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Ultraviolet spectrophotometric validation of dissolution method for Levocetirizine dihydrochloride tablets

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Abstract
Among other antihistamines, Levocetirizine dihydrochloride (LCTZ) is designed to have increased efficacy with less harmful drug reaction. And among other spectrophotometry quantitation methods, ultraviolet spectrophotometry is increasingly becoming the technique of choice for assay and dissolution studies of pharmaceutical formulations. The objective of the present work was to develop and validate a dissolution method for LCTZ tablet using ultra violet spectrophotometry. The dissolution steps were based on the use of the paddle apparatus. The analytical assay was based on absorbance measurements at absorption maximum at 236.5nm Beer's law was obeyed in the concentration range from 2 to 22 mg. The correlation coefficient was 0.9998 with a relative standard deviation (RSD %) of 0.62%. Results of analysis were evaluated statistically and by recovery studies. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness. The proposed methods can be successfully applied in routine work for the determination of LCTZ in tablet dosage form.

Keywords: levocetirizine dihydrochloride, antihistamines, validation, dissolution, ultraviolet spectrophotometry

1. Introduction
Chemically, Levocetirizine dihydrochloride LCTZ, is dihydrochloride salt of (R)-2- (2-(4-((4-chlorophenyl) phenyl methyl) pipeyl) ethoxy) acetic acid and its chemical structure [1] is shown in Figure 1. It is used as antihistamine mediated via selective inhibition of H1 receptors [2]. Levocetirizine dihydrochloride is available in number of combinations with monte- lukast, pseudoephedrine, cefpirome, ambroxol hydro-chloride [3, 4]. LCTZ 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 hay fever [5]. Dissolution studies have emerged in the pharmaceutical field as a very important tool based on the fact that for a drug to be absorbed and available to the systemic circulation, it must previously be solubilized. Therefore the dissolution studies are used not only to assess batch to batch consistency of drug release from solid dosage forms, but they are also essential in several stages of formulation development, for screening and proper assessment of different formulations. Moreover, the *in vitro* dissolution studies obtained from dissolution rate profile has been used for the successful characterization of the in vivo behavior of drugs [6-13]. Some drugs (eg. LCTZ) have no official method and in most cases require development of suitable validated method [9-12] for their assay and dissolution. Several literature review studies were conducted for the determination of LCTZ based on different methods of analytical procedure either chromatographic or UV spectrophotometric [8] analysis methods. The aim of the present study is to develop and validate a new simple, rapid, reliable and precise UV spectrophotometric method to be applied for the dissolution studies of LCTZ from tablet formulation.

2. Materials and Methods
2.1 Instrumentation
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.
PTWS1000, rate power 850 w Temp-rang 10˚ C - 40˚ C Dissolution tester (Pharma test, Germany).

2.2 Materials
LCTZ standard (working standard) and Levohist tablets (5mg) were obtained from Azal pharma LDT, Khartoum, Sudan. All chemicals and solvents used in the spectrophotometric analysis were of analytical reagent grade.

2.3 Methods
2.3.1 Preparation of standard solution
10mg of LCTZ was accurately weighed; then, it was transferred to 100 -ml volumetric flask which had been half-filled with 0.01 M HCl. The mixture was sonicated for 10 minutes, cooled to room temperature and completed to the mark with 0.01 M HCl from which an aliquot of 5ml was diluted to the mark in 50-ml volumetric flask (0.01 mg ml-1).The ultraviolet spectrum of the solution was recorded; and showed a maximum absorption at 236.5nm.

2.3.2 Dissolution studies of LCTZ tablets

One tablet was placed into each six dissolution vessels, individually containing 500ml of the dissolution medium(0.01 M HCl) placed in the dissolution tester after the dissolution system was conditioned (temperature equilibrated to 37˚C±0.5 ˚C,, using paddle and adjusting speed at 50 rpm)for 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).The absorbance of this solution was measured at 236.5 nm to determine the concentration.

2.3.3 Validation of the proposed methods

2.3.3.1 Specificity (selectivity)

10 mg of LCTZ – W.S, 104 mg of placebo equivalent to one tablet and 304 mg from the powder of crushed 20 tablets equivalent to two tablets of levohist were transferred quantitatively to separate 100-ml volumetric flasks which had been half -filled with the dissolution medium (0.01 M HCl). The mixtures were sonicated for 10 minutes to dissolve the powder, cooled to room temperature and completed to the mark with the dissolution medium. Aliquots of 5ml from each solution were pipetted into separate 50-ml volumetric flasks and the volume was completed to the mark with the same dissolution medium. Ultra violet absorption spectra of the solutions was recorded; maximum absorption peak was observed only the spectra of the solution of the standard and the solution of tablets and did not appear in that of the placebo.

2.3.3.2 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. From the 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-1of LCTZ the absorbance of each solution was measured at 236.5nm against the dissolution medium. Range was established through linearity.

Measurement was made from 40.0 % to 400.0% and from 2.0 mg to 20.0 mg LCTZ.

$Y = a + b\,x$

Where Y≡ practical response, a ≡ intercept, b ≡ slope, and x ≡ concentration of the sample.

2.3.3.3 Accuracy recovery

The accuracy /recovery of the dissolution method LCTZ was done in conjunction with the linearity by determining different concentrations of LCTZ from 2.0 to 20.0 µg ml-1 The absorbance of each solution was measured. The recovery of the dissolution method of LCTZ was calculated by using the absorbance of each solution from the linearity determination.

2.3.3.4 Precision

Intermediate precision / repeatability

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

The experiment was also repeated after two days. The percentages of quantities dissolved (Q %) were calculated representing day-to-day precision, respectively.

The repeatability of the dissolution of LCTZ tablet was conducted by performing triplicate dissolution steps on equivalent composites of the ingredient of the tablet. Three composites of the standards and placebo required to produce 50%, 100% and 150% of the tablet content were weighed accurately, transferred quantitatively to 100- ml volumetric flask which had been half -filled with dissolution medium, the mixture was sonicated for 10 minutes to dissolve, cooled to room temperature and completed to the mark with dissolution medium and an aliquot of 5ml was further diluted to the mark in 50-ml volumetric flask. The absorbance of each of these solutions were measured at 236.5 nm.

2.3.3.5 Robustness

For evaluation of robustness for UV spectrophotometric analysis the effect of slightly varying the absorption wavelength (±2 from λ max 236.5nm) was investigated.

10 mg of LCTZ W.S was of weighed accurately and transferred quantitatively to 100- ml volumetric flask which had been 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. An aliquots of 5ml was diluted to the mark in a 50-ml volumetric flask. The absorbance of standard solution was recorded at wavelength 238.5, 236.5and 234.5nm.

2.3.3.6 Stability of solution

The solutions were prepared as in precision day1, the absorbance was recorded in another day and the Q% was calculated

3. Results and Discussion

3.1 Dissolution results

3.1.1 Methods of Dissolution validation

Table (1) shows the RSD% obtained was lower than 2% indicating that UV spectrophotometric validity of the developed dissolution method for determination Q % of LCTZ in Levohist (5 mg).

3.1.2 Specificity/ Selectivity

Specificity of validation dissolution method was ensured by absence of the main absorption peak of the standard LCTZ solution in that of placebo. Table (2) shows that the interference of the absorption of placebo on that of the standard is 0.0%.

3.1.3 Linearity and range

The UV spectrophotometric method for the determination of linearity and range of LCTZ was demonstrated by drawing Beer's calibration curve (Figure.2).which followed the

regression equation:
y=35.868x-0.0018

The linearity range was 2 µg ml-1to 20 µg ml-1 as shown in Table (3).
From calibration curve, the correlation coefficient of the determination was "R²" = 0.9998.
Table (4) shows that the validation method gave a range of 2.0mg (40%) to 20.0 mg (400%) of LCTZ.

3.1.4 Limit of detection (LOD) and limit of quantitation (LOQ)
The limit of detection (LOD) and limit of quantitation (LOQ) were determined by ICH by establishing the maximum level at which the analyte could be reliably detected and the lowest concentration that could be measured, respectively. Then they 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 (5).

3.1.5 Precision
Precision of the result of the developed analytical method for repeatability and intermediate precision/ ruggedness, of different analysts and different days are shown in tables (6) and (7), respectively.
The repeatability studies showed that:

1. For individual preparation, RSD % ranged from 0.00% to 1.33%.
2. For intermediate precision between different analysts, the Q % was100.73% and 99.26 % for analyst 1 and analyst 2 respectively, giving an average of 99.99% and RSD% of 1.04% between the two analysts.
3. For intermediate precision for different days, the Q % was 100.73% and 102.02% for day 1and day 2, respectively, giving an average of 102.99% and RSD% of 1.33% between the two days.

The validation dissolution method showed that the RSD% didn't exceed 1.33% when the absorbance of the test solution was 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.

3.1.6 Robustness
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%).

3.1.7 Accuracy
The accuracy of the method was determined from the weight of LCTZ content from linearity measurements using average absorbance in comparison with those standard solutions to calculated the recovery percentage shown in Table (9) as well as RSD% for the absorbances were less than 2% (0.6 %).
Alternatively, the recovery percentage can also be calculate from precision measurements using prepared standard concentrations and those obtained experimentally concentration giving RSD% of 0.65% Table(10).

3.1.8 Stability of solution
Table (11) shows that the average Q% difference between averages of absorbance measured at day 1 and day 2 as well as RSD% were only 1.74%.

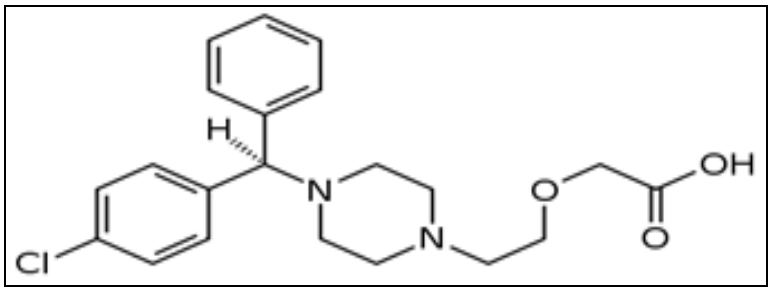

Fig 1: Levocetirizine Dihydrochloride (R)-2-(2-(4-((4-chlorophenyl) phenyl methyl) pipeyl) ethoxy) acetic acid

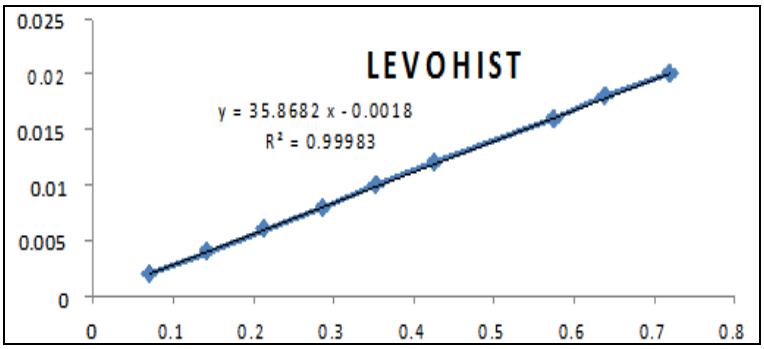

Fig 2: calibration curve

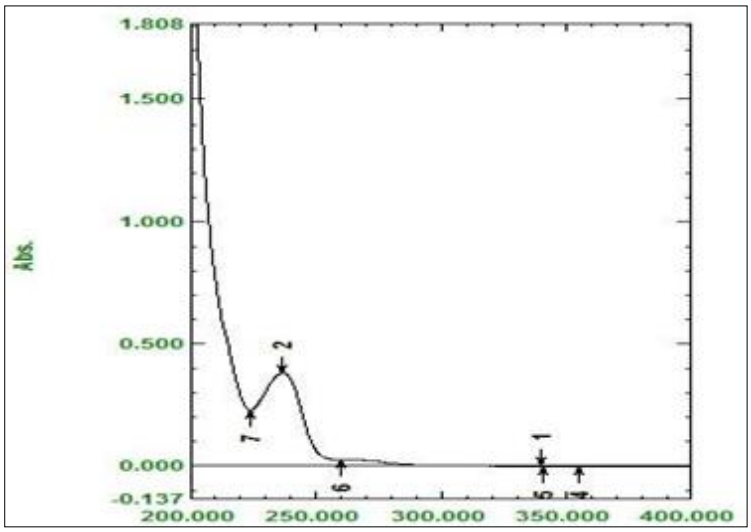

Fig 3: UV spectrum of Identification test

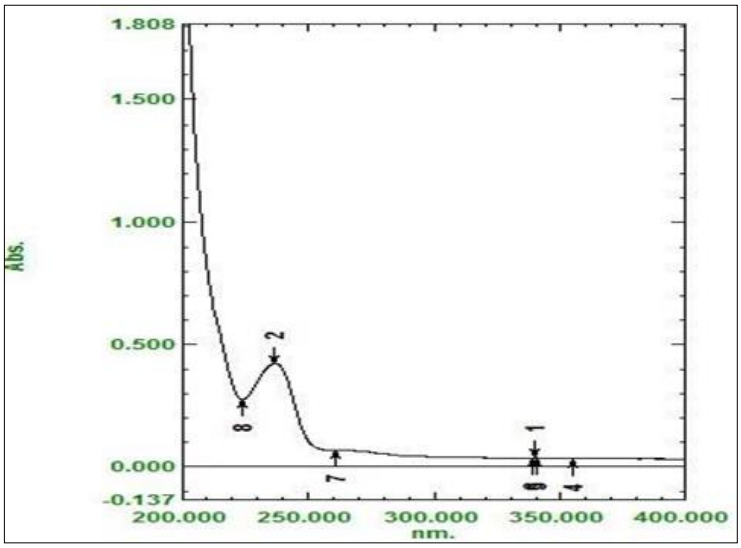

Fig 4: UV spectrum of sample

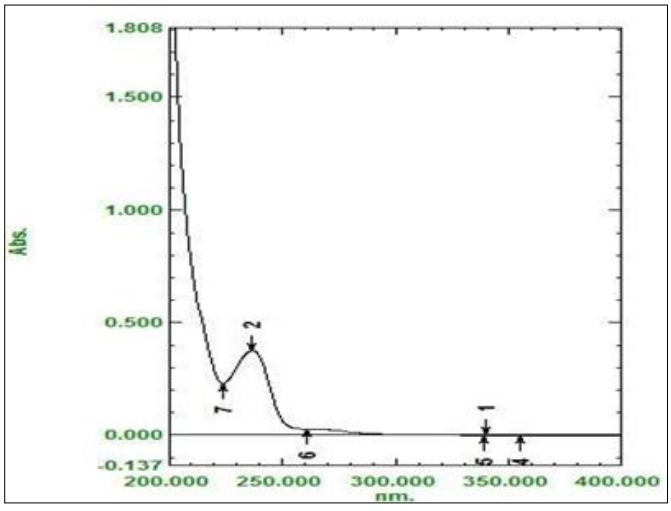

Fig 5: UV spectrum of standard

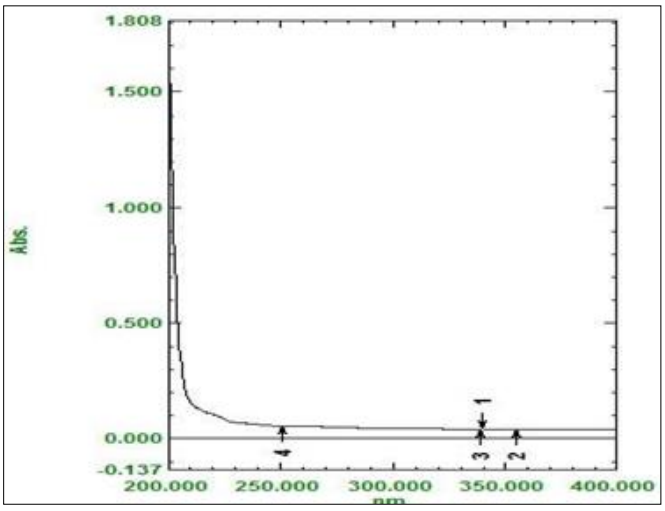

Fig 6: UV spectrum of blank

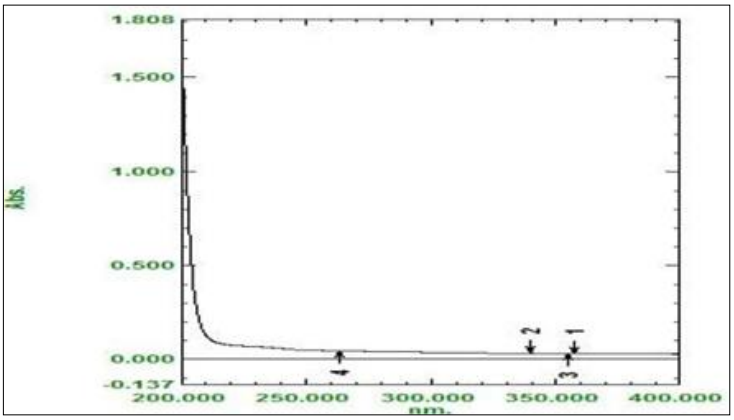

Fig 7: UV spectrum of placebo

Table 1: Dissolution test rustle Q% of LCTZ in levhist 5mg

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

Table 2: 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

Table 3: 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		

Table 4: 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		

Table 5: Parameters for LCTZ of the proposed method

Parameters	Value
Measurement wavelength (nm)	236.5
Linear range	2-20(mg ml-1)
Intercept	0.0018
Standard deviation of the intercept	0.005
Correlation coefficient (r2)	0.9998
Slope	35.868
Limit of detection LOD(µ/ml)	0.5
Limit of quantitation LOQ(µ/ml)	1.52

Table 6: 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 7: 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

Table 8: 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		

Table 9: Accuracy for linearity (Recovery) of LCZT sample

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 10: Accuracy from precision repeatability results

Theoretical concentration of LCTZ	**Measured concentration (µg ml-1)**	**Recovery (%)for the different concentration**	**Recovery (%) for the different concentration depend on standard**	**Average of Recovery (%)**	**RSD %**
LCTZ at 50 %	2.6	52.7	101.96	101.96	0.65
LCTZ at 100 %	4.9	103.22	102.55		
LCTZ at 150 %	7.5	153.01	101.35		

Table 11: 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 Discussion

The spectrum of levocetirizine showed the absorption maxima at 236.5 nm. No effect of dilution was observed on the maxima, which confirmed the maxima at 236.5nm. The statistical analysis of data obtained for the calibration curve of LCTZ in pure solution indicated a high level of precision for the proposed method, as evidenced by low value of coefficient of variation. The coefficient of correlation was highly significant. The linearity range was observed between 2 – 22 µg ml-1. The plot clearly showed a straight line passing through origin (Y =35.868 X + 0.0018). The assay method was validated by low values of standard deviation and standard error, indicating accuracy and precision of the methods. Excellent recovery studies further proves the accuracy of the method. The method was found fulfilling the USP and ICH required guidelines of specificity, linearity, rang, accuracy, precision and stability.

3.4 Equations

By data collected from linearity measurements: "Found contents calculated using Average of absorbance" and Compared with Actual content of prepared solutions.

By data collected from precision; repeatability measurements: between prepared concentrations and result found.

1. LCTZ standard preparations used in linearity with concentrations of 2.1, 4.2, 6.3, 8.4, 10.5, 12.6, 16.8, 18.8 and 20.9 µ ml-1calculated as LCTZ on dried basis and with reference to assay of standard(WS).
   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. Content calculated for each one "as product" from equation:

Found Content = (AT /AS) x C
AT: Absorbance of Sample preparation.
AS: Absorbance of Standard preparation.
C: Concentration of standard in µg ml-1.

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

%Recovery = Found Content X 100 /Actual Content

4. Difference between found content and actual content calculated.
5. Calculated the Q% by equation:

Q% = Found content / L x 100
Q%: Quantity released
L: Labeled claim

4. Conclusions

UV spectrophotometric method was proved to be simple, rapid and cost-effective for the efficient evaluation of the dissolution of LCTZ tablets and its quantitative determination. It fulfilled the USP and ICH required validation guidelines of specificity, linearity, rang, accuracy, precision and stability. It can be applied for the routine estimation of LCTZ in pharmaceutical dosage form.

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