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Development and Validation of a Method for the Simultaneous Estimation of Diltiazem and Lidocaine Using Ultraviolet-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 [1]. 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 has a bitter flavour and appears as a white to off-white crystalline powder. This medicine belongs to a class called calcium-channel blockers. Furthermore, it enhances the circulation of blood and oxygen to the heart [2].

Creating analytical procedures is the first and foremost step in developing pharmaceuticals. The procedure of showing that the developed approach can be used to find the amount or concentration of API in various formulations is also part of it. When it comes to determining the micro- and semi-micro-quantities of analytes in a sample, many great methods have their roots in ultraviolet-visible analysis, one of the very first instrumental analytical techniques. The study's stated objective was to create and test a UV spectrophotometric technique for measuring LDC and DTZ in the same run.

Research Envisaged:

New medications and novel drug combinations are introduced to the pharmaceutical industry each. Typically, only the pharmaceutical corporation has access to analytical methods for these types of medications. Still, it's helpful to have access to numerous analytical methods that can be used for the same medicine or a combination of drugs. Training the analyst in the expert use of complex instruments and a systematic approach to research is another benefit of developing such methods [3]. Such efforts for commercially viable novel pharmaceuticals and their combinations are ongoing in numerous research institutes, according to a review of relevant literature. The current project is structured similarly. The foxed dose forms of diltiazem and lidocaine can be estimated using a variety of approaches.One such combination that has not yet been investigated for the purpose of developing analytical methods using UV and hplc is the one found in the commercially available product crema gel-L.

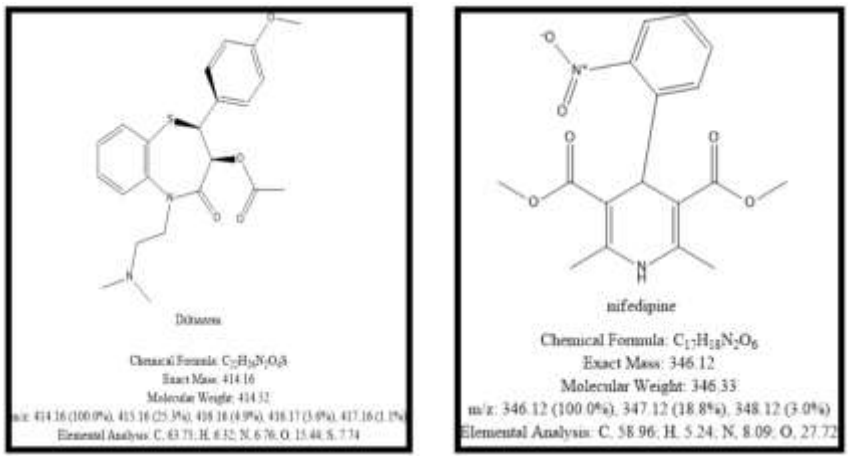

Fig.1 Chemical structure and properties of Diltiazem and Nifedipine

Method development by UV spectrometer

Most analytical uses of molecular spectroscopy are based on the absorption relationship, which is the relationship between analyte concentration and amount of light absorbed. The next approaches for quantitative estimate of multi-component formulations rely on simultaneous spectrometric estimation, which this study lays the groundwork for [4,5]. Advancement of Procedures through UV-visible spectrometer. When it comes to quantitative analysis, absorption spectroscopy is a popular and frequently used method.

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. Because the absorbance of each component in a combination is additive, our technique is based on that principle. [6,7] Consider a sample that includes X and Y, two species that absorb light. Each drug's maximum absorbance (λmax) is used to measure the absorbance of this mixture; for example, λ1 for drug y and λ2 for drugx. Therefore, under specific conditions, the simultaneous approach can be used to determine the individual concentrations of both medications. The necessary data is:

The drug X absorption at λ1 and λ2 are represented by Ax1 and ax1, respectively.
Ay1 and ay2 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.

The tools and techniques Substances and agents:

The ingredients lidocaine and diltiazem hydrochloride were bought from Yarrow chem chemicals in Mumbai. The methanol was bought in Jabalpur from the Sai emporium. We bought distilled water at the corner store. The compounds utilized were all of analytical quality. The methanol was used to newly create all of the solutions.in references [8-10].

Equipment:

The following items are required for this experiment: a UV spectrophotometer (Shimadzu 1700, Japan) linked to a computer program (UV Probe 2.0) that has a 2 nm spectral breadth, a 0.5 nm wavelength precision, and two identical quartz cells, each 1 cm in diameter.[11].

Methods

Physical characteristics of LDC and DTZ

The physical characterization of procured drugs were determined on the basis of following parameters [12]

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

Solubility:
Methods:
In terms of their physical properties, LDC and DTZ
The physical characteristics of the medications that were obtained were assessed using the following criteria:

Organoleptic characteristics:
The organoleptic features of the pure LDZ and LDC samples have been identified, including their colour, flavour, aroma, and nature.

Diluting Power:
Separate flasks were used to dissolve the excess amounts of each medication in 10 ml of methanol until saturated solutions were obtained [13,14] For 48 hours, the drug-saturated solution was swirled at 100 rpm and room temperature (25 ± 1°C) using a magnetic stirrer. A 10-minute centrifugation at 10,000 rpm was then applied to the sample. The bright liquid above the sediment was gathered with a 0.22µm syringe filter and examined with an ultraviolet spectrophotometer. Analysis and notation of the results followed.

Determination of melting point:
The capillary melting point device was used to determine the melting points of DTZ and LDC. Here, the thermometer and a little vial of medication were assembled in a capillary tube with the ends cut off.

Simultaneous estimation of DTZ and LDC using vierodt's method:
The estimation's wavelength range is chosen:
After dissolving DTZ and LDC in separate portions of methanol, the stock solution was used to create suitable dilutions. To determine the drug concentrations, the solutions were scanned at wavelengths ranging from 200 to 400 nm.

Standard stock solutions (1000µg/ml) are prepared [15-18]:
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.

Setting up the calibration curve
A series of 10 ml volumetric flasks were filled with individual aliquots of0.1,0.2,0.3,0.4, and 0.5 ml of the produced stock solution of DTZ and LDC. Until the concentration range of 10–50µg/ml of both drugs was reached, methanol was added to the mixture. The solutions were scanned between 200 and 400 nm with methanol serving as the blank to determine absorbance.

Methodology

LDC and DTZ working standard solutions were scanned under ultraviolet light with a wavelength of 200–400 nm. The wavelengths used for the quantitative measurement of both compounds were found to have the highest absorbance, which was 262 nm for DTZ and 275 nm for LDC. Thus, the simultaneous equation approach (Vierordt's method) was used to determine the concentration of the two components.

UV Method validation:
Checks were made to ensure that the uv spectrophotometric approach was accurate, precise, linear, and robust [19-22].

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 analytical procedure is most precise, accurate, and linear when it encompasses both the greatest and lowest analyte concentrations in the specified range. The concentration range of DTZ and LDC used in the process was 10-50µg/ml. An exact measurement of the standard working solution of LDC and DTZ was made in order to evaluate the range.

Precision:
Accuracy of the approach was achieved by scanning and measuring the absorbance of LDC and DTZ three times (n=6) without changing the parameters of the suggested method. The intraday and interday precision of the recommended procedures were determined by analyzing the reaction three times on the same day and three additional days within a week for all standard solutions ranging from 10µg/ml to 50µg/ml for both medications. The data were reported using standard deviation and percent relative standard (% RSD).

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.[23]

Detection and quantification limits [24]:
In order to identify the limits of detection and quantification of the drugs, the following equation was used to calculate the standard deviation of response and slope of the linearity curve. It was designated by the international conference on harmonization (ICH) rules.
LOD: 3.3 × σ/ slope LOQ : 10 × S.D./slope

the slope of σ Lot size: 10 times the standard deviation divided by the slope.
In this case, ғ reresents the response's standard deviation. A calibration curve's slope is denoted by S.

Result and Discussion

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.

Determination of melting point:
A capillary melting point equipment was used to ascertain the melting point. The melting point of DTZ has been found to be 187°C, while that of LDC is 68°C.

An analytical technique for measuring drug concentrations (UV/visible approach):
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.

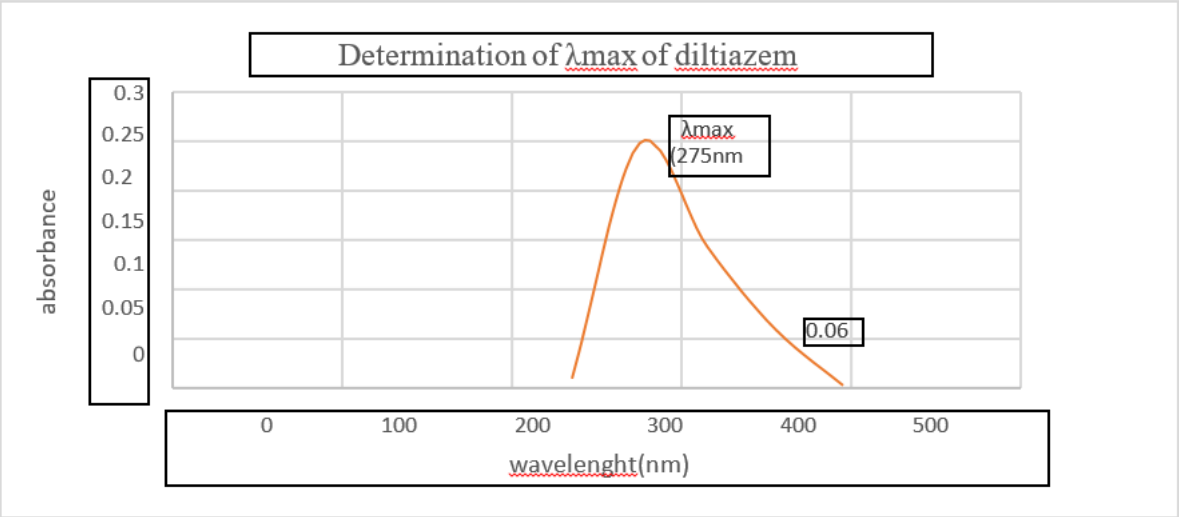

Figure 1: UV Spectrum of Diltiaze

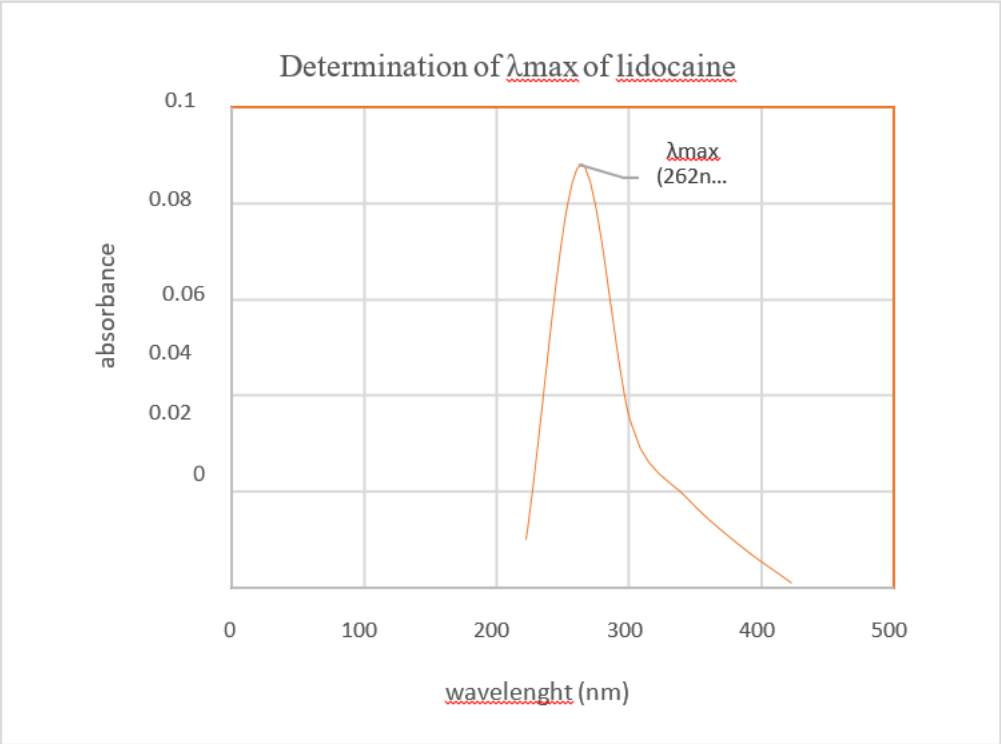

Figure 2: UV Spectrum of Lidocaine

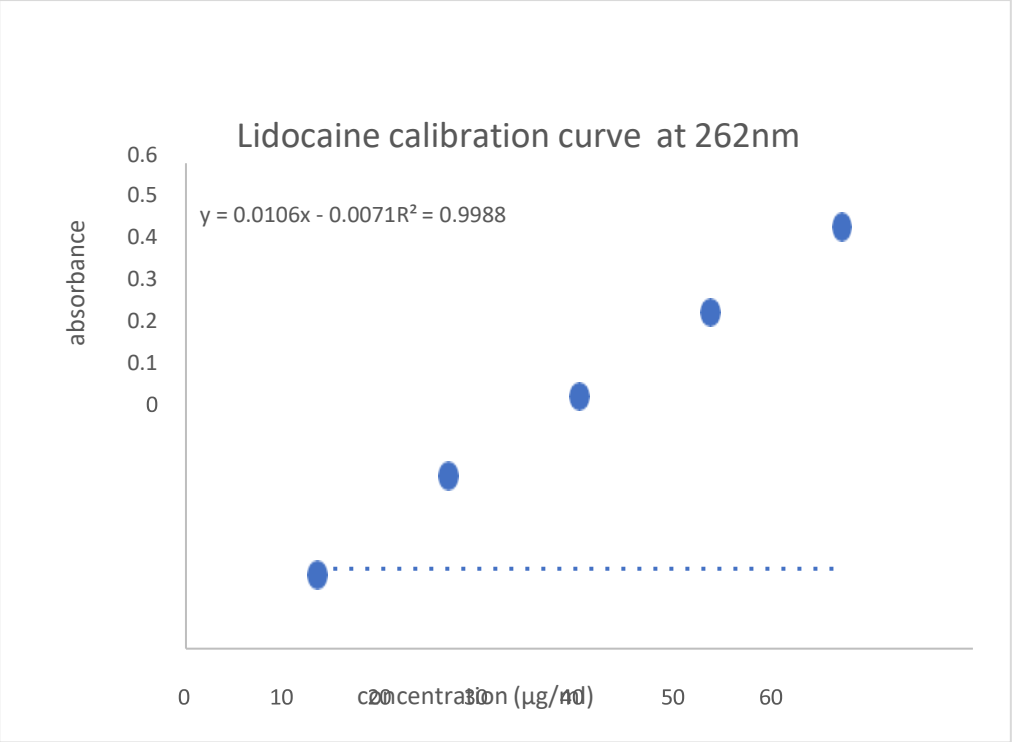

Figure 3: Calibration Curve of Lidocane

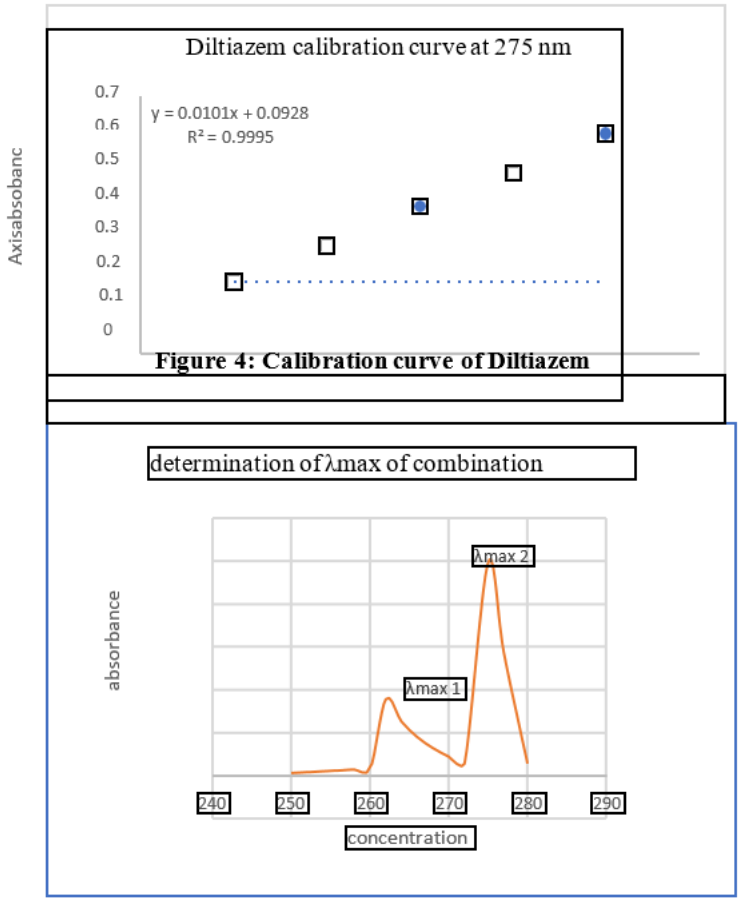

Figure 5: UV spectrum of sample

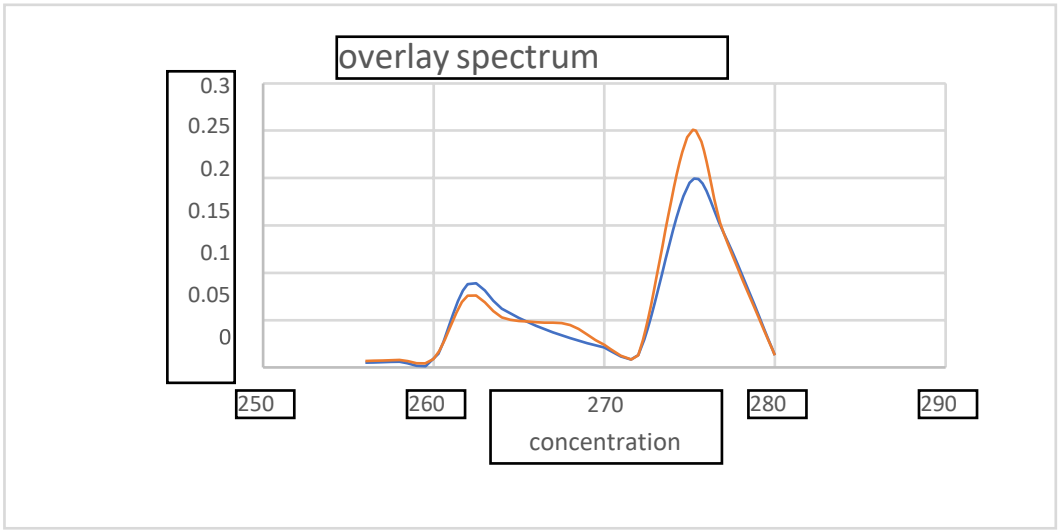

Figure 6: Overlay UV spectrum of the drugs

Method Validation:

Based on the ICH recommendations, the following parameters were used to validate the created method:

Linearity:

Within the concentration range of 10-50µg/ml, DTZ was determined to exhibit linearity. The equation that represents the straight line in this case is y=.0101x +.09228 and the regression

coefficient comes out to be 0.9995.
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
The analytical method's accuracy for DTZ and LDC was assessed at 80%, 100%, and 120% levels of standard solution. Table 2 displays the results for DTZ evaluated at 275 nm, whereas Table 3 displays the results for LDC measured at 262 nm.

Table 1: Recovery studies 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 Lidocane:

% level of recovery	Amount of drug taken (µg/ml)	Amount of drugspiked (µg/ml)	Absorbance	Actual conc. (amt. Recovered)	Mean Concn.	Std. Deviation		Mean% recovery
80	10	8	0.196	17.8	17.77	0.0577 3503	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	0.1527 5252	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	0.2081 666	98.64	97.88
120	10	12	0.237	21.6			98.18	
120	10	12	0.241	21.3			96.82	

For both the intra- and inter-day precision tests, the same optimal circumstances were used. Tables 4, 5, and 6 display the results of the accuracy (inter-day, intra-day, and repeatability) measurements, which demonstrate reliable reproducibility with relative standard deviations (% rsd) below 2%. That the process was so exact is evident from this.

Table 3: intraday precision data of diltiazem and lidocaine:

Sr. No	Sam (µg/ml)	absorbance at		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.	Sample 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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