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Method Development And Validation For Simultaneous Estimation Of Diltiazem And Lidocaine By Uv – Visible Spectroscopy

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Abstract

Diltiazem and lidocaine are two drugs that are used in the treatment of anal fissures. Both of these medications are employed. The objective of this study is to devise analytical procedures that are uncomplicated, precise, and accurate for the purpose of simultaneously estimating the levels of these two drugs. To be more specific, the UV spectrophotometric approach was developed specifically for the aim of being evaluated. In terms of solubility in methanol, both carpets had a good degree of solubility, and linearity was observed between 10 µg/ml and 50 µg/ml for both Lidocaine and Diltiazem. The maximum wavelengths (λmax) of these carpets were measured to be 262 nm and 275 nm, respectively. Comparatively, the recovery percentages for diltiazem and lidocaine were 106% and 96.6%, respectively, when compared to one another. The measurements revealed that the correlation coefficients for diltiazem and lidocaine were 0.999 and 0.996, respectively. These values were determined via correlation analysis. The ultraviolet-visible spectrophotometric approach is better to other methods in terms of accuracy, efficiency, speed, and cost-effectiveness. It is possible that this strategy will prove to be advantageous, taking into consideration the fact that there is not yet an analytical method that has been created for the combination of Diltiazem and Lidocaine.

Keywords : Spectrophotometric method, lidocaine, diltiazem, methanol, LDC, λmax, API ect.

Introduction

Anal fissures are superficial skin tears located distal to the dentate line that often require ER visits. Usually,an injury, constipation, or firm stools induce anal fissures. Anal fissures can happen to adults as well as children, though people who have previously experienced constipation are more likely to have these episodes. While chronic fissures in the jaw take longer to heal, acute fissures can mend in less than six weeks. LDC is a white powder that is soluble in ethyl alcohol and oils but not in water. It is also less lipid-solublethan other local anesthetic agents, which limits its potency. LDC is the monocarboxylic acid amide that is produced when N,N-diethylglycine and 2,6-dimethylaniline are formally condensed. It serves as an environmental contaminant, a xenobiotic, a local anesthetic, an anti-arrhythmia medicine, and a drug allergen. It is a tertiary amino compound that is an amide of monocarboxylic acid and a member of the benzene family. It and glycinamides are functionally similar. DTZ is a white to off white crystalline powder with a bitter taste. It is a member of the calcium-channel blocker drug class. Additionally, improves the heart's blood and oxygen flow.

The most important component of the pharmaceutical development program is the development of analytical methods. Additionally, it is the process of demonstrating that the created method can be applied to determine the concentration or amount of API in different formulations. One of the earliest instrumental analysis techniques is UV-visible, which serves as the foundation for many excellent approaches for figuring out the micro- and semi-micro-quantities of analytes in a sample. Developing and validating a UV spectrophotometric method for the simultaneous measurement of LDC and DTZ was the specific goal of the research project.

Research envisaged

Every year new drugs and newer drug combinations are entering in the market of pharmaceuticals. Analytical methods for such drugs are mostly available only to the manufacturing company. Howeverother accessible multiple analytical methods for the same drug or same drug combination in their formulation is advantageous.

Also, such method development helps in training the analyst for the skillfullhandling of the sophisticated instruments and also the was research approach. After survey of various literature and references reveal that such methods for the new drugs and their combinations available in market are continuously underway in many academic institutions. The present work is also planned in similar way. There are many methods for the estimation of diltiazem and lidocaine in their foxed dosage form.but there are many unexplored drug combinations which are yet to be explored for the analytical method development by uv and hplc , one such combination of them is of lido. and dil.in a marketed product crema gel -L which is used in treatment of anal fissures.

Method development by UV spectrometer

The relationship between analyte concentration and absorbed light amount (absorption) forms the basis of most analytical applications of molecular spectroscopy. This research establishes the fundamentals of subsequent simultaneous spectrometric estimation methods which are employed for quantitative estimation of multi-component formulation[22,23]. Method Development by UV-visible Spectrometer Absorption spectroscopy is one of the preferred and widely used tools available for quantitative analysis.

Simultaneous equation method (vierordt's method)

For spectrophotometric multicomponent analysis, one of the most popular and straightforward techniques is to solve a set of simultaneous equations to determine the concentration of many components in a given mixture, even if their spectra overlap. The concept of this approach comes from the additive natureof each component's absorbance in any mixture. Suppose a sample contains two absorbing species X and Y. Absorbance of this mixture is measured at wavelength of maximum absorbance (λmax) of each drug (λ1 for drug y and λ2 for drug x)

Thus the individual concentrations of both drugs may be determined by using the simultaneous method provided that certain criteria are fulfilled. The information required is :

Ax_1 and ax_1 being the absorptivity of drug X at λ1 and λ2 respectively.

Ay_1 and ay_2 being the absorptivity of drug y at λ1 and λ2 respectively.

Let c_x and c_y be the concentration drug x and drug y respectively in the diluted sample. At λ_1 and λ_2, the absorbance of the mixture in a constant path length b is the sum of the individual absorbance of X and Y.

Simultaneous equations are thereby constructed.

At λ_1 : $A_1 = ax_1bc_x + ay_1bc_y$ .... (1)

At λ_2 : $A_2 = ax_2bc_x + ay_2bc_y$ ....(2)

For measurement in 1 cm cell, b=1

By crammer's rule, c_x and c_y can be determined as follows$C_x = (A_2ay_1 – A_1ay_2) / (ax_2ay_1 – ax_1ay_2)$

$C_y =(A_1ax_2 – A_2ax_1) / (ax_2ay_1 -ax_1ay_2)$

Materials and methods Chemicals and reagents

Diltiazem hydrochloride and lidocaine were purchased from Yarrow chem chemicals, Mumbai. Methanol was purchased from Sai emporium, Jabalpur. Distilled water was obtained from the local market. All the chemicals used were of analytical grade. All the solutions were freshly prepared in methanol.[19,23,24].

Equipment:

UV spectrophotometer – Shimadzu uv, 1700, Japan which is attached to a computer software uv probe 2.0with a spectral width of 2nm, wavelength accuracy of 0.5nm, and a pair of 1 cm matched quartz cell.[23]

Methods

Physical characteristics of LDC and DTZ

The physical characterization of procured drugs were determined on the basis of following parameters**Organoleptic**

properties

The organoleptic properties have been determined for nature, colour,taste and odour of the pure sample ofLDZ and LDC

Solubility

Excess amount of both the drugs were dissolved in 10 ml methanol in separate flask till a saturated solutions [19,20] were obtained. The saturated solution of the drugs were stirred for 48 hrs on magnetic stirrerat 100rpm and room temperature (at 25 ± 1°c). Then the sample was centrifuged for 10 min at 10,000 rpm.Clear supernant was collected using 0.22µm syringe filter and analysed using uv spectrophotometer. The results were analysed and noted.

Melting point

Melting point of DTZ and LDC were determined using capillary melting point apparatus. In this method a small quantity of drugs were filled in a separate capillary tube open both the ends and it was placed along with the thermometer in melting point apparatus.

Simultaneous estimation of DTZ and LDC using vierodt's method

Selection of wavelength range for estimation

DTZ and LDC both were separately dissolved in methanol and appropriate dilutions were prepared by taking aliquots from the stock solution. The drug solutions were scanned from 200 – 400 nm and from thatwavelength ranges were selected for the estimation of drugs.

Preparation of standard stock solutions (1000µg/ml)[4,6,820]

Stock 1: Two separate 100ml volumetric flasks containing 100mg of LDC and 100mg of DTZ were precisely weighed and transferred. To dissolve the medicines, add 10 ml of methanol to each, and then increase the volume to 100 ml to obtain a solution containing either 1 mg/ml or 1000 µg/ml.

Preparation for the calibration curve

Aliquots of 0.1,0.2,0.3,0.4, and 0.5 ml of the prepared stock solution of DTZ and LDC were each placed separately into a series of 10 ml volumetric flasks. Methanol was added to the fluid until the concentrationrange of 10–50µg/ml of both medications was achieved. In order to measure absorbance, the solutions were scanned between 200 and 400 nm using methanol as the blank.

Methodology

The working standard solutions of LDC and DTZ were scanned in ultraviolet light between 200 and 400 nm. The wavelengths with the highest absorbance were found to be 275 nm for LDC and 262 nm for DTZ,and this wavelength was chosen for the quantitative estimation of both substances. Thus, the two components' concentration was determined using the simultaneous equation method (Vierordt's method).

UV Method validation

The uv spectrophotometric method was validated for accuracy, precision, linearity, detectionlimit,quantitation limit and robustness [4,5,6,10,11,12]

Linearity

For both DTZ and LDC, the linearity of the procedure was tested in the concentration range of 10–50µg/ml.The graph of absorbance vs. concentration was plotted in order to create the calibration curves. Over the concentration range, a linear regression equation (y= mx+c) was found.

Range

The range that exhibits the proper level of precision, accuracy, and linearity of the analytical process is the range that contains the highest and lowest concentrations of the analyte. To find the range of the procedure,DTZ and LDC from the concentration range of 10-50µg/ml were utilized. To assess the range, a precisely measured standard working solution of LDC and DTZ was created.

Precision

Without altering the parameters of the suggested method, the precision of the procedure was obtained by repeatedly scanning and measuring the absorbance of LDC and DTZ. three times (n=6) By analyzing the reaction three times on the same day and three other days over the course of a week for all standard solutions of 10µg/ml - 50µg/ml for both medicines, the intraday and interday precision of the suggested procedures was ascertained. Standard deviation and percent relative standard (% RSD) were usedto report the results.

Accuracy

By using the usual addition approach to calculate the recovery of LDC and DTZ, the accuracy of the procedure was ascertained. Pre-quantified sample solutions of diltiazem (10 µg/ml) and lidocaine (10 ug/ml) were mixed with a known quantity of standard solutions at the 80,100.120% level. The resulting values were then applied to the calibration curve's regression equation to estimate the quantities of LDC and DTZ.

Limit of detection and limit of quantification

The international conference on harmonization (ICH) rules designated the following equation, which wasused to calculate the standard deviation of response and slope of the linearity curve, in order to determinethe limit of detection and the limit of quantification of the medications.

LOD : 3.3 × σ/ slope LOQ : 10 × S.D./slope

Where, σ = the standard deviation of the response S = slope of the calibration curve

Result and Discussion

I. Organoleptic properties

DTZ and LDC both were found to be white coloured , non hygroscopic, crystalline powder.

Solubilty

The solubility in methanol of DTZ was found to be 50mg/ml and that of LDC was found to be 40mg/ml.

Melting point

The melting point was determined by capillary melting point apparatus. The observed melting point of DTZ is 187°c and that of LDC is 68°c.

Analytical method for drug concentration measurements (uv/visible method)

DTZ and LDC in bulk and formulation were simultaneously estimated using the UV-visible spectrophotometric technique. This procedure involved scanning the diluted solution of LDC and DTZ from 200 to 400 nm. The two chosen wavelengths from DTZ and LDC, respectively, are 262 and 242 nm. In this case, the absorbance maxima of LDC and DTZ are 275 and 262 nm, respectively.

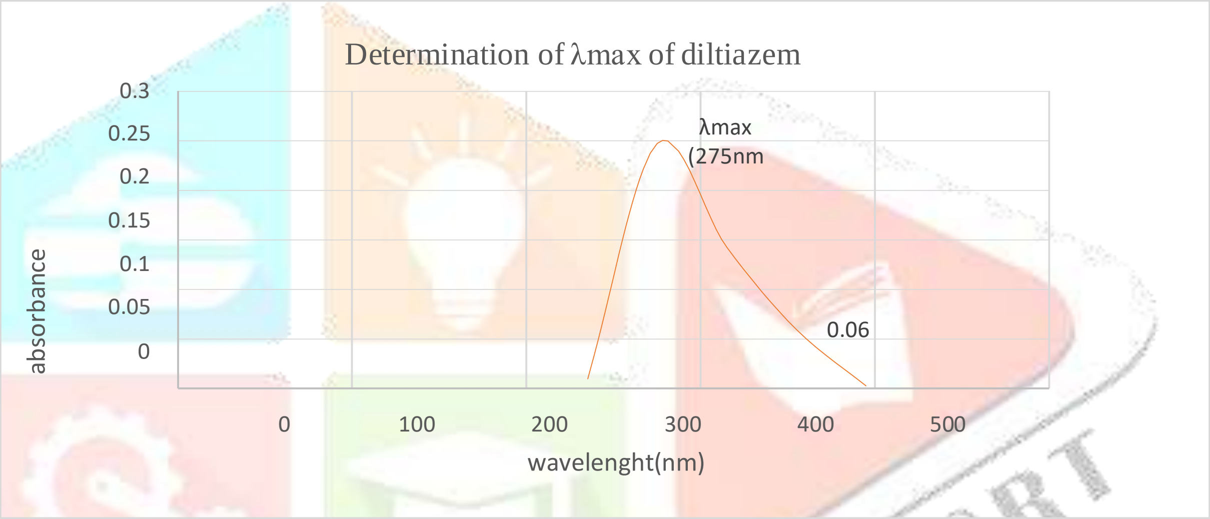

Figure 1: UV Spectrum of Diltiazem

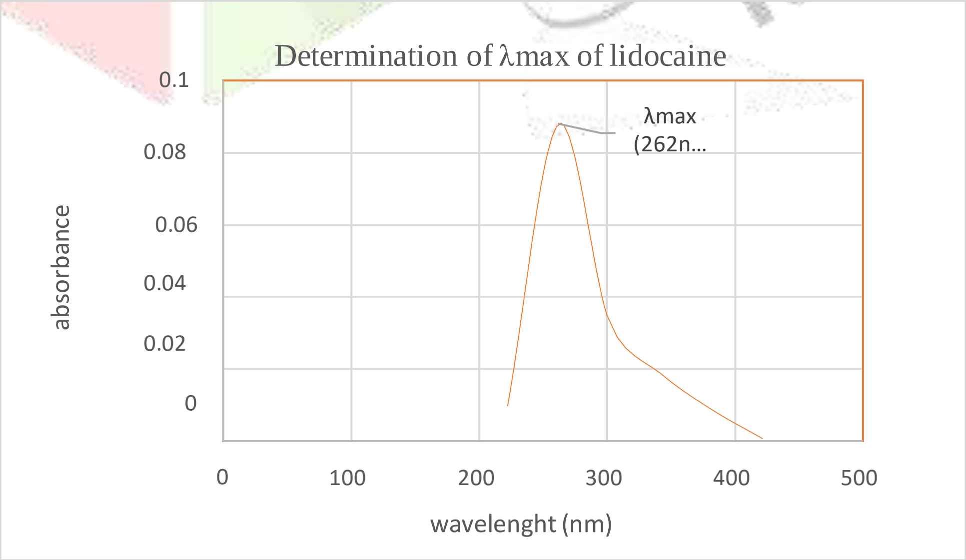

Figure 2: UV Spectrum of Lidocaine

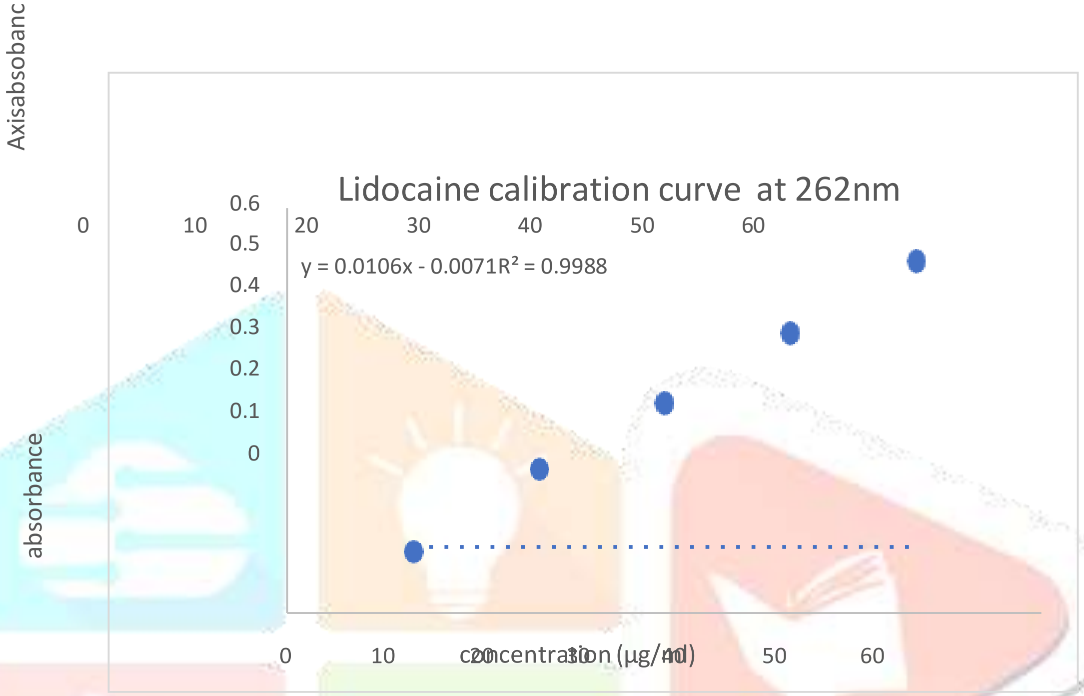

Figure 3: Calibration Curve of Lidocane

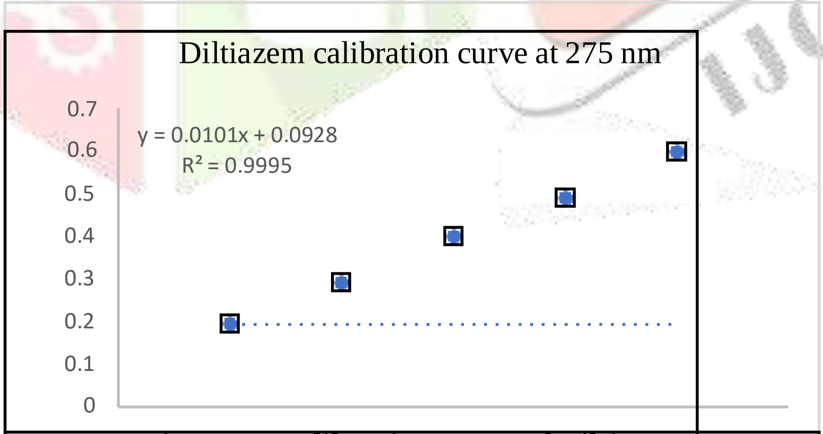

Figure 4: Calibration curve of Diltiazem

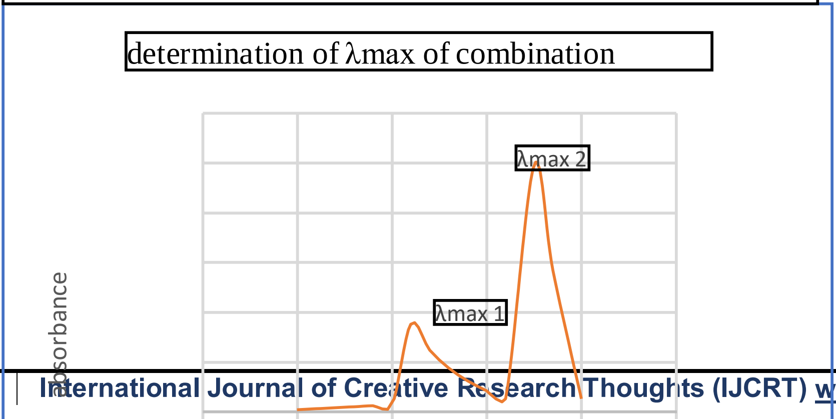

Figure 5:UV spectrum of sample

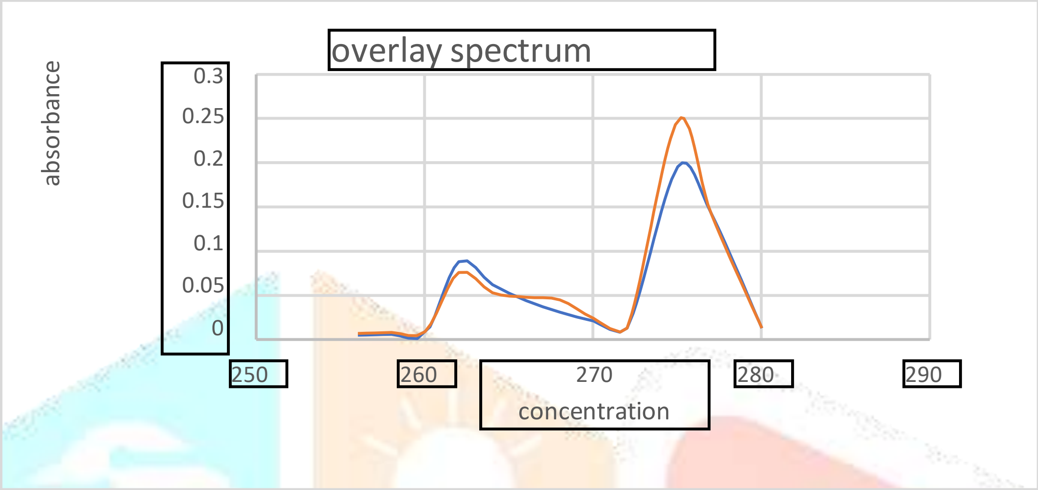

Figure 6: Overlay UV spectrum of the drugs

Method Validation : the developed method was validated as per the ICH guidelines for the following parameters:

Linearity: The linearity for DTZ was found to be linear in the range of 10-50µg/ml . The regression coefficient was found to be 0.9995 and the straight line equation was found to be y=.0101x +.09228.

The linearity of LDC was found to be linear in the range of 10-50µg/ml with the regression coefficient0.9988 and straight line equation was found to be y=0.0106x -0.0071.

Range : the observed range of the sample was found to be 10µg/ml to 50µg/ml

Accuracy : the accuracy of the analytical method for DTZ and LDC was determined at 80%, 100% and120% levels of standard solution. Absorbance was measured at 275nm for DTZ and at 262nm for LDC and the results are expressed in table 2 and 3 respectively.

Table 1: Recovery studied of Diltiazem

% level of recovery	Amount of drug taken (µg/ml)	Amount of drug spiked (µg/ml)	Absorbance	Actual conc.(amt. Recovered)	Mean conc.	Std. Deviation	% recovery	Mean %recovery
80	10	8	0.273	17.8	17.77	0.058	98.8	98.77
	10	8	0.272	17.8			98.8	
	10	8	0.27	17.7			98.7	
100	10	10	0.292	19.6	19.67	0.058	98.5	98.57
	10	10	0.293	19.7			98.6	
	10	10	0.293	19.7			98.6	
120	10	12	0.31	21.6	21.67	0.058	98.5	98.55
	10	12	0.312	21.7			98.6	
	10	12	0.321	21.7			98.6	

Table 2: Recovery studies of Lidocaine

% level of recovery	Amount of drugtaken (µg/ml)	Amount of drug spiked (µg/ml)	Absorbance	Actual conc.(amt. Recovered)	Mean Concn.	Std. Deviation		Mean % recovery
80	10	8	0.196	17.8	17.77	0.05773503	98.89	98.74
80	10	8	0.194	17.8			98.89	
80	10	8	0.196	17.7			98.33	
100	10	10	0.217	19.8	19.63	0.15275252	99.00	98.17
100	10	10	0.215	19.6			98.00	
100	10	10	0.219	19.5			97.50	
120	10	12	0.238	21.7	21.53	0.2081666	98.64	97.88
120	10	12	0.237	21.6			98.18	
120	10	12	0.241	21.3			96.82	

Precision: The intra day and inter day precision were carried out using same optimized conditions. The precision (measurement of inter -day , intra- day, repeatability) results showed good reproducibility with the relative standard deviation (% rsd) below 2% as shown in table 4 and table 5 and table 6 respectively. This indicates that the method was highly precise.

Table 3: intraday precision data of diltiazem and lidocaine

Sr. No	**Sam (µg/ml)**	**absorbanceat**		**label claim(mg/30gm)**		**Label claim estimated**		**% label claim estimated**	
		275	**262**	**Diltiazem**	**Lidocaine**	**Diltiazem**	**Lidocaine**	**Diltiazem**	**Lidocaine**
01	10	0.295	0.240	600	600	635.8	581.8	105.9	96.9
02	10	0.296	0.242	600	600	635.9	581	105.9	96.8
03	10	0.295	0.242	600	600	636	582	106	97
04	10	0.297	0.241	600	600	636	582	106	97
05	10	0.298	0.244	600	600	636	581.7	105.9	96.9
06	10	0.295	0.240	600	600	635.9	581.6	105.6	96.9
Mean								105.95	96.91
Standard deviation								0.054	0.075
Rsd								0.050	0.077

Table 4 : Interday precision data of diltiazem and lidocaine

Sr. No.	ample conc.	Absorbance At		Label claim (mg/30gm)		Label claim estimated		% label claim estimated	
		275	262	Diltiazem	Lidocaine	Diltiazem	Lidocaine	Diltiazem	Lidocaine
1	10	0.294		600	600	635.8	581.8	105.9667	96.96667
2	10	0.295	0.241	600	600	635.9	581	105.9833	96.83333
3	10	0.291	0.241	600	600	636	582	106	97
4	10	0.295	0.24	600	600	636	582	106	97
5	10	0.295	0.24	600	600	636.1	581.7	106.0167	96.95
Mean		0.294	0.2405			635.96	581.7	105.9933	96.95
Standard deviation						0.114018	0.412311	0.019003	0.068718
% rsd						0.017928	0.07088	0.017928	0.07088

Table 5 : Results of repeatability

drug	**Label claim(mg)**	**Amount found(mg)**	**Label claim**	**S.d.**
Diltiazem	600	636	106	0.05
Lidocaine	600	582	97	0.057

Limit of detection and limit of quantification : LOD and LOQ were determined to be 0.52µg/ml and1.5µg/ml for DTZ and 0.53µg/ml and 1.6µg/ml for LDC respectively.

Table7: Validation parameters of Diltiazem and Lidocaine

Parameters	Diltiazem	Lidocaine
Linearity range (µg/ml)	10-50	10-50
Correlation coefficient (r)	0.9995	0.998
Precision (% RSD)	0.055	0.0577
Accuracy (% RSD)	0.053	0.081
Limit of detection (µg/ml)	0.52	0.53
Limit of quantification (µg/ml)	1.5	1.6

Conclusion

When it came to the process of creating and validating the UV spectrophotometric method, the guidelines that were set by the International Council for Harmonisation (ICH) were strictly adhered to throughout the course of the procedure. It is possible to apply the new approach on a daily basis for the research of DTZ and LDC in bulk and combination dose forms. This is due to the fact that it is less complicated, more accurate, more exact, and more cost-effective than other methods. There are additional advantages, one of which is that it is more accurate. This is as a result of the fact that it demonstrates better degrees of precision, accuracy, and accuracy. Further, the results were well within the acceptance standards, which indicates that the method has a high degree of potential for the determination of DTZ and LDC in a wide range of dosage forms. This is because the results were well within the acceptance requirements. One piece of evidence that demonstrates this is the fact that the results fulfilled the standards for acceptance. This is due to the fact that the outcomes were well inside the acceptable range of possibilities, which is the reason for this.

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Conflict of Interests

The authors have no conflict of interests.

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