc.(µg ml^{-1})	Abs
2.1	0.071	12.6	0.426
4.2	0.142	16.8	0.575
6.3	0.214	18.8	0.639
8.4	0.287	20.9	0.720
10.5	0.354		

Figure (3.2.6) Beer Lambert law plot of LCTZ

From calibration curve, coefficient of determination "R²" = 0.9998, Slope "S" = 35.868, Y- intercept "b" = 0.0018, Regression equation Y= mX+C is y = 35.8682X + 0.0018 and concentration" X = (Y+0.0018)/ 35.868.

3.2.2.2 Range:

Range of LCTZ validation method calculated from linearity measurements was found to be for percentage from 40.0 % and 400.0% and from 2.0 mg and 20.0 mg LCTZ is shown in Table (3.2.2.2)

Table (3.2.2.2): Range of LCZT samples

Calculated content " mg "	calculated Q " %	Calculated content " mg "	calculated Q " %
2.10	41.99	12.60	251.93
4.20	83.98	17.00	340.05
6.33	126.56	18.89	377.89
8.46	169.73	21.29	425.80
10.47	209.35		

3.2.3. Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by establishing the maximum level at which the analyte could be reliably detected and determined by establishing the lowest concentration that could be measured according ICH. The limit of detection (LOD) and limit of quantitation (LOQ) were determined according to the following formula

$$LOD=3.3*STDEVa/b$$

$$LOQ=10*SDEVa/b$$

STDEVa: standard deviation of the intercept

b: slope

The limit of detection (LOD) and limit of quantitation (LOQ) were found to be, 0.5 and 1.52 µ/ml respectively Table (3.2.3.1)

Table 3.2.3.1 Parameters for LCTZ of the proposed method

Parameters	Value
Measurement wavelength (nm)	236.5
Linear range	2-20(µ/ml)
Intercept	0.0018
Standard deviation of the intercept	0.005
Correlation coefficient (r^2)	0.9998
Slope	35.868
Limit of detection LOD(µ/ml)	0.5
Limit of quantitation LOQ(µ/ml)	1.52

3.2.4 Precision

Precision of the result of developed analytical method for repeatability and intermediate precision/ ruggedness, of different analysts and different days are shown in Tables (3.2.4.1) and (3.2.4.2), respectively.

Table (3.2.4.1): Intermediate precision / ruggedness of LCTZ samples analyst to analyst

Analyst 1				Analyst 2			
No of test	Weight mg	Absorbance Average	Q %	No of test	Weight mg	Absorbance Average	Q %
T1	155.0	0.287	100.16	T1	155.8	0.282	99.45
T2	157.2	0.290	99.79	T2	163.6	0298	100.08
T3	156.4	0.296	102.38	T3	161.6	0.294	99.96
T4	162.3	0.302	100.66	T4	160.7	0.284	97.10
T5	160.5	0.299	100.77	T5	158.8	0.286	99.33
T6	157.0	0.292	100.61	T6	158.8	0.288	99.26

Table (3.2.4.2): Intermediate precision / ruggedness of LCTZ samples day to day

	Day 1				Day 2		
No of test	Weight mg	Absorbance Average	Q %	No of test	Weight mg	Absorbance Average	Q %
T1	155.0	0.287	100.16	T1	154.3	0.275	102.61
T2	157.2	0.290	99.79	T2	160.9	0.286	102.33
T3	156.4	0.296	102.38	T3	158.9	0.284	102.90
T4	162.3	0.302	100.66	T4	156.7	0.278	102.14
T5	160.5	0.299	100.77	T5	162.9	0.289	102.14
T6	157.0	0.292	100.61	T6	160.0	0.278	100.03

The repeatability studies showed that:

1.   For individual preparation, the relative standard deviation percentage (RSD %) ranged from 0.00% to 1.33%.

2.   For intermediate precision between different analysts, the quantity released percentage (Q %) were 100.73% and 99.26 % for analyst1and analyst2 respectively, giving an average of 99.99% and RSD% of 1.04% between the two analysts.

3.   For intermediate precision for different days, the quantity released percentage (Q %) were 100.73% and 102.02% for day 1and day 2, respectively, giving an average of 102.99% and RSD% of 1.33% between two days.

The validation dissolution method showed that the RSD% didn't exceed 1.33% when the absorbance of the test solution were measured. The method was also proved to be precise as the RSD % didn't exceed 1.04% when the intraday and intra analyst precision were tested.

3.2.5. Robustness

The robustness of the validation method of LCTZ tablet was checked by remeasuring of the absorbance of the sample solutions at 2nm above and below their maximum absorption at 236.5nm and the reading was recorded in Table (3.2.5.1).

Table (3.2.5.1): Robustness of LCZT samples in different wavelengths

Wavelength	234.5 nm	236.5 nm	238.5 nm
Average for absorbance	0.323	0.334	0.329
RSD%	1.7		

The robustness of the method for LCTZ was asserted by slight change in the average absorbance of sample solutions due to a variation of as much as ±2nm from the maximum absorbance (table 8) as well as RSD% between the three absorbances are 1.7% (not more than 2%).

3.2.6. Accuracy

The accuracy of the method was determined from weighed of LCTZ content from linearity measurements using average absorbance in comparison with those standard solutions to calculate the recovery percentage show in Table (3.2.6.1).Alternatively, the recovery percentage can also be calculated from precision measurements using prepared standard concentrations and obtained concentration results Table (3.2.6.2)

1. LCTZ standard Preparations Used in Linearity with concentrations of 2.1, 4.2, 6.3, 8.4, 10.47, 12.60, 17.00, 18.89 and 21.29 µg ml^{-1} calculated as LCTZ on dried basis and with reference to Assay of standard.

By using absorbance of thus solutions against standard, content can be calculated form equation**:**

Actual Content: weight * P/100 * (100-WC)/100

Weight: weight of sample**.**

P: Standard Assay**.**

WC: Standard water content**.**

2.   Placebo spiked with Standard Preparations "as Product" used in Repeatability with concentrations of 50 %, 100 % and 150 % Assay. A. Content Calculated for each one "as product" from equation:

$$\text{Found Content} = (AT /AS) \quad x \ C$$

AT: Absorbance of Sample preparation.

AS: Absorbance of Standard preparation.

C: Concentration of standard in mg/ml.

3.   For both kind of solutions; %Recovery calculated by equation:

$$\%\text{Recovery} = \text{Found Content X } 100 /\text{Actual Content}$$

Difference between found content and actual content calculated.

Calculated the Q% by equation:

Q% = Found content / L x 100

Q%: Quantity released

L: Labeled claim

Table (3.2.6.1): Accuracy from linearity (recovery) of LCZT samples

Actual content in mg	Found content in mg	Recovery (%)	Actual content in mg	Found content in mg	Recovery (%)
2.09	2.10	100.28 ± 0.28	12.56	12.66	100.28 ± 0.28
4.19	4.20	100.28 ± 0.28	16.77	17.00	101.41 ± 1.41
6.28	6.33	100.75 ± 0.75	18.84	18.89	100.28 ± 0.28
8.37	8.49	101.34 ± 1.34	20.94	21.29	101.69 ± 1.69
10.47	10.47	100.00			

Table (3.2.6.2): Accuracy from precision repeatability results

Theoretical concentration of LCTZ	Measured concentration (µg/mL)	Recovery (%)for the different concentration	Recovery (%)for the different concentration depend on standard	Average of Recovery (%)	RSD%
LCTZ at 50 %	0.0026	52.7	101.96		
LCTZ at 100 %	0.0049	103.22	102.55	101.96	0.65
LCTZ at 150 %	0.0075	153.01	101.35		

From Linearity and within 9 different concentration from 41.99 % to 425.80 %;
recovery found to be 100.70 % +/- 0.70%, but from repeatability and within 3 different
concentrations from 50%, 100% and 150% through 3preparations; % Recovery found to
be 101.96 % with +/- 1.96.

It was found that the average recovery percentage from linearity (100.7%) and
repeatability (101.96%) was 101.32 % ± 1.325 %.

The results confirmed that the method of dissolution of LCTZ was accurate because it
lied between the standard limit range from 95.0% to 105.0% and below the maximum
limit of RSD 2.0%.

3.2.7 Stability of solution

For solutions prepared in precision day 1 read the absorbance in another day and calculate the Q%, deference in the Q% between two days is 1.74% as shown as below in Table (3.2.7.1)

Table (3.2.7.1): Stability of solution

Day 1				Day 2			
No of test	Weight mg	Absorbance Average	Q %	No of test	Weight mg	Absorbance Average	Q %
T1	155.0	0.287	103.38	T1	155.0	0.274	101.77
T2	157.2	0.290	103.00	T2	157.2	0.275	100.71
T3	156.4	0.296	105.67	T3	156.4	0.277	101.96
T4	162.3	0.302	103.89	T4	162.3	0.285	101.10
T5	160.5	0.299	104.01	T5	160.5	0.281	100.79
T6	157.0	0.292	103.84	T6	157.0	0.279	102.31

3.3. Conclusion and recommendations:

The validated developed analytical method proved to be rapid, simple, specific, accurate and cost effective. It can be considered reliable and suitable for the routine quality control analysis of Levocetirizine dihydrochloride in tablet dosage form.

 The presence of such validated, simple and reliable dissolution method especially by U.V spectrophotometry encourages further dissolution method studies on drugs dosage forms by veterinarians, pharmacists and scientists. The method was found fulfilling the ICH required guidelines of specificity, linearity, accuracy and precision

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